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Dairy Foods Symposium: Todd R. Klaenhammer Memorial Symposium—Contributions to Our Understanding of Lactic Acid Bacteria

2000 Extending the Klaenhammer legacy by engineering probiotics using CRISPR. R. Barrangou*, *North Carolina State University, Raleigh, NC.*

The advent of CRISPR-based technologies has revolutionized our ability to manipulate the genetic content of organisms across the tree of life, and democratized genome editing across the globe. Repurposed from obscure adaptive immune systems in bacteria, CRISPR molecular machines have been broadly deployed in academia and industry in the past 10 years, to manipulate the genomes of organisms relevant to medicine, biotechnology and agriculture. We now have access to a portable CRISPR toolbox enabling flexible editing from a single nucleotide to large scale genome manipulation in diverse microbes, including lactic acid bacteria widely used as starter cultures and probiotics in the food supply chain. Extending the legacy of Todd R. Klaenhammer, I will discuss recent advances in engineering of starter cultures and probiotic bacteria spanning 1nt base editors from single Cas effectors, to >100,000 bp manipulations and island engineering using Cascade-Cas3 and CRISPR-associated Tn7-like transposons, spanning CRISPR-Cas systems classes and types, in endogenous and exogenous contexts. I will also highlight the practical limitations and implications of scaling up genome editing and deploying an expanded CRISPR toolbox, showcasing the path to engineering next-generation lactobacilli and bifidobacteria used as probiotics, to enhance human health and a more sustainable food supply chain.

Key Words: CRISPR, probiotic, genome editing

2001 Bacteriophage-bacterial interaction, then and now. C. Hill*, *University College Cork, Cork, Ireland.*

Todd Klaenhammer was a principal investigator at NCSU for almost 40 years. In that time he conducted pioneering work on probiotics, lactic acid bacterial (LAB) genetics and bacteriophages. In this presentation I will reflect on the advances in bacteriophage biology over the course of a single professional life, particularly focusing on areas where Todd made an outstanding contribution. Todd's career spans from a time where no single LAB bacteriophage had ever been sequenced (and sequencing even a single gene was the work of many months) to a time where thousands of bacteriophage genomes can be determined in one metagenomic sequencing run. Science has of course benefitted enormously from these advances, but something has also been lost in an era where overwhelming amounts of data can be generated in real time with little effort.

Key Words: bacteriophage, genomes, lactic acid bacteria

2002 Probiotics and prebiotics: A healthy gut and healthy aging. M. A. Azcárate-Peril*[1,2], M. Aljumaah[2], J. Gunstad[3], A. J. Ritter[4], D. A. Savaiano[5], J. W. Arnold[1], and J. M. Bruno-Barcena[2], *[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]North Carolina State University, Raleigh, NC, [3]Kent State University, Kent, OH, [4]Myosin Therapeutics Inc., Jupiter, FL, [5]Purdue University, West Lafayette, IN.*

Advancing age coincides with declines in gut homeostasis and cognitive ability. Our studies aimed to assess probiotics for mild cognitive impairment (MCI) and prebiotic galacto-oligosaccharides (GOS) in the aging gut. The first study investigated the gut microbiome of middle-aged and older adults with MCI compared with neurologically healthy individuals and the impact of probiotic *Lactobacillus rhamnosus* GG (LGG) in a double-blind, placebo-controlled, randomized clinical trial. Microbiome analysis identified *Prevotella ruminicola*, *Bacteroides thetaiotaomicron*, and *Bacteroides xylanisolvens* as taxa correlated with MCI. Differential abundance analysis at baseline identified *Prevotella* as significantly more prevalent in MCI subjects than cognitively intact subjects. A decrease in the relative abundance of *Prevotella* in response to LGG in the MCI group was correlated with an improved cognitive score. Our second study aimed to confirm and extend our previous reports that a 30-d administration of pure GOS significantly reduced symptoms and altered the fecal microbiome in patients with lactose intolerance (LI). We assessed the fecal microbiome of 377 LI patients randomized to one of 2 GOS doses or placebo. Taxa impacted by treatment and subsequent dairy consumption included lactose-fermenting species of *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, and *Streptococcus.* Secondary fermentation microorganisms included *Coprococcus*, *Ruminococcus*, *Blautia*, and *Methanobrevibacterium*. Finally, tertiary fermenters (use acetate to generate butyrate) included *Faecalibacterium prausnitzii* and *Roseburia faecis*. Microbiome analysis at 16 and 22 weeks after treatment further suggested relatively long-term benefits when individuals continued consumption of dairy products. Finally, we evaluated GOS in a pre-clinical study comparing gut aging in young versus old mice. Old animals had a distinct microbiome characterized by increased ratios of non-saccharolytic versus saccharolytic bacteria and a low abundance of β-galactosidases. We found that GOS reduced diversity and increased the abundance of specific saccharolytic and non-saccharolytic bacteria and β-galactosidases in young and old animals; however, a strong, homogeneous bifidogenic effect was not observed. Galacto-oligosaccharides reduced intestinal permeability and increased *MUC2* expression and mucus thickness in aged mice. Our studies show that probiotic and prebiotic interventions targeting the gut microbiome can preserve and restore cognitive health protecting the aging brain via the gut-brain axis, improving gut dysbiosis, reducing intestinal permeability and inflammation, and ameliorating digestive issues.

Key Words: prebiotics, probiotics, aging gut microbiome

2003 Innovation toward a dairy-based platform for effective next-generation probiotics. D. Mills*, *University of California, Davis, CA.*

Milk is well known to direct the enrichment of the neonatal gut microbiome, an assembled "organ" with important consequences on host health. A key driver of that guided enrichment are the glycans present in milk including free oligosaccharides, glycoproteins, and glycolipids. In the last decade, much research has focused on the molecular mechanisms by which free oligosaccharides enrich a beneficial gut microbiome in infants. Milk also contains an abundance of glycoconjugates presumed to participate in a similar enrichment of microbes in the gut, yet the molecular nature of this interaction is poorly defined. The dairy industry produces abundant glycoconjugate-rich streams that can be examined for their interaction with glycan-consuming commensal microbes. Recently, we employed a culturomics approach to identify specific bifidobacteria that grow on media containing whey protein phospholipid concentrate (WPPC), a glycoconjugate-rich byproduct of cheesemaking. Fecal enrichments with WPPC identified several bifidobacterial strains that acidified WPPC-containing media, suggesting it could grow on the

glycans in WPPC. Comparative genomics of *Bifidobacterium longum* strains that grew well on WPPC with strains that grew poorly identified unique genes putatively associated with WPPC growth. Transcriptomic analysis of one such strain, *B. longum* YK1048, revealed differential upregulation of carbohydrate-active enzymes on WPPC including a β-glucuronidase present in only WPPC fermentable strains. Determination of the molecular mechanisms responsible for robust growth of bifidobacterial strains on WPPC advances novel WPPC-based synbiotics for use in animals and humans. More importantly, precise chemical characterization of dairy glycoconjugates and their molecular interactions with beneficial gut commensals set the stage for tailored use of these byproduct streams to increase the efficacy of "next-generation" live bacterial therapeutics and probiotics.

Key Words: *Bifidobacterium*, whey protein phospholipid concentration, probiotics

ADSA-EAAP (European Federation of Animal Science) Speaker Exchange Symposium: Building a Resilient Dairy Sector—Circular Economies of Dairy Production and Dairy Foods

2120 Nutritional strategies to minimize carbon footprint and maximize nitrogen efficiency in dairy systems. N. I. Nielsen*[1], M. Ø. Kristensen[1], and M. Larsen[2], [1]*SEGES Innovation, Aarhus N, Denmark,* [2]*Aarhus University, Foulum, Denmark.*

Previous studies have shown that abomasal infusion with amino acids or casein in the first 28 DIM increase daily milk yield by 5–7 kg. The aim was to investigate the effect of alleviating the amino acid deficiency in the first 4 weeks (wk) after calving on performance in 15 wk using phase feeding strategies with feeds high in metabolizable protein (MP). Thirty-six multiparous Holstein cows were used in a randomized block design with 3 treatments and repeated measurements for the first 15 wk of lactation. At the day of calving, cows were randomly assigned to 1 of 3 treatments: 1) a control diet with 17 g of MP/MJ NEL fed for 15 wk (TMR1), 2) phase feeding with 20 and 16 g MP/MJ NEL (TMR2) fed wk 1 to 4 and wk 5 to 15 postpartum, respectively, and 3) phase feeding with 20, 17 and 15 g MP/MJ NEL (TMR3) fed wk 1 to 4, wk 5 to 10, and wk 11 to 15 postpartum, respectively. To achieve diets with high MP, soypass and corn gluten meal substituted partly barley, soybean meal, and canola meal. Treatments started on d 4 postpartum and all cows were fed the same diet until d 4 after calving. Data was analyzed with treatment (TMR1; TMR2; TMR3), period (period 1: wk 1–4; period 2: wk 5–10; period 3: wk 11–15) and their interaction as fixed effects, cow as random effect, and wk in milk within cow as repeated measurement. In period 3, dry matter intake was higher for TMR2 (26.2 kg/d) compared with TMR1 (24.9 kg/d) and TMR3 (24.1 kg/d) but did not differ in period 1 and 2 ($P < 0.05$; interaction). ECM yield was higher in period 1 for TMR2 (48.6 kg/d) and TMR3 (48.1 kg/d) compared with TMR1 (44.2 kg/d) whereas ECM yield did not differ among treatments in period 2 and 3 ($P < 0.05$; interaction). In period 1, N efficiency was higher for TMR1 compared with TMR2 and 3 and did not differ among treatments in period 2 and 3 ($P < 0.05$; interaction). In conclusion, increasing the postpartum MP supply using commercially available protein feeds starting at d 4 postpartum appeared to have limited effect on milk production and N efficiency after the transition period. However, it can be speculated that the extra MP supply should have been initiated before d 4 postpartum.

Key Words: phase feeding, protein deficiency, amino acids

2121 Understanding the production of beef from dairy systems in the UK: An analysis of trends. J. Gordon*[1], K. Glenk[1], V. Eory[1], E. Wall[1], and D. Moran[2], [1]*Scotland's Rural College, Edinburgh, United Kingdom,* [2]*University of Edinburgh, Edinburgh, United Kingdom.*

The breeding technologies and programs available to the dairy sector are allowing more dairy beef cross cattle (DBX) to be produced annually in the United Kingdom. This increase in DBX production is being driven by many factors including stakeholders, policy and public demand for high welfare and sustainable UK cattle production systems. This paper draws on comprehensive data from the UK cattle sector to analyze how DBX production has changed over time and what role the different actors play in the breeding decisions. It uses an interdisciplinary approach based on 47 semi structured interviews with farmers and stakeholders in the UK, and an analysis of the UK Cattle Tracing System database. The aim of the study is to provide further insights into risks and opportunities for future development of the cattle industry and to provide an insight to identify trends in the sector and underlying influencing factors. The results show that DBX are becoming an increasingly important production system in the UK cattle sector. The number of DBX animals slaughtered increased by 31% from 2010 to 2020. DBX animals are embedded in systems ranging from terminal intensive production to extensive breeding farms. The growth in DBX was accompanied by a growing reliance on two key breeds: the Aberdeen Angus and British Blue. Using interview data, we identify factors that influence breed selection for DBX. In the UK, DBX offers an opportunity to increase value of dairy calves to be marketed as beef animals. Farmers emphasize calving ease and ensuring marketability of the DBX calf as important considerations for breeding selection. Our analysis highlights both the challenges and opportunities for optimizing the DBX production to meet demand for high welfare and sustainable cattle. This includes achieving a better consensus in the breeding strategies, considering marketability and profitability both for the dairy (breeding) and beef (rearing) farmers involved in DBX production, and an increased involvement of genomic selection for better beef production traits in DBX animals.

Key Words: breeding, breed selection, beef on dairy

2122 Dairy coproducts can be useful feedstocks for the circular bioeconomy. J. Lucey*, *University of Wisconsin–Madison, Madison, WI.*

The dairy processing sector generates large volumes of byproducts from cheesemaking and Greek yogurt products. Dairy processors routinely extract the proteins from cheese whey due to its high value, but that leaves large volumes of a byproduct called permeate. The solids in this permeate consist of primarily lactose and some salts. Currently permeate is mostly used as an animal feed, some is used to make various grades of lactose. Greek yogurt production results in the generation of acid whey, which lack significant protein. It's high ash and lactic acid contents makes drying a challenge so much of it is land spread, risking potential negative environmental impacts due to possible leaching of nitrogen, phosphorus and other materials into soil and the groundwater. The large volumes, simple sugars (most lactose), and relatively consistent composition of these dairy byproducts makes them attractive as potential feedstock for biofermentation processes. Researchers are exploring the microbial engineering of microorganisms to efficiently ferment lactose into a range of chemicals. Some potential target chemicals include pure lactic acid, medium chain fatty acids, polyhydroxyalkanoates, various organic acids and various platform chemicals. Potential applications include bioplastics (currently only around 1% of plastics are biobased) and biochemicals to replacing fossil derived bulk/specialty chemicals (which are about 90% of currently used chemicals). Reactor conditions would need to be identified and optimized, and separation/purification methods would need to be developed to isolate the chemicals of interest to the required level of purity. Successfully achieving these steps could facilitate the dairy industry become a significant partner in the emerging circular bioeconomy, where dairy byproducts are no longer viewed as a type of "waste product" but instead become an important source of renewable feedstocks for biofermentation.

Key Words: circular bioeconomy, feedstocks, permeate

2123 Upcycling strategies of dairy byproducts and waste for value-added applications. S. I. Martínez-Monteagudo*[1,2,3], [1]*Family and Consumer Sciences, New Mexico State University, Las Cruces, NM,* [2]*Department of Chemical and Materials Engineering, New Mexico State University, Las Cruces, NM,* [3]*Center of Excellence in Sustainable Food and Agricultural Systems, New Mexico State University, Las Cruces, NM.*

The generation of waste and byproducts is ubiquitous in the dairy industry. Instead of landfilling or recycling, these streams can be turned into value-added products, such as sweeteners, micronutrients, ingredients, and additives. Valorization of these materials is multidisciplinary since it involves applying chemical and physical methods to create a desirable modification. This presentation summarizes our research efforts in creating value from dairy waste and byproducts. Upcycling is a guiding philosophy for the work presented here—a combination of processes that convert materials into new ones of higher quality and increased functionality. The upcycling strategies are illustrated under 4 scenarios: 1) converting lactose streams into sweeteners via heterogeneous catalysis; 2) extraction and fractionation of milk phospholipids with switchable solvents; 3) converting ice cream wastewater into functional peptides through subcritical hydrolysis; and 4) converting ice cream waste into biochar and crude oil via hydrothermal liquefaction. Illustrating upcycling in dairy streams has significant potential for improving circularity in the dairy industry.

Key Words: dairy streams, value-added, transdisciplinary

ADSA-GSD Competition: Dairy Foods Oral Presentations

2124 Impact of protein content and pH on the properties of microwaved shelf-stable cheese puff snacks. J. Pronschinske*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], R. A. Ibáñez[2], M. E. Johnson[2], and J. A. Lucey[1,2], *[1]University of Wisconsin–Madison, Madison, WI, [2]Center for Dairy Research, Madison, WI.*

Certain cheeses (e.g., Mozzarella) can be stretched and molded when heated. If a cheese can stretch, perhaps it can also be expanded during heating/drying to produce a crunchy, shelf-stable snack. To investigate this, we made a model system of pasteurized process cheese product (PPCP) and microwaved these PPCPs to produce a shelf-stable puff. Nine different PPCP treatments were made (n = 6) in a process cheese cooker using micellar casein powder, anhydrous milkfat, lactose, maltodextrin (MD), NaCl, trisodium citrate (TSC), citric acid (CA), and water. The protein (PN) content of PPCP varied from 28% (0% MD), 24% (~8.3% MD), and 20% (~16.8% MD); pH varied 5.8, 5.4, and 5.0 in a 3 × 2 factorial design. Water (35%), NaCl (1.1%), TSC (2.5%), and PN to fat ratio (1.12:1.00) were fixed for all treatments. Ingredients were mixed in the cooker and heated to 82°C for 2 min. Results were analyzed with SAS software and Tukey's HSD test. During heating, all PPCP had maximum loss tangents < 1 (i.e., did not melt) when measured via small-strain oscillatory rheology. Cylinders (9 mm high × 13 mm diameter) were cut from PPCPs, comingled (n = 3), and expanded in a 900-W microwave oven for 45–70 s at 100% power to attain 1.91 ± 0.54% moisture and 0.24 ± 0.05 A_w. 28% protein puffs (PP) expanded more (to ~6.5 mL, $P < 0.05$) than 24% PP and 20% PP (to ~4.2 mL and ~3.7 mL, respectively) when measured via glass bead displacement. Expansion was unaffected by pH for all PN levels. Puffs were evaluated at 1wk by trained panelists (n = 8–10) using a 15-point QDA scale for texture and flavor. Crunchiness, tooth-packing, and hardness were lower (~10, ~5.3, ~6.2, respectively, $P < 0.05$) for 28% PP than 20% PP. Tooth cling (TC), evaluated on a 5-point scale, was highest for 20% PP (~4.5, $P < 0.05$). Puff texture was affected by PN content rather than pH. All flavor attributes (except salt) were < 3.0. Scanning electron microscopy showed thicker cell walls for 24% and 20% PP. Our model PPCP system could puff/expand, in which MD added to control PN negatively impacted expansion/texture. PN content of the PPCP was more important than pH for its expansion properties.

Key Words: dairy snack, microwave, shelf-stable

2125 Sensory analysis, shelf stability, and cost analysis of manufacturing microwave vacuum dried cheese snacks. B. Gong*, A. Stelick, and C. Moraru, *Cornell University, Ithaca, NY.*

Microwave vacuum drying (MVD) can produce shelf stable cheese snacks through rapid, volumetric heating and reduced drying temperature. Depending on protein matrix strength and initial sample shape, the final product can be a puff or crisp. The desirability and viability of MVD cheese snacks are paramount for the dairy industry to know before investment, but information in literature is lacking. Therefore, the objective of this study was to assess the consumer acceptance, shelf stability, and cost of manufacturing MVD cheese snacks. A consumer panel (n = 86) of self-identified cheese snack consumers (age 18–73) evaluated cheese puffs (Mozzarella, mild Cheddar, and commercial Moon Cheese Cheddar) and crisps (Colby Jack, extra-sharp Cheddar, and commercial Parm Crisps). Participants rated different attributes on a 9-point hedonic scale and Just-About-Right (JAR) scale and picked their favorite puff and crisp. For the shelf-life study, Mozzarella puffs and extra-sharp Cheddar crisps were gas-flushed and stored at 21 ± 2°C or 35°C for 6 mo in metallic pouches. Water activity, texture, and color were measured monthly and peroxide value semi-monthly (for lipid oxidation). Analyses were performed in triplicate and data analyzed for statistical significance ($P < 0.05$) using the Tukey HSD test. The total cost of production was estimated on the capacity of a pilot-scale nutraREV 10 kW MVD unit (EnWave Corporation, BC, Canada) and average rates for cheese, labor, electricity, and packaging in NY state in 2022. Experimental samples had much higher overall liking and purchase likelihood than commercial samples ($P < 0.001$). Panelists favored mild cheddar puffs (57%) and extra-sharp cheddar crisps (48%). Texture was stable and lipid oxidation negligible over the storage period, but all samples browned over time ($P < 0.001$). Browning was highest in extra-sharp cheddar and slightly accelerated at 35°C. The total cost to produce a 50 g bag of MVD cheddar crisps was $2.98. Overall, this study demonstrates that MVD cheese snacks are a viable option with good consumer acceptability, shelf-life, and reasonable costs for dairy processors.

Key Words: microwave vacuum drying, cheese, snack

2126 Milk phospholipids protect *Bifidobacterium infantis* ATCC 15697 during in vitro digestion through changes in bacterial cell surface. E. Kosmerl*[1], B. Gonzalez-Orozco[1], I. García-Cano[2], J. Ortega-Anaya[3], and R. Jiménez-Flores[1], *[1]The Ohio State University, Columbus, OH, [2]National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico City, Mexico, [3]Arla Innovation Centre, Aarhus, Denmark.*

Bifidobacterium infantis is associated with the gut microbiota of breastfed infants and promotes intestinal and immune function through several proposed mechanisms, including by interactions between their surface polysaccharides and the host. Dairy foods and ingredients may provide benefits for their delivery and efficacy in the gut. Milk phospholipid (MPL)-rich ingredients have been increasingly recognized for their versatile benefits to health, including interactions with the gut microbiota and intestinal cells. Therefore, our objective was to investigate the capacity for MPL to promote survival of *B. infantis* during simulated digestion and identify bacterial surface changes in the presence of MPL. To achieve these aims, *B. infantis* was incubated with or without 0.5% MPL in MRS media at 37°C under anaerobiosis. All experiments were performed at least in triplicate using Student's t-tests ($\alpha = 0.05$). Survival across digestive phases using in vitro digestion was measured using plate count, along with adhesion to goblet-like intestinal cells. MPL significantly increased *B. infantis* survival at the end of the intestinal phase by 7% and decreased adhesion to intestinal cells. The bacterial surface characteristics, which may contribute to these effects, were assessed by ζ-potential, changes in S-layer proteins using comparative proteomics, and production of capsular polysaccharides (CPS). MPL significantly decreased the surface charge of the bifidobacteria from −17 to −24 mV. We identified a 57-kDa protein with at least a 2-fold expression change in the MPL treatment which appears to play a role in stress resistance mechanisms, potentially through CPS induction. The production of CPS was characterized using FTIR, HPLC, and TEM imaging. These techniques all suggest a significant increase in CPS production at least 1.7-fold in the presence of MPL. Prolonged survival during simulated digestion combined with the increase in bacterial polysaccharides suggest that MPL may be capable of promoting desirable functional properties through bacterial CPS production in foods and/or the digestive tract.

Key Words: digestion, probiotic, polysaccharide

2127 Evaluation of affinity between buttermilk proteins to hydroxyapatite and influence of physicochemical parameters.

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The volumes of buttermilk have increased markedly in the last decades. Cream churning release milk fat globule membrane (MFGM) residues in buttermilk. The MFGM is rich in health-promoting components such as phospholipids and membrane proteins, but these molecules impair cheese and yogurt properties. The isolation of MFGM would improve the techno-functional properties of buttermilk while recovering the MFGM bioactive components. Hydroxyapatite (HA) possess charged sites known to interact with MFGM compounds. The affinity of the main buttermilk proteins [casein micelles (CM), β-lactoglobulin (β-lg) and a-lactalbumin (a-lac)] and the MFGM to HA was studied. For each component, a quartz crystal microbalance diffusion analysis was performed. A maximum adsorption time and a attached mass density on the HA-coated gold sensor was determined. The results showed that β-lg and a-lac adsorbed readily with the adsorption equilibrium reached within 5 min with 10 and 12 $\mu g/cm^2$ attached mass density, respectively. For the CM and MFGM, the attached density versus time profile showed 2 phases characterized by a first adsorption phase reaching a first plateau, followed by an increase until reaching the maximal adsorption equilibrium. While the adsorbed mass densities of CM (26 $\mu g/cm^2$) and MFGM (22 $\mu g/cm^2$) were not significantly different, the adsorption time to reach equilibrium was significantly longer for the MFGM (700 min) than the CM (5 min) ($P < 0.05$). Therefore, the absorption rate was by increasing order: CM* > β-lg, a-lac* > MFGM*(*$P < 0.05$, ANOVA). Then, the influence of pH, ionic strength, and temperature on the affinity of each buttermilk component for the HA particles was assessed using a 3-levels and 3-factors Box-Behnken design, for which a full quadratic (second order) model was fitted for the response surface. The model showed that only the adsorption of the CM was affected by the temperature ($P < 0.05$) suggesting the possibility of extracting specifically the MFGM by adjusting the physicochemical conditions of the medium. Experiments are ongoing to determine the conditions allowing to fractionate MFGM directly from buttermilk.

Key Words: hydroxyapatite, milk fat globule membrane, adsorption

2128 Characterizing micellar casein and kappa-carrageenan gels: Ultrastructure, textural, and rheological analysis.

N. Pougher*, A. Vollmer, and P. Sharma, *Utah State University, UT.*

Highly Concentrated Micellar Casein Concentrate (HC-MCC) is a dairy ingredient with 17–23% casein in solution and is known to form a weak gel at cold temperatures (<10°C). HC-MCC requires a high protein content to form this gel. Kappa-Carrageenan (KC) is a common dairy stabilizer and could potentially assist gel formation. The aim of this study was to investigate whether KC could reduce the minimum protein content required for gelling, and its effect on textural properties in conjunction with pH adjustment and calcium chelation. Samples were diluted from 19% protein to 10% via deionized water and had 4 levels of KC added (0–0.3% wt/wt). Additional samples were adjusted to pH 7.0 or had 25mM trisodium citrate (TSC) added. Sample gels were mixed and tested using a 3-stage oscillatory rheological protocol. Gelation point was determined using a multiwave method named Winter and Chambon criterion during a temperature sweep from 40 to 5°C, where the loss tangent became independent of the frequency measured. Samples after rheological tests were harvested for TEM ultrastructure analysis at 2000x and 20,000x magnification. Additional gels were made for compressional texture profile analysis testing. HC-MCC diluted to 10% protein without any treatments did not form a cold gel. Addition of KC in increasing concentrations (from 0.1 to 0.3%) significantly raised ($P < 0.05$) gelling temperatures (19.9°C at 0.1% KC to 29.5°C at 0.3% KC) and storage modulus values (27.1 Pa to 1.74×10^3 Pa for the same samples). Samples with added TSC or pH adjustments formed a stronger gel than unmodified samples regardless of KC content level. However, texture analysis data shows that unmodified with 0.3% KC samples have the highest hardness (2.85 N) among all the treatments. TEM micrographs exhibited minimal interaction between casein and carrageenan within solution, with KC absorbing high amounts of moisture and concentrating the pockets of casein. These concentrated casein pockets increase protein-protein interactions and encourage gelation. This work will help develop an understanding of factors which could improve cold gelling ability of HC-MCC at lower concentrations. Further research in this area will create new opportunities for alternative uses.

Key Words: casein, rheology, gel

2129 Nutritionally enhanced, in-mouth, self-disintegrating milk protein puffs for infants and elderly.

J. Uhrin* and S. Rizvi, *Cornell University, Ithaca, NY.*

The objective of this study was to develop in-mouth, self-disintegrating puffs that are nutritionally enhanced with in process polymerization of galactose into galacto-oligosaccharides (GOS). The market of disintegrating puffs is dominated by carbohydrate-based puffs lacking protein. These puffs only cater to infants and do not address the needs of the elderly who require high protein foods and may also suffer from swallowing difficulties and lactose intolerance. To address these issues, lactose hydrolyzed (LHSMP) or unhydrolyzed skim milk powder (SMP) were combined with 80% milk protein concentrate (MPC80) powder and a calcium chelator, then processed using low-temperature, low-shear supercritical fluid extrusion (SCFX) with carbon dioxide (CO_2) to produce protein-rich puffs that disintegrate similarly to the commercial puffs. Calcium chelators and CO_2 induced acidity are known to prevent excessive protein interactions and thus helped in the creation of in-mouth, self-disintegrating puffs. Disintegration was evaluated using constant stress uniaxial texture analysis; the MPC80-based puffs had a faster disintegration rate (−0.610 mm/s) than the commercial puffs (−0.250 mm/s) ($P < 0.04$); while MPC80-LHSMP and MPC80-SMP puffs had disintegration rates equivalent to the commercial puffs ($P > 0.05$). Galactose in LHSMP as a precursor to the formation of GOS during extrusion was also investigated through gas chromatography, since GOS is an important class of prebiotics that exerts beneficial effects on intestinal microbiota and promotes gut health. As hypothesized, the low pH and controlled temperature enhanced the polymerization of galactose into GOS. The MPC80-LHSMP puffs had the highest content of short chain GOS with 2.3%wt GOS. The current study provides first valuable insights for the utilization of LHSMP and SMP to produce in-mouth, self-disintegrating value-added protein puffs for both the infant and elderly populations.

Key Words: lactose hydrolyzed and unhydrolyzed skim milk powder, self-disintegrating puffs, milk protein concentrate

2130 Processing effects on buttermilk fractionation. R. D. Melendrez-Alvarez* and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

Buttermilk (BM) is the by-product obtained during the butter manufacturing process. BM is an underutilized resource despite its high nutritional quality and composition. In addition to its nutritive value, the milk fat globule membrane (MFGM) contained in BM is well-known for its human health impact due to its phospholipids (MPL) and protein content. From literature and due to the interference of casein micelles, MFGM concentrates are obtained from liquid BM by pH control, sodium citrate addition, and membrane filtration. The present study aimed to obtain an enriched MFGM ingredient from the centrifugation of BM. The tri-factorial design of our experiments included BM from 4 different sources: commercial production (CP), pilot plant scale (PP), bench-top scale (BT), and resuspended commercial BM powder (BP); 2 temperatures of centrifugation: 4°C and 55.5°C; and 2 storage conditions before centrifugation: fresh liquid BM and BM subjected to one cycle of freeze-thaw; the whole procedure was repeated 3 times. To determine how MFGM MPL and proteins disperse during fractionation, high-performance liquid chromatography (HPLC) and sodium dodecyl sulfate-PAGE (SDS-PAGE) techniques were used. We observed that during the fractionation of BM, MPL and proteins aggregate, thus, increasing their density and forming sediment in the bottom of the separator. The yield of sedimentation increased significantly ($P < 0.05$) after BM was subjected to freeze-thaw, so we hypothesize it was due to MPL changes in chemical conformation. Separation treatments at 55.5°C resulted in a significant ($P < 0.05$) higher ratio of polar lipids (30.70 ± 9.84%) over fat ratio compared with 4°C separation (12.83 ± 6.68%). MPL profile was standard for 3 BM sources, showing changes only in the BP treatment attributed to the spray drying process during its manufacture. The resulting sediment was significantly enriched in the content of MUC1, XO, CD36, ADPH, BTN, and lactadherin only in the frozen batches ($P < 0.05$). Our results proved that with the centrifugation of freeze-thaw BM, an ingredient rich in MFGM can be recovered from centrifugation sediment.

Key Words: separation, milk fat globule membrane, phospholipids

2131 The impact of polymerized whey protein on the microstructure, probiotic survivability, and sensory properties of goat milk yogurt infused with hemp extract. H. Shi*[1], K. Freeman[2], E. Kawka[3], and M. Guo[1], [1]*Department of Nutrition and Food Sciences, University of Vermont, Burlington, VT,* [2]*Department of Emergency Medicine, University of Vermont, Burlington, VT,* [3]*Cattis LLC, Hardwick, VT.*

Hemp extract (HE) is a source of various bioactive compounds, including cannabidiol (CBD), terpenes and fatty acids. The natural astringency from hemp terpenes and the unpleasant goaty flavor of goat milk may negatively affect the sensory properties and popularity of HE-infused goat yogurt. Heat-induced polymerized whey protein (PWP) has positive effects on structure and flavor-masking properties. This study aimed to explore the effects of PWP on the physicochemical properties, microstructure, probiotic survivability, and sensory properties of HE-infused goat milk yogurt. Goat yogurts were prepared with ABY-3 starter cultures (0.1%, wt/vol) containing 2 probiotics *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12. Yogurt samples infused with HE, HE plus whey protein (0.5%, vol/vol), and HE plus PWP (0.5%, vol/vol) were made and compared with the control (plain). Hemp extract (0.264 g/L) containing 25 mg CBD was added to yogurt samples (160 mL). Cow yogurts were also prepared as references with the same fortifications. Addition of PWP did not change the chemical composition of yogurts in 4 groups ($P > 0.05$), but it significantly increased the viscosity of HE-infused goat yogurts (88.11 ± 29.57 Pa•s) compared with the control (25.76 ± 14.07 Pa•s) ($P < 0.05$). The microstructure photographs of HE-infused goat yogurts with PWP showed dense gel networking. The viability of *Lactobacillus acidophilus* LA-5remained above 10^6 cfu/g for the first 5 weeks in both goat and cow milk yogurt and then decreased quickly, while *Bifidobacterium* BB-12 maintained above 10^6 cfu/g for all samples during 9 weeks of the study. The preliminary results of the sensory evaluation indicated that PWP reduced the hempy flavor in HE-infused cow and goat yogurts and mitigated the goaty flavor in goat milk yogurts. In conclusion, PWP not only can improve the texture of goat milk yogurt but also can mitigate hempy and goaty flavors for the HE-infused goat milk yogurt and help improve consumer acceptance. Further work will focus on systemic sensory evaluation of these products.

Key Words: polymerized whey protein, hemp extract, goat yogurt

2132 Withdrawn.

2133 Effect of micellar calcium phosphate adjustment on casein micelle structure. E. Ahmadi*[1], T. Huppertz[2], and T. Vasiljevic[1], [1]*Victoria University, Melbourne, Victoria, Australia,* [2]*Wageningen University, Wageningen, the Netherlands.*

Micellar calcium phosphate (MCP) plays an important role in structural organization of casein micelle as well as its behavior during processing and digestion. The study aimed to establish how MCP content governs structural characteristics of the casein micelle. MCP content was adjusted using pH modulation, by either lowering the pH with glucono delta-lactone or increasing it by adding slowly 1.0M NaOH, followed by dialysis against 2 × 20 volumes of original skim milk for 72 h at 5°C. The MCP content was adjusted to 7, 26, 31, 42, 58, 67, 100 (control), 113, and 129% of the control milk, respectively. The turbidity, zeta potential, and particle size distribution of the casein micelles as well as the partitioning of calcium and milk proteins between colloidal and soluble phases were investigated. Changes in the secondary structure of proteins were characterized using Fourier Transform Infrared (FTIR) spectroscopy. The data were replicated 3 times on 3 different occasions. A one-way ANOVA followed by Tukey's multi-comparison of the means was applied to separate the means. The level of significance was pre-set at $P < 0.05$. The results indicate 2 defined areas in which substantial changes occurred regarding the micelle integrity. First, by lowering the MCP content from 100% to 42%, the samples underwent a considerable change depicted by a decrease in turbidity. Interestingly, lowering the MCP content to a half of the original amount did not result in a noticeable change in the average particle size. A second notable change was observed between samples with 42% and 31% of MCP content, when the casein micelle underwent several considerable physicochemical changes including a decline in the average particle size, turbidity accompanied with a remarkable rise in the concentration of individual caseins in the serum, which may indicate the starting point of disintegration of the casein micelle. FTIR analysis confirmed the substantial changes in the secondary structure of the casein micelle. The skim milk sample, which still contains 67% of initial MCP content appeared to be the lowest MCP amount attainable to maintain the casein micelle integrity. The amounts of calcium associated with proteins elevated in MCP enriched skim milk samples and enhanced the integrity of casein micelles. Such changes due to adjustment of MCP content in the casein micelle would likely lead to different skim milk functionalities.

Key Words: micellar calcium phosphate, casein micelle, pH modulation

ADSA-USD Competition: Dairy Foods Oral Presentations

2134 Dairy products in a pandemic culture. J. Bratton* and D. Olver, *Pennsylvania State University, University Park, PA.*

The COVID-19 pandemic played a major role in shifting markets for dairy products. Since the pandemic began, there was a transition from purchasing dairy products in-store to ordering a much larger portion online. In March 2022, the at-home food sales in the United States were 57% higher than that of the same week in 2020 (USDA). From March to August 2022, the amount of dairy products sold online increased by 200% in relation to the same time period in 2019. Many of the customers who purchased dairy products online claimed that it was a much more convenient and efficient option. Additionally, the constant threat of COVID-19 exposure as well as the "ease of price comparisons" added to the motivation to start purchasing products online early in the pandemic. This trend was consistent across age, gender, and income. A survey of dairy consumers by McKinsey revealed that experimentation with new dairy products increased during the pandemic, and many of these consumers thought that this trend would continue. Although there were sharp declines in food away-from-home spending during March and April of 2020, USDA's ERS stated that away-from-home spending returned to pre-pandemic levels by March of 2021. The demand for dairy products remains strong. Dairy food sales in October of 2022 were over 10% higher compared with October of 2021, indicating that consumers still have a strong interest in dairy products 2 years after the pandemic began.

Key Words: dairy products, pandemic, consumer preferences

2135 The saturated fat content in dairy products: A controversial look into the low-fat dairy food group recommendations in the USDA Dietary Guidelines for Americans. R. Hutton* and J. Bohlen, *University of Georgia, Athens, GA.*

Every 5 years, the United States Department of Agriculture and the Department of Health and Human Services release an updated version of the Dietary Guidelines for Americans. The current 2020–2025 version is a resource along with USDA MyPlate used by healthcare professionals and registered dietitians to provide the most recent and healthful nutrition information to the public. One of the core guidelines is to limit saturated fat content such as the fat in coconut oil, vegetable oil, fried foods, processed meats, fatty cuts of red meat, and full-fat dairy products. These saturated fats increase low-density lipoprotein levels in the blood and can increase the risk of stroke and cardiovascular disease. However, recent research concerning the saturated fat content in whole milk might contradict this notion. Studies are now showing evidence that dairy products high in saturated fat, such as whole milk, might have neutral and even positive effects on cardiovascular health and do not increase serum cholesterol levels. In addition, fat in milk and milk products increases the availability and absorption of fat-soluble vitamins such as vitamins A, D, and K compared with nonfat milk and dairy alternatives fortified with these vitamins, showing that plant-based alternatives such as soy, MyPlate's only comparable alternative to dairy, may not provide as many bioavailable nutrients as thought. Further research regarding the impact of saturated fats from dairy products on cardiovascular health and nutrient absorption is needed. This research may lead to future modifications of the USDA dietary guidelines.

Key Words: saturated fat, low-density lipoprotein, dietary guidelines

2136 Maximizing the potential of dairy as a functional food. J. Marston* and D. Winston, *Virginia Tech, Blacksburg, VA.*

Dairy products are a major player in the functional foods market and have the potential for continued growth. Research surrounding novel products such as yogurt with added bioactive components and fortification of milk with functional components provides insight into the health enhancing characteristics of dairy as a functional food. Functional foods are rapidly gaining popularity in the United States for their ability to promote health. They are valuable for more than the basic nutrient value and contain components that act to prevent disease and enhance health through unique properties such as antioxidative and anti-inflammatory properties. Common bioactive components found in functional foods include probiotics, catechins, and carotenoids. Several dairy products, such as yogurts, are considered functional foods. In fact, a large, growing portion of the functional foods market is dairy. Per capita consumption of yogurt has increased from 2.0 pounds in 1975 to 14.3 pounds in 2021. Potential novel products using chia seed extract, red ginseng extract, carotenoids or phenolics all present opportunities for the industry that have a potential to introduce new ways for consumers to enjoy dairy products. These products may have the ability to positively affect human health. Additionally, milk proteins can be used in many functional foods as they increase the bioavailability of certain functional components within foods. The food and beverage markets are constantly evolving as novel products are introduced. The dairy industry has the potential to utilize the growth of the functional food market to increase demand for dairy products through introduction of novel, health targeted products and marketing of the functionality of dairy products.

Key Words: functional foods, novel products, bioactive compounds

2137 The effects of using milk as a sports drink. M. Rossborough*, E. Lindner, and E. Miller-Cushon, *University of Florida, Gainesville, FL.*

While milk and dairy products are considered a core component of a healthy diet, milk is not commonly viewed as a sports drink despite potential positive attributes. Sports drinks are consumed to improve hydration when there is lower fluid availability or lack of replenishment fluid following exercise. Additionally, sports drinks help stimulate fluid ingestion, reabsorption, and retention. Current research suggests that milk is an excellent recovery drink after strenuous activity. Benefits of consuming milk post workouts with resistance training included an increase in muscle hypertrophy and lean mass. Research has found that the consumption of milk increases the rate of muscle protein synthesis which results in better net muscle protein balance. This effect of milk consumption is due to an increase in muscle protein synthesis which results in a better net muscle protein balance. Although milk is not the conventual post-workout drink, beverages containing milk products have been considered as an alternative to traditional carbohydrate and electrolyte-based sports drinks and may improve fluid retention. Milk also contains a higher volume of electrolytes and carbohydrate content compared with other sports drinks. Continued research is examining benefits of consuming milk post-workouts, specifically because of milk's beneficial elements such as high energy density, electrolytes, carbohydrates, and fat content. Consuming milk, or drinks containing milk products such as milk permeate, following a workout has the potential to improve fluid retention and muscle composition.

Key Words: milk, sports drink, human performance

ADSA-USD Competition: Dairy Production Oral Presentations

2138 Use of clove oil for disbudding in dairy calves. K. Brody*, M. Ellett, K. Daniels, and D. Winston, *Virginia Tech, Blacksburg, VA.*

In the US, dairy calves are commonly disbudded before 8 wk of age. Disbudding destroys horn-producing cells, preventing subsequent horn growth. A downside is that the procedure can cause pain. Currently, provision of pain relief during disbudding is recommended but not required in the US. New or refined disbudding techniques could improve calf welfare by minimizing pain. Research in other countries with clove oil (CLOV) shows promise in both disbudding efficacy (cytotoxic effect on horn-producing cells) and pain mitigation (analgesic effect). When used for disbudding in research settings, CLOV has been injected under or in the horn bud with or without prior administration of local anesthetic. In calves disbudded <7 d of age, Sutherland et al. (2018) reported an increase in laying time, increased pain sensitivity threshold, and decreased number of head shakes in CLOV disbudded calves (0.5 mL of CLOV under each horn bud) when compared with cautery disbudded calves. Efficacy of CLOV at stopping future horn growth was not examined. In a follow-up study, Sutherland et al. (2019) reported that 0.5 mL of CLOV under each horn bud (administered <4 d of age) was only 32% effective at preventing horn or scur growth whereas cautery disbudding was nearly 100% effective when examined at 16 mo of age. Dose of CLOV was examined in more recent work. Schoiswohl et al. (2020) found that efficacy of CLOV for disbudding is dose dependent, with 1.5 mL injected into the horn bud being as effective as cautery disbudding at preventing horn growth up to 9 mo (Schoiswohl et al., 2022). Efficacy of CLOV at mitigating pain was not examined in Schoiswohl et al. (2020, 2022). Despite showing some promise, it must be mentioned that CLOV is not currently an FDA-approved therapeutic in the US. To attain approval as both an analgesic and chemical agent that destroys horn-producing cells, US-based research with CLOV products must incorporate validated methods of pain assessment, along with measures of disbudding efficacy. Until such research is conducted and regulatory approval is attained, it is unlikely that use of CLOV for disbudding will be commonplace in the US.

Key Words: clove oil, disbudding, horn

2139 Changes to industry efficiency through genetic management plans. R. Finchum*, J. Beever, and E. Eckelkamp, *University of Tennessee, Knoxville, TN.*

Artificial insemination (AI) became widely implemented on American dairy farms in the 1940s. This technology broadened the genetic base as elite germplasm became more readily accessible. Genetic improvement slowed as AI became a mainstay in dairy herd reproductive and genetic management plans (GMP). Improvement decline could be combatted through dynamic GMPs using genomic testing (GT) in conjunction with AI. Genomic tests were first utilized in the US to evaluate sire merit in 2008 and have become advanced enough to analyze performance of both sires and dams. Through GT, generational intervals decreased from 7.0 to 2.5 yr for bull sires and from 4.0 to 2.5 yr for bull dams. This dramatic decrease demonstrated that GT provided more information for both parents compared with traditional AI matings. Genomic tests have increased selection intensity for low-heritability traits such as fertility, lifespan, and udder health from almost unmeasurable to favorable. The use of GT has remained limited due to cost, but implementation has improved dairy herd genetic merit, particularly in GMPs that used AI with sexed and beef semen. High performing animals, most often heifers, bred to sexed semen produced an appropriate number of high genetic merit replacement females while multi-parous and low-performing animals bred to beef semen produced crossbred progeny. These higher-value calves demonstrated heterosis, improved carcass traits, and prevented lower-quality genetics from entering the herd. An obstacle preventing GMP adoption was fear that beef-on-dairy crossbreds may flood the existing beef market. However, animals entering the beef market remained consistent but had improved feed efficiency and carcass quality. Considering these advantages, GMPs that selectively implemented sexed and beef semen based on dam GT resulted in the most potential for genetic, sustainability, and economic improvement.

Key Words: genomic testing, sexed semen, beef semen

2140 Have you heard? Hearing loss in the agriculture workforce. K. Jenkins* and D. Olver, *The Pennsylvania State University, University Park, PA.*

Agricultural workers face the risk of hearing loss due to prolonged noise exposure and other factors. A 2017 article by Masterson et al., found the Agriculture, Forestry, Fishing, and Hunting (AFFH) sector's audiograms indicated that hearing loss in the cattle rancher sector was 19%. AFFH overall hearing loss was found more frequently in younger individuals, with 13% more workers having hearing loss in the 18 to 35 age range compared with other industries. Farm workers in the dairy industry also face this issue. In a 1991 article, Marvel et al., found 65% of dairy farmers experienced hearing loss in the higher sound frequencies, but only 37% of nonfarm workers had this issue. Noise is the most common cause of agriculture work related hearing loss. To prevent this, the National Institute of Occupational Safety and Health recommends that workers not be exposed to more than 85 dB for a duration of 8 h. The ceiling for noise is 140 dB. Dairy farms are no stranger to loud noises. Areas with cattle can reach 70 dB, closed-cab tractors can reach 92 dB, and ventilation fans can reach 80 dB. In a 2022 article, Fetzer et al., recommends the use of personal protective equipment such as ear plugs and ear muffs to prevent the effects of loud noise in agriculture. Noise exposure is not the only cause of hearing loss in agriculture. A Nebraska study found exposure to ototoxic chemicals such as pesticides, detergents, and fumes from operating engines can cause damage to inner ear cells. Another study at Ohio State University found that exposure to organophosphate pesticides had a positive correlation with hearing loss. Hearing loss can cause a variety of issues such as sensorineural hearing loss and tinnitus. Sensorineural hearing loss is caused by loud noise exposure or chemical inhalation that causes damage to inner ear nerves or hair cells. Prolonged noise exposure can also lead to tinnitus, the perception of sound without external stimuli characterized by buzzing or ringing. Following proper hearing protocols on a dairy will ensure that dairy producers can maintain a safe hearing environment for themselves and employees.

Key Words: hearing loss, ototoxicity, employee safety

2141 Evaluating correlations between blood and urine in young dairy calves to develop reference ranges for non-invasive disease testing. R. Powers* and S. I. Kehoe, *University of Wisconsin-River Falls, River Falls, WI.*

On farm testing for specific diseases in calves is not common due to the timely and costly nature of the tests themselves needing expensive laboratory equipment. According to the 2014 National Animal Health Monitoring System, 33.8% of calves enrolled in the study had at least one morbidity event with 6% experiencing more than one event. In calves with a morbidity event, 73.8% of those presenting signs of illness received antibiotics however 9.8% received no treatment at all. Many farms treat calves based on symptoms without diagnosing the illness due to a lack of non-invasive and affordable tests. In a study done in 2008 by Maruta et al., urinary pH in 1.5-year-old steers was a good indicator of blood pH and base excess, but in younger calves, Mohri et al. (2006) reported different reference ranges. More research is needed to determine the correlation between urine and blood components in young dairy calves at this vulnerable stage of life. The development of correlations and definite reference ranges between blood and urine could be used in future studies to find ways to test for clinical or subclinical diseases on farm in young dairy calves. Providing farmers with an easy and inexpensive method of testing could help reduce morbidity and mortality in young dairy calves by being able to test non-invasively via urine on farm.

Key Words: dairy calves, urine, blood

2142 Poop for profit: An analysis of the use of anaerobic digesters. I. Revere*, E. Lindner, and A. De Vries, *University of Florida, Gainesville, FL.*

With global warming and climate change rapidly occurring, the agriculture sector has received criticism from the public surrounding carbon emissions. In the dairy industry, manure is produced at a large rate and releases methane gas when broken down, causing public concerns regarding greenhouse gases. Recently, farms have adopted the use of anaerobic digesters to reduce greenhouse gas emissions. Anaerobic digesters collect manure and harvest the biogas that is produced. Biogases consist of methane and carbon dioxide, which can be combusted to generate power or further processed into a form of renewable natural gas. Methanation of biogas allows it to be used by pipeline companies as a source of renewable natural gas. Besides the biogas, remaining digested materials can be used as bedding material or crop fertilizer around the farm. Previously, anaerobic digesters were mainly feasible for larger-scale farms, but recent research shows that co-digestion of manure with other organic material can significantly increase biogas yield, making anaerobic digesters more accessible and sustainable for smaller-scale farms. Although the anaerobic digestion process can be expensive and complicated for the typical dairy farm, the recent government push for reducing greenhouse gas emissions may make this process more accessible to farmers. Additionally, with the help of carbon credits and other programs, producers could make a profit from their methane production. In conclusion, the use of anaerobic digesters could aid the dairy industry toward a greener future, both financially and environmentally.

Key Words: dairy production, anaerobic digestion, sustainability

ADSA-USD Competition: Original Research Oral Presentations

2143 Effect of dietary replacement of alfalfa hay with sericea lespedeza hay on intake, performance, and milk composition of lactating dairy goats. T. Broussard, N. Mendez*, T. H. Terrill, B. Kouakou, and A. A. Pech-Cervantes, *Fort Valley State University, Fort Valley, GA.*

Sericea lespedeza (SL; *Lespedeza cuneata*) is a low-input forage legume with condensed tannins commonly used in small ruminant diets. However, there is little research on its effect in the diet of lactating small ruminants. Thus, the present study was conducted to evaluate the effect of replacing alfalfa (AL; Medicago sativa) hay SL hay on the performance and milk composition of early lactation dairy goats. Thirty early lactation dairy goats (<21 d in milk; DIM) were stratified by milk yield (MY) and DIM and randomly assigned to 2 (AL = TMR with 51% alfalfa and SL = TMR with 49% lespedeza) isocaloric (2.5 ± 0.02 Mcal/kg) and isonitrogenous (16.3 ± 0.05% CP) dietary treatments in a continuous lactation trial for 9 weeks. Dry matter intake (DMI) was adjusted daily (10% refusal), and MY was recorded daily. Feed, milk, and blood samples were collected weekly to estimate chemical composition, milk composition, and blood metabolites. Data were analyzed with the GLIMMIX procedure of SAS with the effect of treatment, week, interactions, and the random effect of the animal. No differences were observed between AL and SL in DMI (2.53 vs. 2.54 kg/d), MY (1.1 vs. 1.08 kg/d), milk fat (3.6 vs. 3.3%), milk protein (3.4 vs. 3.5%), lactose (4.3 vs. 4.3%), and milk solids (12.2 vs. 11.9%). Compared with AL, dietary SL increased ($P < 0.05$) blood urea nitrogen (2.69 vs. 4.2 mmol/g), but no differences were observed in blood glucose (75.3 vs. 71.9 mg/dL), and NEFA (0.13 vs. 0.13 mmol/g) concentrations. Compared with AL, dietary SL increased long-chain fatty acids (35.6 vs. 43.4%) but reduced ($P < 0.05$) short-chain fatty acids (63.3 vs. 56.8%), and polyunsaturated fatty acid concentrations (8.5 vs. 7.4%) in dairy goat milk. Compared with AL-based diets, dietary SL increased ($P < 0.05$) stearic acid (13.1 vs. 14.3 g/100 FAMES) and oleic acid (22.1 vs. 28.4 g/100 FAMES). In conclusion, the dietary replacement of AL hay with SL hay increased oleic acid in milk without affecting the intake and performance of lactating dairy goats.

Key Words: sericea lespedeza, dairy goats, milk yield

2144 Novel aspects of probiotics for improving cattle gut health. M. Moran*, A. Widenmann, M. Zhou, and L. Guan, *University of Alberta, Edmonton, AB, Canada.*

Antimicrobial resistance is an ongoing issue in dairy cattle due to the usage of prophylactic antimicrobials for promoting growth rates and reducing pathogen susceptibility. A possible substitute to reduce antimicrobial usage is probiotics; however, long-term and effective probiotic products are lacking due to the limited understanding of their mode of action in vivo. In addition, most of the probiotic products are not isolated from the target host resulting in these bacteria being unable to adapt to and colonize their gut. The objective of this research is to determine if probiotic bacteria isolated from the neonatal calf can interact with other commensal bacteria through participation in a cross-feeding pathway that promotes gut microbiome health. Two *Limosilactobacillus reuteri* strains were isolated from the cecum and colon of 2-d-old healthy calves and, total DNA was extracted from the pure culture, and subjected to whole genome sequencing using the shotgun method. The *pduCDE* operon was identified in both strains using NCBI's blast2 function by comparing against the annotated genome of *Lm. reuteri's pduC* (DQ223722.1), *pduD* (KJ435308.1), and *pduE* (DQ233724.1). Strain 1 and 2 are 83.46%, 100%, 89.21%, and 83.46%, 100%, and 89.27% similar to the *pduC*, *pduD*, and *pduE* genes, respectively, providing the first evidence of this microbial pathway within cattle. The *pduCDE* operon is required to metabolize 1,2-propanediol (1,2-PD), a by-product from dietary fiber digestion by other commensal bacteria, and to produce acetate and propionate. Future studies are needed to assess the effect of 1,2-PD on these 2 *Lm. reuteri* strains' growth and metabolism. This information will provide a scientific foundation to discover effective probiotics to promote gut microbiome health and improve dairy production.

Key Words: probiotics, gut microbiome, 1,2-propanediol

2145 Understanding the functional properties of dairy powders with varying protein, lactose content and particle sizes. N. Pace*, A. Parhi, and P. Sharma, *Utah State University, Logan, UT.*

High protein dairy powders are an excellent source of protein and an essential component of numerous commercially available food products. Understanding the physical attributes helps create an ideal product for consumers which is easier to produce, package, and transport. Dairy powders consist of multiple components such as protein, lactose, and fat. The storage, packaging, and transportation of these are affected by their physicochemical properties, thus necessitating an understanding of their relationship. In this study, lactose-rich dry whey (DW) (12.3% protein: 77.6% lactose), whey protein-rich WPC 80 (79.84% protein: 9.3% lactose), and casein-rich micellar casein concentrate (MCC) (87.14% protein: 2.1% lactose) were the 3 dairy powders used. Various physico-chemical tests were conducted, including loose, tapped, and particle densities, occulated air content, wettability, sink-ability, insolubility index, particle size distribution, and water activity. The Carr index and Hausner ratio for determining flowability were calculated from the bulk density measurements. Powders were subjected to particle size measurement using an Anton Paar particle size analyzer. Mean particle size (d43) of DW, WPC 80 and MCC were 115, 113, 86 µm, respectively. WPC 80 powders had a significantly higher ($P < 0.05$) tapped bulk density (1.03 g/mL) followed by DW (0.9 g/mL) and lastly MCC (0.42 g/mL). These differences could be attributed to the different extent of presence of monodisperse particles in the powders. Concurrently, DW powders exhibited better reconstitution properties with a lower sinking time, wetting time, and insolubility index. This may be attributed to the higher moisture affinity of lactose crystals. WPC 80 and MCC powder showed slightly lower reconstitution properties, which could be related to stronger protein-protein interactions taking place from exposure to heat during evaporation and drying. Furthermore, the Hausner ratio and Carr index for DW were lower than WPC 80 and MCC (1.17 and 14.7 respectively) indicating a higher flowability. This could be attributed to the presence of lactose crystals in DW. Overall, this study would further the understanding of dairy powder manufacturers about the effects of powder structure on functional properties, thus reducing wastage, improving sustainability and consumer appreciation.

Key Words: dairy powder, functional property

2146 Fit or flabby: Can we simplify the body condition scoring system? E. M. Schafer* and B. A. Wenner, *The Ohio State University, Columbus, OH.*

Body condition scoring (BCS) on a 1–5 scale is a common method of assessing dairy cow body fat reserves, serving as a management tool to consistently identify patterns and address herd nutrition, reproduction, and health. However, the efficacy of scoring in different BCS point increments has not been well described. Our objective was to evaluate the variation in BCS based on scoring in half- (HALF) or quarter-point (QRT) increments and to evaluate the accuracy of categorically assessing cows as fat, thin, or ideal (CAT) for current lactation stage compared with assigning traditional BCS. Our hypothesis was that HALF scoring would not increase variation of managerial value. We further hypothesized that CAT score would be interpretively similar to traditional BCS. Jersey cows (n = 20) balanced by lactation stage were scored by undergraduate students (n = 14) of varying experience for 6 weeks. Before trial initiation, all participants received BCS training and were provided an on-farm scoring guide. Each week, participants assigned QRT, HALF, and CAT scores. Statistical analysis included a SAS mixed-model approach (fixed effects: scoring method, lactation stage, week, scorer experience; random effects: scorer, cow) with repeated week as appropriate; residuals were regressed against predicted BCS from HALF, centered to the mean. CAT method was on a 2–4 scale; QRT and HALF were on a 1–5 scale. Regression of the residuals indicated a mean bias ($P < 0.01$) where HALF underestimates herd BCS by 0.018 and a slope bias ($P < 0.01$) where HALF increased by 0.110 points greater than QRT for every whole point BCS. Scoring method influenced mean score ($P < 0.01$) where CAT was lower than QRT or HALF by 0.055. Novice scorers estimated cows 0.24 points lower than more experienced scorers ($P < 0.01$). Variance of QRT and HALF were not different ($P = 0.73$). These data indicate that HALF score could be equally accurate as QRT, while potentially decreasing producer effort and time to score. Further work is needed to support these results on farms with different breeds and management styles and with scorers of different ages and backgrounds.

Key Words: nutrition, cow grouping, transition

2147 Evaluating the influence of heritable metabolic and biological factors during the periparturient period on resumption of cyclicity postpartum.

M. Shaffer*, S. Johnson, T. Marins, S. Tao, and J. Bohlen, *University of Georgia, Athens, GA.*

Genetic, metabolic, and environmental variables have large implications for the reproductive success of dairy cattle. While some of these factors are well documented, the interplay of genetic and environmental influence is still in need of further discovery. Therefore, the aim of this study was to investigate a metabolic marker for energy partitioning (insulin) and a fertility factor (anti-Müllerian hormone (AMH), and their influence on resumption of cyclicity postpartum. For this study, Jersey (n = 13) and Holstein cows (n = 22) were enrolled −14 d before calving (d 0). Blood samples, milk production, and body condition scores (BCS) were collected at −14 d, −7 d, 0 d, 7 d, and 14 d. Blood samples were analyzed for NEFA (µEq/L), Insulin (µg/L), and glucose (mg/dL) with insulin sensitivity calculated using the RQUICKI method, with a lower value indicative of higher insulin resistance. All cows were fitted with a DeLaval activity meter and monitored from day of calving until first estrus. An estrous event was determined using the program algorithm with a ++ and +++ and/or estrous intensity (EI) of >120% with EI and DIM recorded for the event. As expected, glucose values decreased in the postpartum period while NEFA levels rose ($P < 0.05$). Insulin levels decreased postpartum in all animals ($P < 0.05$). Concurrently, RQUICKI rose during the postpartum period with d −14, d −7, and d 0 values all being lower than those at d 7 ($P < 0.05$). RQUICKI was not influenced by BCS ($P > 0.05$). Interestingly, Jerseys had higher insulin concentrations at all time points ($P < 0.05$) compared with Holsteins though there was no breed effect on RQUICKI. The d 14 RQUICKI tended to have a negative correlation with estrous intensity ($P = 0.08$); however, there were no indications that glucose, NEFA, or RQUICKI had any effect on DIM at first estrous event ($P > 0.05$). Finally, AMH was higher in animals that had a normal resumption of cyclicity (214% ± 31.7) compared with delayed (110% ± 25.1) ($P < 0.05$). Metabolic tendencies were as expected however breed differences in insulin and its implication for reproduction is an avenue for future investigation.

Key Words: insulin resistance, nonesterified fatty acid, periparturient

2148 Pre-partum anti-inflammatory therapies in high-priority cow groups: Effects on metabolic status, systemic inflammation, and daily milk production.

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The objective of this study was to assess the effects of pre-partum administration of anti-inflammatory therapies on body condition score (BCS), β-hydroxybutyrate (BHB) concentration, haptoglobin (HP) concentration, and daily milk yield in high-priority cow groups (over conditioned cows [≥3.75 pts.], primiparous cows, and cow with calving disorders [i.e., stillbirth, dystocia, and/or twins]). At 14 d before the expected calving date, cows (n = 170) and heifers (n = 63) were blocked by BCS category (optimal = 3–3.5; high ≥3.75) and parity (nulliparous; parous), and randomly allocated to one of 3 treatment groups: 1) ASA (n = 80): receive one oral administration of acetylsalicylic acid (4 boluses; 480 grain/bolus; 125 g/d); 2) MEL (n = 76): receive one oral administration with meloxicam (1 mg/kg of BW), or 3) PLC (n = 77): receive one oral treatment with gelatin capsules. Body condition score was assessed, and blood samples were collected (i.e., BHB and HP concentration assessment), weekly starting 1 week before treatment until 3 weeks after calving. Daily milk yields were collected for the first 150 DIM from on farm computer records. The data were analyzed using MIXED procedure of SAS as a randomized complete block design. There was no difference on BCS and BHB concentrations in the first 21 DIM between treatment groups. Cows and heifers treated with ASA tended ($P = 0.06$) to have lower HP concentration compared with cows and heifers treated with MEL and PLC (ASA = 20.52 µg/mL, 95% CI = 9.87–32.97; MEL = 34.47 µg/mL, 95% CI = 20.32–51.29; PLC = 28.43 µg/mL, 95% CI = 16.09–42.94). Primiparous cows treated with MEL produced 4.67 kg/d and 4.92 kg/d more milk compared with ASA and PLC cows, respectively, during the first 150 DIM (ASA = 35.93 ± 1.39 kg/d; MEL = 40.62 ± 1.49 kg/d; PLC = 35.70 ± 1.51 kg/d; $P = 0.02$). These findings suggest that treatment with pre-partum anti-inflammatory therapies may have positive effects on systemic inflammation and daily milk yield in high priority cow groups.

Key Words: prepartum anti-inflammatory therapies, systemic inflammation, daily milk yield

2149 Effect of cooling and *Moringa oleifera* supplementation on milk composition of heat stressed sows.

E. M. Tobolski*[1], L. M. Beckett[1], W. Ogundare[2], M. Stansberry[1], T. M. Casey[1], A. Schinckel[1], and R. C. Minor[2], [1]*Purdue University, West Lafayette, IN,* [2]*North Carolina Agricultural and Technical State University, Greensboro, NC.*

Heat stress increases oxidative stress and is linked to decreased milk production. *Moringa oleifera* is a high fiber, high antioxidant feedstuff. We conducted a 2 × 2 factorial study to determine the effect of conductive

cooling and 4% Moringa supplementation on milk composition and litter growth across lactation of heat stressed sows. We hypothesized that Moringa supplementation and cooling will increase fat and protein percent in milk. Sows (n = 48) blocked by parity and back fat thickness were assigned to 1 of 4 treatments: heat stressed (HS), heat stress + cooling (C), HS + Moringa (M), C + M. Sows were moved to farrowing crates at 100 d of pregnancy and limit fed control gestation or gestation diet + 4% M and exposed to ambient room temperature. At 110 d of gestation animals were changed to a control or 4% M lactation diet, and rooms were heated to 33°C. Gestation and lactation diets were formulated to be isoenergetic. Half of the sows in each diet had a conductive cooling pad installed in the floor of the farrowing crate. Colostrum (d0) was manually collected within 3 h of initiation of farrowing and milk was collected on d3 and d14 of lactation after the administration of oxytocin. Percent of fat and protein in colostrum and milk samples were measured using Creamatocrit and Bicinchoninic Acid (BCA) assays. Mixed model analysis indicated average daily gain (ADG) was not affected by treatment, and a tendency for a diet effect ($P = 0.07$) on milk fat, with the C+M and HS+M having the highest percent across all days. There was an effect of day ($P = 0.04$) on fat percent, with d0 lower than d3 and d14, but d3 and d14 did not differ from each other. Cooling did not affect percent fat of milk. There was an effect of day ($P < 0.0001$) on protein percent with d0 > d3 and d14, and d3 > d14. There was an interaction between cooling and diet ($P = 0.0013$) on d14 protein, with HS+C and C+M being the highest. Our study revealed that Moringa effects milk fat percent across all days which supports our hypothesis. However, treatment effects on milk macronutrient content did not significantly influence ADG of piglets.

Key Words: heat stress, *Moringa*, milk composition

Animal Behavior and Well-Being Symposium: Hot Topics in Calf Management—Welfare Considerations from Birth to Transport

2150 Cow-calf separation: Public acceptance and scientific evidence. M. A. G. von Keyserlingk* and D. M. Weary, *University of British Columbia, Vancouver, BC, Canada.*

Early cow-calf separation is standard practice on most dairy farms around the world. However, a growing body of evidence of public discomfort with this practice, undermining the dairy industry's social license. Proponents of early separation argue that artificial rearing improves health, prevents the development of abnormal behavior in calves and results in improved milk production. During this presentation, we review the latest available evidence on public acceptability of cow-calf separation and discuss reasons why this practice fails to resonate with societal values. We also summarize the findings of 2 recent systematic reviews that synthesized the available research on the effects of prolonged cow calf contact on 1) acute responses of cows and calves to separation at different times, and long-term effects on calf behavior, welfare and productivity (calf growth and cow milk yield) and 2) cow and calf health, including calf scours, Cryptosporidiosis, Johne's disease, pneumonia, passive transfer, mortality, and the dam's udder health. We conclude that there is little evidence that early separation improves the health of dairy cows or calves, or that this practice improves whole-lactation milk yield. Indeed, the available evidence indicates that a period of extended cow-calf contact can have positive effects on calf behavior and growth. We also highlight areas requiring further research to help farmers interested in adopting prolonged cow-calf contact systems.

Key Words: cow-calf contact, public attitudes, nurse cows

2151 Calf housing and social impacts. E. K. Miller-Cushon*, *University of Florida, Gainesville, FL.*

Housing practices for calves vary widely in the dairy industry today and an understanding of dairy calf behavior is driving changes to refine calf rearing. Calf management influences early life experiences, having implications for behavioral development, cognition and response to novelty, and abnormal behaviors. Social housing supports the development of social bonds between calves and eases adaptation to novel social groups. Social housing is particularly beneficial when introduced early, with behavioral benefits of social housing evident in the first weeks of life. Social housing also influences the development of both feeding patterns and competitive behavior. Cumulative effects of calf social housing on social and feeding behaviors may mediate longer-term effects on behavior and performance and there remains a need to examine these effects in adulthood. Beyond influences on social development, socially reared dairy calves are less reactive to novelty and may have improved ability to navigate management transitions. Social housing, in addition to other aspects of environmental complexity, also relates to the development of abnormal oral behaviors. While cross-sucking is a concern in socially housed calves, the duration of individual abnormal oral behaviors is greatest in individually housed calves raised in relatively restrictive environments. Providing opportunities for dairy calves to perform a range of motivated natural behaviors, including social interaction and feeding behavior, is key for calf behavioral development and likely to have long-term welfare implications.

Key Words: dairy calf, social housing, social behavior

2152 Transportation impact on preweaned calves. C. Cramer*, *Colorado State University, Fort Collins, CO.*

An important animal welfare concern across the US dairy industry is the transportation of preweaned calves from the source dairy to a calf-raising facility (e.g., calf ranches, heifer raising facilities, veal operations), auction, livestock market, or directly to slaughter. The increased adoption of breeding dairy cows with beef semen has resulted in more surplus calves being transported within the first week of life, garnering additional attention to calf transportation practices. Transportation stressors include limited (if any) access to food and water, commingling, environmental temperature changes, and a variety of handling techniques. Neonates are particularly vulnerable to transportation stressors due to their decreased ability to thermoregulate, underdeveloped immune system, and immature physiologic stress responses. Given that calves in the US are often transported at an average age of 3 d, and in many cases, less than 24 h of age, there is a critical need to address these welfare concerns. In addition to age, fitness for transport is a key welfare consideration; recent data from the US demonstrates that some source dairies transport compromised calves (i.e., dehydrated, diarrhea, navel inflammation, etc.), leading to significant welfare challenges during transportation. Calves arriving at US veal facilities have been reported to be dehydrated, lethargic, hypoglycemic; and/or have poor body condition, navel inflammation, and diarrhea. It is also possible that calves become compromised in transit to their destination. Thus, there is ample opportunity to target decision-making and producer-focused education not only at the source dairy, but at each stage of transportation to address critical welfare concerns. This presentation will summarize relevant research and key welfare issues relative to calf transportation and identify current gaps in knowledge.

Key Words: surplus calves, transport, welfare

2153 Thermal stress impact on calves. J. Van Os* and K. Reuscher, *Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI.*

Aspects of calf welfare, such as the need for social contact and thermal comfort, have the potential for synergy or conflict. Calves expend energy to produce heat and maintain homeothermy at higher ambient temperatures relative to older cattle. Cold stress negatively affects growth performance and health. Mitigation strategies largely focus on nutritional interventions or by limiting heat loss with resources such as bedding or jackets. A yet-undocumented potential strategy to conserve body heat is for calves to huddle together in pair or group housing. Numerous other benefits of social housing have been documented in terms of calf social and cognitive development, welfare, and growth. Conversely, in the warm season, close social proximity could perhaps exacerbate heat stress, but this has not been investigated. Relative to older dairy cattle, less attention has been given to heat abatement strategies for pre-weaned calves. Providing supplemental shade (e.g., shade cloth over outdoor hutches) can reduce environmental heat gain. Strategies to promote heat dissipation include providing fans in indoor housing or increasing the apertures on outdoor hutches to improve air exchange and airflow. In Wisconsin, we conducted studies of calves housed in outdoor hutches, where they are exposed to seasonal extremes. We evaluated how social contact, when calves are housed in pairs of adjacent plastic hutches connected with an outdoor area, interacts with thermal comfort in

winter vs. summer. In summer, we additionally investigated the effects of hutch ventilation to mitigate heat stress. Preliminary results show that pair-housed calves prefer to spend most of their time together across weeks of life, regardless of season. In winter, ambient temperature inside the hutch increases more when there are 2 calves vs. 1 calf inside. In summer, hutch ventilation, but not the number of calves, affects the hutch microclimate; furthermore, ventilation reduces calves' thermoregulatory indicators of heat stress. These findings suggest that pair housing does not conflict with thermal comfort in either winter or summer, further building the case for promoting good calf welfare through social housing.

Key Words: cold stress, heat stress, heat abatement

Animal Health 1

2154 Bovine monocyte-derived macrophages exhibit endotoxin tolerance after repeated stimulation with lipopolysaccharide. H. L. Reisinger*, L. K. Mamedova, and B. J. Bradford, *Michigan State University, East Lansing, MI.*

Prolonged infection or repeated exposure to bacterial endotoxins can lead to the development of endotoxin tolerance in immune cells, and in some cases, the animal as a whole. Endotoxin tolerance occurs when cells become less responsive to endotoxin stimulation, resulting in an immune-tolerant state. To study endotoxin tolerance, bovine monocytes from healthy mid- to late-lactation dairy cows (n = 3) were isolated from whole blood by density and plate adherence and maintained in cell culture media for 7 d until differentiated into macrophages. On d 7, cells were stimulated with 0 or 100 ng/mL (HI) of lipopolysaccharide (LPS) for 24 h then re-stimulated for an additional 6 h with 0, 10 (LO), or 100 (HI) ng/mL LPS (3–6 replicates per treatment). RNA was harvested following 6-h restimulation to analyze mRNA abundance via RT-qPCR. Control genes were *UXT* and *YZ*; the geometric mean Ct value was unaffected by treatment and used as a control to determine target gene relative transcript abundance. Data were analyzed to assess the fixed effect of treatment while accounting for the random effect of source cow (JMP). Cell viability, measured by resazurin metabolism, was unaffected by treatment ($P = 0.36$). Following a single stimulation with 100 ng/mL LPS for 24 h, *TNFα* and *IL-6* abundance were similar to unstimulated cells but elevated for *IL-1β* by 33 ± 16-fold and *IL-10* by 1.7 ± 0.25-fold (both $P < 0.05$), suggesting that some but not all targets returned to baseline. Cells treated with HI then LO LPS exhibited signs of endotoxin tolerance; transcript abundance of proinflammatory cytokines *TNFα* and *IL-6* were reduced after LO restimulation by 85 ± 33% and 68 ± 36%, respectively, compared with LO LPS without pre-treatment (both $P < 0.05$). LO restimulated cells had a 71 ± 12% reduction in *IL-10* mRNA compared with LO LPS without pre-treatment; HI restimulation also reduced *IL-10* mRNA by 53 ± 14% compared with HI LPS without pre-treatment (both $P < 0.05$). Reduced cytokine mRNA abundance after repeated LPS stimulation in bovine monocyte-derived macrophages suggests that prolonged Gram-negative infections may reduce immune cell function.

Key Words: immune memory, immune response, innate immunity

2155 Effects of β-caryophyllene supplementation on dry matter intake and productivity of late-lactation dairy cows through repeated lipopolysaccharide challenges. J. Fehn*[1], K. Krogstad[1], L. Mamedova[1], Maya Zachut[2], H. Reisinger[1], and B. Bradford[1], [1]*Michigan State University, East Lansing, MI,* [2]*Agricultural Research Organization, Volcani Center, Rishon LeZion, Israel.*

β-caryophyllene (BCP) is a terpenoid phytochemical that is relatively abundant in plants such as black pepper, oregano, and cinnamon, and is a ligand for cannabinoid receptor 2. Cannabinoids such as BCP reduce inflammation, but these effects have not been tested in dairy cattle with purified BCP. Twenty lactating Holstein cows (240 ± 60 DIM; 33 ± 6.9 kg/d of milk) with SCS <4 were enrolled in a randomized complete block experiment lasting 70 d. Cows received 5 mg/kg BW liquid BCP or no top-dress (CON) for 42 d. On d 28 and 56, cows were challenged in the right rear quarter (RR) with 100 µg of lipopolysaccharide (LPS). Quarter-level milk (RR) was collected at 0, 8, 16, 24, 48, 72, 96, 120, 144, and 168 h after each LPS challenge. Data were analyzed with linear repeated mixed models which included fixed effects of treatment, time, parity, and their interactions; block and cow were random effects. Before LPS, BCP did not affect DMI (24.4 vs. 24.9 ± 0.85 kg/d for BCP vs. CON), milk yield (35.5 vs. 37.0 ± 1.6 kg/d), milk composition, SCS, or plasma glucose concentration ($P > 0.10$). After each LPS challenge, BCP did not affect DMI, milk yield or composition, but reduced composite milk SCS in first-lactation animals (1.59 vs. 3.23 ± 0.39 SCS, $P < 0.01$; treatment × parity $P = 0.01$). A treatment × time interaction ($P < 0.001$) for SCS revealed a tendency for more complete resolution in BCP-treated cows at d 52 (0.87 vs. 2.42 ± 0.49 SCS, $P = 0.06$). Conversely, SCS in the RR quarter was greater for BCP vs. CON (12.6 vs. 10.9 ± 0.50, $P = 0.02$) at 16 h after challenge 2 (treatment × time $P < 0.001$). First and second challenges did not result in different responses, failing to demonstrate tolerance to intramammary LPS; furthermore, treatment did not significantly influence this result. BCP supplementation blunted the SCS response to intramammary LPS in first-parity cows and may have promoted more complete resolution of inflammation in the 4 weeks after challenge, while causing a greater peak SCS in challenge 2, but did not have clear effects on productivity.

Key Words: cannabinoid, endotoxin, mastitis

2156 Effects of dietary rumen-protected choline supplementation on choline metabolites and inflammatory markers in mammary tissue during an intramammary lipopolysaccharide challenge. T. Swartz*[1,2], B. Bradford[1], L. Mamedova[1], and K. Estes[3], [1]*Michigan State University, East Lansing, MI,* [2]*South Dakota State University, Brookings, SD,* [3]*Balchem Corporation, Montvale, NJ.*

The objective of this study was to examine the effects of supplementation and dose of rumen-protected choline (RPC) on choline metabolites and inflammatory markers in mammary tissue during an intramammary lipopolysaccharide (LPS) challenge. Parous Holstein cows were blocked by calving month and randomly assigned within block to receive 45 g/d of RPC (20.4 g/d of choline ions; CHOL45, n = 18), 30 g/d of RPC (13.6 g/d of choline ions; CHOL30, n = 21), or no RPC (CON, n = 19) as a top-dress starting 24 d before expected calving until 21 d postpartum. Cows were alternately assigned within treatment group to either receive an intramammary LPS challenge (200 µg in each rear quarter) or not at 17 DIM. Mammary biopsies were conducted at 8 h relative to the LPS challenge. Transcript abundances were determined using NanoString's nCounter technology. Metabolites were quantified using LC-MS/MS. Data were analyzed using linear mixed models including the fixed effects of treatment, LPS, and the interaction. For metabolomics analyses, treatment false discovery rates (FDR) were calculated using the Benjamini-Hochberg method. Transcript abundance of inflammatory markers and cytokines in mammary tissue including *HP*, *SAA3*, *LTF*, *CXCL8*, *IL1B*, *IL6*, *TLR4*, and *TNFA* were unaffected by treatment (all $P \geq 0.33$). The predominant choline metabolite in mammary tissue was choline, followed by betaine, total lysophosphatidylcholine (LPC), total sphingomyelin (SM), and total phosphatidylcholine (PC). All choline metabolite concentrations (choline, betaine, total SM, total PC, total LPC, and total choline) were unaffected by treatment (FDR >0.10). The LPS challenge reduced concentrations of total LPC ($P < 0.001$; unchallenged vs. LPS, 61 vs. 28 nmol/g, SE = 4.6), total SM ($P = 0.01$; unchallenged vs. LPS, 3.5 vs. 3.2 nmol/g [natural log], SE = 0.11), and total PC ($P < 0.01$; unchallenged vs. LPS, 2.8 vs. 2.4 nmol/g [natural log], SE = 0.085), but did not impact choline, betaine, or total choline concentrations in mammary tissue. Taken together, dietary RPC supplementation did not alter inflammatory markers during an intramammary

LPS challenge, possibly because dietary RPC supplementation did not impact concentrations of most choline metabolites in mammary tissue.

Key Words: methyl donor, mastitis, inflammation

2157 Short- and long-term consequences of postpartum inflammation associated or not with clinical disease on feeding behavior, metabolism, and performance in dairy cows.

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Our objectives were to evaluate the short- and long-term consequences of postpartum inflammation associated or not with clinical diseases on feeding behavior, energy metabolism, antioxidant responses, and performance of dairy cows. Cows (n = 250) were classified based on incidence of postpartum clinical disease and concentration of haptoglobin in serum into one of 3 experimental groups: 1) cows diagnosed with clinical diseases in the first 21 DIM (ClinD21, n = 68); 2) cows with no ClinD21 and high concentration of haptoglobin in serum (HHp, n = 68); or 3) cows with no ClinD21 and low concentration of haptoglobin in serum (LHp, n = 114). Thresholds for haptoglobin classifications were 0.8 g/L on d 0, 3, 7 relative to calving and 0.4 g/L on d 10 and 14 relative to calving. Cows were followed from 42 d before calving to 98 DIM. Blood metabolites were evaluated on d −21, −10, −3, 0, 3, 7, 10, 14, 23 and 65 relative to calving. Statistics was performed using GLIMMIX of SAS and considered the effects of group, parity, dietary treatment, their interactions, season, and the random effect of cow. By design, the concentration of haptoglobin was lower ($P < 0.01$) in LHp than in HHp and ClinD21, and it did not differ between the latter 2 groups. Plasma concentrations of ceruloplasmin followed a similar pattern of haptoglobin, and serum concentrations of albumin followed the opposite pattern of haptoglobin, thus confirming the differences in systemic inflammation between groups. In the first 3 wk after calving, DMI differed between all groups and was lower for ClinD21, followed by HHp and LHp (14.9 ± 0.3 vs. 15.9 ± 0.3 vs. 16.8 ± 0.2; $P < 0.01$). Milk yield was lower for ClinD21 but did not differ between HHp and LHp (29.4 ± 0.7 vs. 31.4 ± 0.6 vs. 31.6 ± 0.5). For blood Ca, cows that had ClinD21 had the lowest concentration, followed by HHp and then LHp cows. In addition, cows that had ClinD21 had greater activity of glutathione peroxidase than LHp cows. Blood metabolites, feeding behavior, and performance in cows with HHp differed from cows with LHp and resembled, to some degree, the differences observed in cows with ClinD21.

Key Words: postpartum inflammation, clinical disease

2158 The impact of prepartal liver glutathione on milk performance parameters and welfare of peripartal dairy cows.

A. F. Souza Lima*[1], G. Goncalves Begalli[1], M. H. Oliveira[1], R. C. Barcellos Grazziotin[1], J. Halfen[1], E. Trevisi[2], and J. Osorio[1], [1]*School of Animal Science, Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Our objective was to determine the effects of prepartal liver glutathione (GSH) concentration on milk performance parameters and welfare in transition dairy cows. Fifty-three Holstein dairy cows were enrolled at −21 d relative to calving and remained on trial until 30 d in milk (DMI). All cows received the same close-up diet from −21 DIM until calving (1.59 Mcal/DM and 14.63% CP) and lactation diet from calving until 30 DIM (1.82 Mcal/DM and 18.39% CP). A liver biopsy was performed in all cows at −10 d relative to calving and total GSH was measured. This metric was used to perform retrospective analysis and assign cows to either high prepartal liver GSH (HGSH; n = 24, 1.43 ± 0.1 m*M*) or low prepartal liver GSH (LGSH; n = 29, 0.51 ± 0.1 m*M*) group. Blood and liver samples were collected at various time points between −21 and +21d relative to parturition to evaluate oxidative stress, metabolism, inflammation, and liver function biomarkers. Statistical analysis was performed using the MIXED procedure of SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. There was a trend ($P = 0.09$) for increased milk yield (+2.91 kg/d) and milk protein ($P = 0.07$) in HGSH cows in comparison to LGSH, but no differences were observed for milk fat ($P = 0.35$). There was a Group × Wk interaction ($P = 0.09$) in postpartal BCS, where HGSH cows had greater ($P < 0.01$) BCS than LGSH at 1 wk postpartum followed by a trend ($P \leq 0.08$) for greater BCS in HGSH than LGSH at 2 and 3 wk postpartum. This effect was reflected in an overall greater ($P = 0.01$) postpartal BCS in HGSH cows than LGSH. These effects in postpartal BCS were preceded by a trend ($P = 0.06$) for greater prepartal BCS change ($\text{BCS}_{-1\text{wk}} - \text{BCS}_{-3\text{wk}}$) in HGSH cows than LGSH (+0.1 vs. –0.1 BCS). Overall, our results suggest that prepartal liver glutathione has a positive effect on milk yield and milk protein. Prepartal liver glutathione seems to be linked to BCS changes around parturition. Further analysis from this project will help us understand the role of GSH on peripartal lipid mobilization and oxidative stress in transition dairy cows.

Key Words: peripartum period, oxidative stress, glutathione

2159 Greater prepartal liver glutathione is associated with improved liver function and milk production in transition dairy cows.

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Glutathione is a potent antioxidant that dairy cows can store in the liver and use later during high oxidative stress periods, such as the transition from late pregnancy to lactation. This project aimed to evaluate the effects of prepartal liver glutathione (GSH) levels on milk performance parameters, dry matter intake (DMI), and blood biomarkers profile. Data from 72 Holstein dairy cows from 2 published transition cow experiments were used. Liver glutathione (GSH) concentrations from −10 d relative to calving were averaged and then used to classify cows into high prepartal liver GSH (HGSH; n = 36, 1.86 ± 1.1 m*M*) or low prepartal liver GSH (LGSH; n = 36, 0.54 ± 0.5 m*M*) group. Blood samples were collected at −10, + 7, and + 21 d relative to calving. Data were analyzed using the MIXED procedure of SAS. Daily milk yield and DMI data during the transition period were analyzed. There was a trend ($P = 0.09$) for greater postpartal DMI (18.27 vs. 16.86 kg/d) in HGSH cows compared with LGSH. Greater milk yield was observed in HGSH cows compared with LGSH, resulting in 4.54 kg/d ($P < 0.01$; 44.23 vs. 39.69) increase in milk yield. Compared with LGSH, HGSH cows have a greater ($P = 0.02$; 36.54 vs. 35.47 g/L) albumin concentration, a biomarker related to liver function. A trend ($P = 0.06$; 4.50 vs. 4.69 U/L) for a lower gamma-glutamyl transferase (GGT) in HGSH cows compared with LGSH was observed. A Group × Time interaction ($P = 0.01$) was observed in cholesterol, where HGSH cows have greater ($P < 0.01$; 4.93 vs. 4.29 mmol/L) cholesterol concentrations than LGSH

at 21 DIM. A greater ($P = 0.02$) concentration of BHB was observed in HGSH cows compared with LGSH. These results suggest that prepartal liver glutathione positively affects liver function in early lactation cows allowing them to sustain greater milk production and partially improving DMI postpartum.

Key Words: peripartum period, oxidative stress, glutathione

2160 Association of health, survival, and performance of dairy heifers with the postpartum health status of their dams. I. Avalos Rosario*, G. Lu, M. R. Carvalho, and E. S. Ribeiro, *Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.*

Our objective was to investigate the associations of health, survival, and performance of dairy heifers from birth to end of first lactation with the postpartum health of their dams. Heifers (n = 1,811) were categorized according to postpartum health of their dams as daughters of dams that had no clinical disease (NoClinD21), a single clinical disease (SClinD21), or multiple clinical diseases (MClinD21) in the first 21 DIM. Postpartum diseases included retained placenta, metritis, mastitis, lameness, and digestive and respiratory problems. Data from daughters included birth body weight (BBW), morbidity, reproductive performance, milk production, and culling. Statistical analyses were performed in SAS using GLIMMIX and PHREG procedures. Statistical models included the effects of group, parity of the dam, and season. Orthogonal contrasts evaluated the effects of ClinD21 (NoClinD21 vs. SClinD21+MClinD21) and number of ClinD21 (SClinD21 vs. MClinD21). BBW (38.4 ± 0.2 kg) did not differ between groups. Daughters of ClinD21 dams had lower ($P = 0.02$) preweaning morbidity than daughters of NoClinD21 dams, however, number of ClinD21 did not influence morbidity (NoClinD21 = 44.9 ± 1.5 vs. SClinD21 = 37.4 ± 2.4 vs. MClinD21 = 34.3 ± 6.8%). This result was mostly explained by digestive problems, which presented the same association (NoClinD21 = 24.9 ± 1.3 vs. SClinD21 = 17.6 ± 1.8 vs. MClinD21 = 16.1 ± 5.6%). The rate of morbidity was also lower for daughters of ClinD21 dams than for daughters of NoClinD21 dams (AHR = 0.78; 95% CI: 0.66–0.91). No differences in reproductive outcomes were observed. Compared with daughters of NoClinD21 and SClinD21 dams, daughters from MClinD21 dams had lower milk (11,287 ± 48 vs. 11,372 ± 80 vs. 10,620 ± 256 kg; $P = 0.02$) and protein (344 ± 1.3 vs. 346 ± 2.1 vs. 324 ± 6.8 kg; $P = 0.01$) production in the first lactation, and a greater proportion left the herd from birth to 305 DIM (33 ± 1.4 vs. 34 ± 2.4 vs. 48 ± 7.2%; $P = 0.05$). In conclusion, daughters of cows that developed ClinD21 had lower risk of preweaning health problems, but those born from cows that had MClinD21 specifically had lower milk production and survival by the end of first lactation.

Key Words: DOHaD, transition

2161 Association between residual feed intake and ex vivo immune cell response in mid-lactating Holstein dairy cows. M. H. De Oliveira*[1,2], T. C. Michelotti[3,4], N. Carpinelli[3,5], J. Halfen[1], E. Trevisi[6], and J. S. Osorio[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*State University of São Paulo, Botucatu, SP, Brazil,* [3]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [4]*INRAE, UMR Herbivores, Saint-Genès-Champenelle, France,* [5]*Nutricorp, Araras, SP, Brazil,* [6]*Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Residual feed intake (RFI) is a well-known parameter for feed efficiency; however, its correlation with health status through the lactation of dairy cows is limited. The objective of this study was to evaluate the effect of RFI level in mid-lactation dairy cows on blood biomarkers of metabolism and ex-vivo immune cell response via whole blood stimulation assay (WBSA). Sixty-two Holstein dairy cows (26 to 195 DIM) were fed a basal diet (1.70 Mcal of NEL/kg and 16.3% CP) once daily over a 12 wk period. Milk samples were analyzed weekly for fat, protein, SCC, and MUN. At 0, 6, and 12 wk, blood samples were collected for the evaluation of a) energy and muscle mass catabolism biomarkers, b) WBSA with LPS, c) polymorphonuclear leukocyte isolation and LPS stimulation for gene expression. The RFI was estimated as the difference between a cow's actual feed intake and its predicted feed intake based on its ECM, metabolic BW, age, DIM, and ADG. Then, cows were divided into high RFI or low efficient cows (LE; n = 31, 1.89 ± 1.99, 116 ± 46 DIM) and low RFI or high efficient cows (HE; n = 31, −1.89 ± 1.71, 104 ± 43 DIM). Extreme phenotypes were selected from each group to increase the statistical power compared with random sampling. Therefore, we selected 12 cows with the lowest (−2.53 ± 0.35) and highest (+3.86 ± 2.79) RFI values. Compared with LE, HE cows had lower DMI (23.2 vs. 26.9 ± 0.62) ($P < 0.01$) and greater ($P < 0.01$) milk efficiency (milk yield/DMI). Blood urea tended ($P = 0.09$) to be lower in HE cows than in LE. Results from WBSA showed a decrease in myeloperoxidase and IL-6 in HE cows compared with LE. In contrast, WBSA urea was greater ($P = 0.01$) in HE cows than in LE. An RFI × Time interaction ($P \leq 0.02$) was observed in glucose and IL-1β, where a contrasting effect was observed; while both biomarkers decreased over time in HE cows, there was an increase over time in LE. Overall, our results suggest that while HE and LE cows show similar metabolic conditions, there is a possible differential cellular programming of the immune system. The HE cows seem to have a lower response to LPS stimulation and such response seems to decrease throughout the lactation.

Key Words: immune response, residual feed intake, lipopolysaccharide challenge

2162 Predicting calcium status in early lactation multiparous Holsteins using milk constituent analysis. J. A. Seminara*[1], K. R. Callero[1], C. R. Seely[1], M. Van Althuis[3], S. An[3], C. M. Salpekar[3], D. M. Barbano[2], and J. A. A. McArt[1], [1]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [2]*Department of Food Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY,* [3]*College of Agriculture and Life Sciences, Cornell University, Ithaca, NY.*

Subclinical hypocalcemia is common among early lactation dairy cows. Cows that have not recovered from this condition by 4 DIM develop dyscalcemia, a metabolic state associated with negative health and production outcomes. Despite relatively high prevalence, identifying cows with dyscalcemia remains a challenge on dairies. Our objective was to develop an algorithm for predicting dyscalcemia status using Fourier-transform infrared spectroscopic (FTIR) analysis of milk samples at 3 and 4 DIM, through a prospective cohort study. We collected blood from multiparous Holsteins (n = 453) on 4 herds in NY at 4 DIM for total calcium concentration (tCa) analysis and classified cows as eucalcemic (tCa ≥2.2 mmol/L; n = 353) or dyscalcemic (tCa <2.2 mmol/L; n = 100). Proportional milk samples were collected at 3 and/or 4 DIM and analyzed via FTIR for the following constituents: lactose, protein, fat, de novo fatty acids (FA), mixed origin FA, and preformed FA; relative percentages of each FA group; individual FA including C16:0, C18:0, and C18:1 *cis*:9; milk urea nitrogen, milk acetone, milk β-hydroxybutyrate, and milk predicted blood nonesterified FA. Milk weights were also collected at each milking. Partial least squares regression models were fit for each DIM separately and were used to predict calcium status at

4 DIM using individual daily estimated milk constituents, daily milk yield and parity group (2, 3, and ≥4). Receiver operating characteristic curves were generated using predictions from each model. Area under the curve was 0.76 and 0.81 for 3 and 4 DIM respectively. Sensitivity, specificity, positive and negative predictive values for the models were 0.57, 0.84, 0.51, and 0.88 respectively at 3 DIM, and 0.62, 0.86, 0.55, and 0.89 respectively at 4 DIM. Our results indicate that FTIR milk constituent analysis at 3 and 4 DIM can accurately identify cows that are eucalcemic at 4 DIM and therefore may be useful as a tool to optimize the management of early lactation cows to improve health and production outcomes.

Key Words: dyscalcemia, milk Fourier-transform infrared spectroscopy, subclinical hypocalcemia

2163 A predictive model for hypocalcemia in dairy cows utilizing behavioral sensor data combined with deep learning.

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(Sub)clinical hypocalcemia occurs frequently in the dairy industry, and is one of the earliest symptoms of an impaired transition period. Calcium deficiency is accompanied by changes in cows' daily behavioral variables, which can be measured by sensors. The goal of this study was to construct a predictive model to identify cows at risk of hypocalcemia in dairy cows using behavioral sensor data. For this study 133 primiparous and 476 multiparous cows from 8 commercial Dutch dairy farms were equipped with neck and leg sensors measuring behavioral elements, including eating, ruminating, lying and walking behavior of the 21 d before calving and the day of calving. From each cow, a blood sample was taken to measure their blood calcium value. Cows with a blood calcium value ≤2.0 mmol/L, within 48 h after calving, were defined as hypocalcemic. To correct for dependencies in the data, a second way of dividing the blood calcium status into 2 groups was proposed, using a linear mixed-effects model with a k-Means clustering (absolute and residual calcium). Three possible binary predictive models were tested; a logistic regression model, a XgBoost model and a LSTM deep learning model. Additional features as input variables were added to all models; parity (1, 2, or 3+), calving season, day of blood measurements (0, 1, or 2), BCS and Locomotion score. Of the 3 models, the deep learning model performed best with an AUC of 0,66 and an average precision of 0.53. This final model was constructed with the addition of the static features, since they improved the model's tuning AUC by 12%. The calcium label with the cut-off value proved to be easier to predict for the neural network and the XgBoost, while the logistic regression model performed better using the clustered labels. An ameliorated version of the deep learning model proposed in this study could serve as a tool to monitor herd calcium status and to identify animals at risk for associated transition diseases.

Key Words: dairy cattle, hypocalcaemia, sensor

2164 Withdrawn.

Joint Breeding and Genetics and Lactation Biology Symposium: Genomics and Phenomics of Lactation

2165 Use of milk mid-infrared spectral data in dairy genetics: Past, present and future. N. Gengler*, *University of Liège-GxABT, Gembloux, Belgium.*

For 5 decades, major milk components such as fat and protein contents have been routinely predicted using mid-infrared (MIR) spectrometry, a nondestructive analysis technology, based on the absorption of MIR light by milk, associated to the Fourier transform generating whole MIR spectra. The historic use of MIR spectra in animal breeding was until recently restricted to its indirect use through MIR-predicted major milk components. A reason why additional potential for the use of MIR spectra data was overlooked for such a long time, was its unavailability outside used spectrometers. But this bottleneck was resolved and since around 2006, published research has been exploding leading to the present-day increasing availability of novel MIR-based phenotypes based on predictions developed by associating relevant reference data and corresponding MIR spectra. Key factors affecting the quality of predictions were identified and animal breeders have today access to many MIR-based phenotypes. These does not only include direct fine milk composition traits as fatty acids, but also indirect traits to assess quality of milk and milk products, technological properties, animal health and status, and emissions and interactions with the environment. Current situation is that breeders but also farm advisors, are feeling overwhelmed with many traits. Only slowly industry is taking up the generated opportunities for animal breeding and management. From an animal breeding perspective integrating even only some of these MIR-predicted traits in sustainable breeding goals is currently a challenge. In the future an alternative view on this situation should be considered. As a matter of fact, the high-dimensional MIR spectral data space, defined by the underlying absorbances along the range of wavenumbers but also across lactations and lactation stages, is also describing a global milk phenome. Direct use of MIR data as phenotypes has been proposed. These high-dimensional traits could allow breeders to rapidly assess underlying genomic information, however important theoretical and computational improvements are still needed to deal with them properly in the future.

Key Words: milk components, novel traits, high-dimensional traits

2166 Phenotypic and genomic modeling of lactation curves: A longitudinal perspective. H. R. Oliveira*, G. S. Campos, and L. F. Brito, *Purdue University, West Lafayette, IN.*

Lactation curves, which describe the pattern of milk production over time, provide critical insights about animal health, milk production efficiency, and the overall profitability of the dairy industry. Consequently, understanding the longitudinal perspective of lactation curves can help dairy producers optimize management practices, improve milk production efficiency, and reduce animal health risks. Both phenotypic and genomic modeling have been used to study lactation curves, providing valuable information for dairy producers and breeders. Phenotypic modeling involves the analysis of data on milk production collected from individual animals, which can be used to estimate parameters of phenotypic lactation curve models, such as lactation peak and persistency. On the other hand, genomic modeling also incorporates genetic marker information to predict the genetic basis of lactation curves, which allows the identification of genetic variants associated with milk production. Random regression models provide a powerful tool for modeling lactation curves, allowing for the estimation of both genetic and environmental effects on milk production over time. Furthermore, the use of random regression models allows for a more flexible and robust modeling of the lactation curve compared with traditional models based on fixed time points. In this invited talk, we will discuss the importance of the longitudinal perspective in the study of lactation curves and the various statistical and mathematical models used to analyze longitudinal data. We will also highlight the key factors that influence milk production over time, and the potential applications of longitudinal analysis of lactation curves in improving animal health and milk production efficiency. Overall, analyzing the longitudinal perspective of lactation curves will continue to play a crucial role in improving the production efficiency and sustainability of the dairy industry, and the methods and models developed will be applied to other longitudinal traits.

Key Words: milk production, profitability, random regression model

2167 Mitochondrial bioenergetics of extramammary tissues during lactation and in response to heat stress. A. Skibiel*[1], M. Zachut[2], G. Dahl[3], A. Kavazis[4], and W. Hood[4], *[1]University of Idaho, Moscow, ID, [2]ARO Volcani Center, Rishon Lezion, Israel, [3]University of Florida, Gainesville, FL, [4]Auburn University, Auburn, AL.*

Heat stress has detrimental impacts on agricultural operations by reducing livestock productivity and quality. In the US dairy industry, heat stress results in financial costs that exceed $2 billion. Heat stressed dairy cows produce less milk, and have altered energy metabolism, and higher morbidity and mortality rates, which occur when cows are heat stressed when dry and when lactating. However, gaps in knowledge remain regarding the cellular and molecular mechanisms underlying heat effects on energy metabolism and consequently milk production. Mitochondria produce around 90% of cellular ATP through oxidative phosphorylation, generate precursors for milk synthesis, and modulate the energetic response to stress. Yet, little information exists regarding mitochondrial bioenergetics during lactation and in response to heat stress in dairy cows, particularly for extramammary tissues with supporting roles in milk synthesis. Here, I will focus on our studies in dairy cows exploring hepatic mitochondrial adaptations across lactation and impacts of heat stress on expression of genes and proteins involved in mitochondrial function as well as mitochondrial bioenergetics and structure. Our studies involved observational and experimental approaches, including proteomics analysis of liver tissue from dairy cows that were heat stressed or actively cooled and mitochondria functional assays conducted across a typical lactation and in mid-lactation cows that were heat stressed or normo-thermic. Expression of nuclear genes involved in mitochondrial biogenesis and function were quantified using qRT-PCR. Mitochondrial morphology was assessed through transmission electron microscopy. Mitochondrial respiration using NADH or $FADH_2$-linked substrates was quantified using an electrode-based system to measure mitochondrial oxygen consumption. Results from this work indicate that mitochondrial efficiency is enhanced as milk yield increases during lactation. Additionally, while moderate to high levels of heat stress reduce expression of hepatic mitochondrial proteins involved in energy production, mild heat stress appears to have little direct impact on hepatic mitochondrial function. Further research is necessary to investigate mitochondrial responses to moderate and high heat loads in dairy cows both during the dry and lactating periods.

Key Words: proteomics, energy metabolism, liver

2168 The microbiome of mammary gland: Implications for udder health and therapeutic potentials. H. Derakhshani*[1], J. C. Plaizier[1], H. W. Barkema[2], J. De Buck[2], E. Khafipour[3], and M. G. Surette[4], [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*University of Calgary, Calgary, AB, Canada,* [3]*Cargill Animal Nutrition and Health, Minneapolis, MN,* [4]*McMaster University, Hamilton, ON, Canada.*

The advent of culture-independent metagenomics has revolutionized our understanding of the role of commensal microbiota in immune homeostasis and disease susceptibility of humans and animals. In the context of the bovine mammary gland, several studies have linked the composition of milk microbiota to both the physiological status of cows and the farm environment. Factors such as stage of lactation, metabolic status, incidence of clinical and subclinical mastitis, cows' genotype, and the type of bedding material used on farms have all been related to the composition of milk and teat canal microbiota. The notion of a viable intramammary microbiota, if proven valid, could have important implications for development of novel microbial ecosystem therapeutics for improving mastitis resistance. However, strong debates exist on the permissiveness of the mammary gland for a rich and diverse microbiome, which usually contradicts the very low bacterial biomass detected in milk samples collected from healthy udders. Culture-independent metagenomic approaches alone cannot establish whether the identified microbial communities are viable, and if so, can play a role in udder physiology and mastitis susceptibility. In this presentation, we will provide a snapshot of the current state and limitations of the mammary microbiome research. In addition, we will describe how recent advances in high-throughput culturing and genome sequencing of microbiota has enabled isolation and identification of previously uncultured lineages of the host-associated microbiotas, including strictly anaerobe species isolated from the bovine mammary gland. Particularly, we will discuss how the application of genome mining tools and high throughput functional screening assays could facilitate identification of potential therapeutic targets, including novel probiotic candidates and microbial natural products with potent activities against mastitis pathogens.

Key Words: mammary microbiome, microbiome therapeutics, mastitis

Dairy Foods Symposium: Managing the Risks—Lessons from the Infant Formula Crisis

2169 Current perspectives on the US infant formula crisis. C. Galer*, *Dairy Management Inc., Rosemont, IL.*

Cronobacter sakazakii, the organism at the heart of the infant formula crisis, is a ubiquitous, opportunistic pathogen that has been found in dairy powders. The US Food and Drug Administration (FDA) conducted a sampling assignment in 2014 to understand the prevalence of *Cronobacter* spp. and *Salmonella* in 55 US dry dairy facilities. *Cronobacter* was detected in 69 percent of the facilities with the highest rates in zone 4 (14.3%). The positive rates decreased progressively, with 8.7 percent in zone 3, 4.5 percent in zone 2, and the fewest positives found in zone 1 with only a 1.1% positive rate. By contrast, *Salmonella* was found in only 3 (5.5%) of the 55 facilities sampled. The dairy industry has been proactive to control pathogens in dry dairy processing environments, but this data indicates more can be done to control *Cronobacter* and there will be more scrutiny in the future. This presentation will highlight what the dairy industry is doing, areas for dry dairy manufactures to focus on, and what resources exist for the dairy industry to learn more about controlling *Cronobacter.* These programs include dry sanitation, environmental monitoring programs, and finished product testing to name a few. Ultimately, we intend to introduce to you a framework to safely implement a control program that provides *Cronobacter sakazakii* free products to markets and consumers.

Key Words: *Cronobacter*, dairy powders

2170 Determining power of sampling and testing plans for detecting *Cronobacter* by simulating powdered infant formula batches with different sampling plans and hazard profiles. M. Kim and M. J. Stasiewicz*, *University of Illinois at Urbana-Champaign, Urbana, IL.*

Simulating bulk product sampling and testing for foodborne pathogens shows (i) taking more individual samples increases the power to detect small clusters of contamination and (ii) taking a larger total sample mass increase the power to detect low-level, widespread contamination. We adapted this sampling simulation tool for powdered infant formula (PIF) sampling to determine the power of industry-relevant sampling plans. We evaluated performance to detect published *Cronobacter* contamination profiles for a recalled PIF batch [42% prevalence, −1.8 ± 0.7 log(cfu/g)] and a reference, non-recalled, PIF batch [1% prevalence, −2.4 ± 0.8 log(cfu/g)]. Simulating a range of numbers of grabs [n = 1–22,000 (representing testing every finished package)] with 300 g total composite mass showed that taking 30 or more grabs detected contamination reliably (>99% median probability to reject the recalled batch). Benchmarking representative sampling plans ([n = 30, mass grab = 10 g], [n = 30, m = 25 g], [n = 60, m = 25 g], [n = 180, m = 25 g]) showed that all plans would reject the recalled batch (>99% median probability to reject) but would rarely reject the reference batch (>50% median probability of acceptance, all plans). Further, power difference between sampling patterns decreases as the number of clusters increase. These simulations could help industry stakeholders design science-based sampling and testing plans by entering parameters describing hazard scenarios and sampling plans of interest. Yet key questions remain for accurate simulations. What is the real variability in contamination profiles for *Cronobacter*? Or *Salmonella*? Including overall contaminated proportion, levels when contaminated, and likelihood of these contamination events – published data are limited, and only for *Cronobacter*. How would sampling excess product, then testing only a well-mixed sub-sample, impact performance? Our work clarifies what specific data are required to better assess the power, and therefore risk management role, of using product sampling and testing to improve powdered infant formula safety.

Key Words: sampling, *Cronobacter*, powdered infant formula

2171 Dry sanitation in dairy powder processing facilities. M. Bohanan*, *Leprino Foods, Denver, CO.*

Traditional cleaning and sanitizing in dairy processing facilities has utilized wet chemistry and water to remove soils and mitigate against microbiological hazards. There are parts of facilities processing dairy powders, however, where it is now accepted that Dry Clean ONLY is the best approach. This presentation will focus on the philosophy behind undertaking Dry Clean ONLY, the methodologies employed to implement Dry Clean ONLY, and some techniques to assist in the successful implementation of Dry Clean ONLY in dairy powder processing facilities as part of overall food safety programs.

Key Words: war on water, *Salmonella*, *Cronobacter*

2172 Underlying issues and security of national infant formula supplies. D. Clark*, *Bovina Mountain Consulting LLC, Englewood, FL.*

The infant formula supply crisis dominated the headlines in the US from February 2022 until early 2023. Desperate parents, if not confronted with empty shelves, could only purchase very limited quantities of infant formula from retailers. Following emergency intervention, not least the execution of 'Operation Fly Formula,' the supply situation eased but had not been totally resolved by the beginning of 2023. Legislation and regulations have been proposed, reviewed and some have been passed with the aim of prevention of a recurrence of the 'formula crisis' but do they go far enough? Many questions still remain unanswered including: What caused the supply chain of infant formula to be so vulnerable? Are the weaknesses confined just to the manufacturers of the finished product or do they extend further through the supply chain? Why are there so few manufacturers and production facilities for infant formula in the US? What barriers are preventing new entrants? What impact does the infant formula product mix in the US market have on supply chain security? Insights into the crisis and answers to these questions are necessary to provide guidance to legislators, industry and academia as to how they can contribute to strengthen the security of the infant formula supply chain for the future.

Key Words: infant formula, supply chain, production

Production, Management, and the Environment 1

2174 In-barn and outdoor temperature-humidity index conditions in Canadian dairy barns in current and future climates. A. VanderZaag*[1], E. Le Riche[1], H. Baldé[1], S. Kallil[1], V. Ouellet[2], É. Charbonneau[2], T. Coates[3], T. Wright[4], P. Luimes[5], R. Gordon[6], W. Smith[1], and B. Qian[1], [1]*Agriculture and Agri-Food Canada - Ottawa, Ottawa, Ontario, Canada,* [2]*Université Laval, Québec, Quebec, Canada,* [3]*Agriculture and Agri-Food Canada-Lethbridge, Lethbridge, Alberta, Canada,* [4]*Ontario Ministry of Agriculture, Food, and Rural Affairs, Guelph, Ontario, Canada,* [5]*University of Guelph Ridgetown Campus, Ridgetown, Ontario, Canada,* [6]*University of Windsor, Windsor, Ontario, Canada.*

Canada's climate is warming faster than the global average, however, the warming is not uniformly distributed spatially nor seasonally. Likewise, dairy farming is also unevenly distributed across the country. Increased occurrence of heat stress conditions could negatively impact milk production. This study evaluated dynamics of the temperature-humidity index (THI) in the past and in future projections, considering both outside and in-barn conditions in key regions across Canada. In-barn THI was measured in 9 barns in 4 provinces. These data showed in-barn THI exceeded outdoor THI and enabled equations to be derived depending on ventilation type (linear in natural ventilated, and nonlinear in mechanically ventilated barns). Climate changes were evaluated using historical hourly data to compare the frequency, extent, and duration of THI above critical levels from 1960 to 2019, and future projected changes were evaluated using 5 global climate models under 3 emission pathways (low, moderate, and high). Projections were grouped in three 30-yr periods targeting the 2030s, 2050s, and 2070s. Historical climate trends show an increase in THI exceedance in most locations on the west coast and eastern Canada, affecting about 84% of the national dairy herd. Future scenarios project that 90% of the national herd will experience a large increase in the frequency, severity, and duration of THI exceedance in the future under all but the most optimistic scenario. In the moderate scenario, the number of days with THI >65 is projected to increase by 35.7, 32.6, 25.0, 31.0, and 28.1 d between the 2030s and 2070s for Abbotsford BC, Red Deer AB, Windsor ON, Quebec QC, and Halifax NS, respectively. Overall, both historical observations and future projections indicate the largest increases in the magnitude and duration of heat stress occur in Ontario, while central Alberta and Newfoundland will have the least expected change. These findings highlight the need for Canadian dairy farmers to consider strategies to adapt to elevated THI.

Key Words: temperature-humidity index, heat stress, climate change

2175 A model for integrating rumination time measurements by two commercial wearable biosensors estimated actual rumination time with high accuracy in Holstein dairy cows. H. Cho*, M. Lee, and S. Seo, *Chungnam National University, Daejeon, Yuseong-gu, Korea.*

The objective of this study was to develop a model to integrate sensor measurements for measuring rumination time (RT) by 2 wearable biosensors (i.e., an ear-tag sensor [ETS; CowManager, Agis Automatisering BV, Netherlands] and a neck collar sensor [NCS; cSense Flex tag, SCR by Allflex, Israel]) in Holstein dairy cows. A total of 13,647 RT measurement pairs over 2-h windows by 2 sensors were collected from 9 lactating cows and 3 nonlactating cows over 4 mo. After removing outliers due to the physical location or signal transmission issues in either sensors, 9,470 data pairs were used for subsequent analysis. A classic statistical linear regression (LR) and a Support Vector Regression (SVR), were applied to construct models. The model development and evaluation were conducted independently using the 10-fold cross-validation. The model adequacy was evaluated based on the coefficient of determination (R^2), root-mean-square prediction error (RMSPE), concordance correlation coefficient (CCC). The model-predicted values were also independently evaluated against actual rumination data. The chewing activity of 5 lactating cows equipped with both sensors was video-recorded for 3 d. Actual rumination time data over 2-h windows (ART; n = 60) were measured manually by visual observation of the video recordings. Since SVR did not improve the model performance, LR was used to develop a final equation to convert the RT values of ETS to those of NCS (NCS = 4.382 (±0.0312) + 0.919 (±0.0007) × ETS; R^2 = 0.65, RMSPE = 14.06, CCC = 0.79). The CCC between ART and NCS was higher (0.82) than between ART and ETS (0.52). Nevertheless, when conversion of the ETS values into the NCS values was performed using the linear equation, the converted ETS values estimated ART with moderate accuracy (R^2 = 0.64, RMSPE = 15.30, CCC = 0.73). In conclusion, we developed a model that integrating RT measurements collected from different sensors and showed that ART can be estimated by integrated RT measurements.

Key Words: lactating dairy cows, modeling, rumination time

2176 Behavioral changes of heat stressed lactating dairy cows during lipopolysaccharide challenge. I. M. Toledo*, O. Martinez, F. Saputra, A. Fraz, C. Nelson, and G. E. Dahl, *University of Florida, Gainesville, FL.*

Controlled studies have extensively documented that exposure of dairy cows to heat stress negatively affects health, behavior, reproductive and productive performance during all stages of the lactation cycle. In the present study, cows were either heat stressed (HS) or cooled (CL) during lactation. After 4 weeks of treatment, cows from both groups were challenged with LPS. Automated monitoring devices (Nedap, Netherlands) were used to assess behavioral activity of CL (n = 6) and HS (n = 6) mature lactating Holstein dairy cows during days −2, −1, 0, 1 and 2, relative to the LPS challenge. Cows received a leg tag to measure daily lying time, number of steps and standing bouts and a neck tag to measure eating and rumination time. Statistical analysis were performed using mixed procedure. All cows were housed in a sand-bedded free-stall barn. The CL pen was equipped with cooling soakers and fans. Eating time was reduced in cows exposed to heat stress (HS) compared with CL cows (101.0 ± 17.3 vs. 186.0 ± 14.6 min/d; $P < 0.01$). Rumination time significantly decreased in HS compared with CL cows (367.7 ± 50.2 vs. 529.0 ± 50.2 min/d; $P = 0.05$). Daily inactivity during the LPS challenge increased in HS relative to CL (922.2 ± 61.5 vs. 698.0 ± 61.2 min/d; $P = 0.03$). In addition, lying time was reduced in HS compared with CL cows (537.0 ± 21.3 vs. 724.0 ± 24.4 min/d; $P < 0.01$). Standing time and stand bouts did not differ between the treatments. A better understanding on how the development of intramammary infections affect the behavior of lactating dairy cows in heat stress conditions may contribute to the development of more effective management strategies to decrease the negative effects of heat exposure during challenging periods of the lactation cycle.

Key Words: heat stress, lipopolysaccharide challenge, behavioral activity

2177 Reducing water use to cool cows using "smart" technologies. L. T. Casarotto*, K. A. Forbes, J. M. Bobel, D. Martinez Cabrera, and G. E. Dahl, *University of Florida, Gainesville, FL.*

When trying to achieve optimum production, lactating and dry dairy cows must be provided with relief from heat and humidity. The most practical and common methods to alleviate the negative effects of heat stress on lactating and dry dairy cows are grouped into 3 main areas: shade, ventilation, and active cooling. Our objective was to determine if an automated "smart" system (Agpro, Paris, TX) for control of soaker output is as effective as the conventional approach to control of soakers that relies on set timing after a threshold temperature is reached. Thirty multiparous pregnant Holsteins cows were dried off ~45 d before expected calving and randomly assigned to one of 3 treatments: 5 min interval (30 s on, 5 min off) cooling period with shade, fan, and soakers (CL, n = 10), Agpro smart cooling with shade, fan and soakers (SS, n = 10), and heat stress with only shade (HT, n = 10) during the entire dry period. Daily dry matter intake (DMI) of individual cows, pen water intake and water usage from soakers were measured daily. Blood samples for hematocrit were taken weekly. Respiration rate (RR) and rectal temperature (RT) were measured thrice weekly. Calf birthweight and gestation length were recorded upon parturition. Data were analyzed using the GLIMMIX procedure in SAS. No differences were observed for gestation length, calf birthweight, dry period length and hematocrit among the treatments. However, SS cows had a greater overall DMI than HT (10.1 vs. 8.6 ± 0.4 kg/d; $P = 0.01$) and CL tended to increase DMI relative to HT (9.5 vs. 8.6 ± 0.4 kg/d; $P = 0.11$). Water usage per cow was higher in the CL compared with SS and HT groups (469.1 vs. 148.6 vs. 168.1 ± 102.1 L/cow/d). Despite lower water usage in SS, RR (48 vs. 53 vs. 68 ± 1.2 bpm; $P < 0.001$), RT (38.3 vs. 38.4 vs. 38.8 ± 0.03°C; $P < 0.001$) and vaginal temperatures (38.7 vs. 38.6 vs. 38.9 ± 0.09) were affected by treatment with HT having the higher values relative to CL and SS. Compared with conventional cooling, the SS system decreased the total water usage per cow, effectively cooled the animals, and maintained animal welfare.

Key Words: hyperthermia, dry period, heat abatement

2178 Association of Ontario compost-bedded pack barn characteristics and herd-level cleanliness, lameness, milk volume and bulk tank somatic cell count. A. M. Wilson*[1], D. B. Haley[2], G. W. Price[3], T. C. Wright[4], D. F. Kelton[2], C. Wand[4], R. J. Gordon[5], G. LaPointe[6], and R. Bergeron[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Department of Engineering, Faculty of Agriculture, Dalhousie University, Truro, NS, Canada,* [4]*Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada,* [5]*University of Windsor, Windsor, ON, Canada,* [6]*Dairy at Guelph, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to investigate compost-bedded pack (CBP) conditions and management associated with herd-level cow cleanliness, lameness, milk volume, bulk tank somatic cell count (BTSCC), and bulk tank milk (BTM) aerobic and coliform counts. Commercial CBP farms (n = 25) in Ontario, Canada, were visited 3 times every 2 mo during the summer and fall of 2022. Farms averaged (±SD) 83 ± 47 lactating cows on the packs with an average (±SD) space of 123.8 ± 43.79 ft^2 per cow. Twelve spots across the pack were measured for depth temperature at 30 cm and sampled for moisture content (MC) and total coliform and aerobic counts. A subset of lactating cows was scored for cleanliness (rear upper limb, ventral abdomen) and gait. General management practices (bedding material, tilling equipment, milking system, pack size and number of cows on the pack) were recorded. Milk volume and BTSCC records from the 4 milk pickups closest to each visit were obtained. Milk aerobic and coliform counts were determined from BTM samples. All outcome variables were reported on a herd level, with the farm as the experimental unit. The proportion of clean cows (score $\geq$3) and lame cows (score $\geq$3) were analyzed using multivariable β regression models. Milk volume (liters/cow/day), BTSCC and BTM bacterial counts were analyzed using multivariable linear regression models. All models were constructed using PROC GLIMMIX in SAS 9.4 and included the repeated measure of visit with subject as farm. Herds with parlor milking systems were associated with lower milk coliform count [0.5 vs. 3.5 ± 0.66 ln colony-forming units (cfu)/mL, $P = 0.004$] and lower aerobic count (6.4 vs. 7.5 ± 0.24 ln cfu/mL, $P = 0.003$) than herds with automatic milking systems. The proportion of the herd with lame cows was positively associated with pack aerobic bacterial count ($P = 0.040$). The proportion of the herd with clean upper limbs was negatively associated with MC ($P = 0.008$). The results suggest that pack conditions may be associated with herd cleanliness and lameness in Ontario CBP barns.

Key Words: housing, milk quality, compost-bedded pack

2179 Description of local immune responses within the pulmonary tract of dairy calves exposed to wildfire smoke. A. Pace*, K. Mirkin, M. Larson, D. Konetchy, P. Rezamand, and A. L. Skibiel, *University of Idaho, Moscow, ID.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).

Wildfires are a major source of air pollution, particularly in the western US. Inhalation of particulate matter ($PM_{2.5}$) from wildfire smoke poses health and performance concerns in dairy calves. Our group previously found that wildfire-$PM_{2.5}$ exposure is linked to metabolic and systemic inflammatory responses and clinical respiratory symptoms in calves. This study aimed to elucidate the effects of wildfire-$PM_{2.5}$ on the calf respiratory tract and health. Holstein heifer calves (n = 17) were monitored in summer 2022, from birth to early post-weaning. Hourly $PM_{2.5}$ and meteorological data, used to calculate temperature-humidity index (THI), were retrieved from a local monitoring station. Respiration rates (RR), rectal temperatures (RT), heart rates (HR), and health scores were recorded 3X weekly, and thoracic ultrasound scores (TUS) were recorded 1X weekly. Blood was collected 5X for hematology analysis. Trans-tracheal washes (TTW) were performed on a subset of calves (n = 13) to differentiate pulmonary leukocyte populations before and during a wildfire event. General linear mixed models with lags of up to 7 d were used to analyze all data, except TTW, which was analyzed with generalized linear mixed models. During the study, $PM_{2.5}$ reached 113.5 μg/m^3. On lag d 0, greater $PM_{2.5}$ and THI reduced RR, but increased RT and HR ($P < 0.04$). Elevated $PM_{2.5}$ and THI also reduced white and red blood cell counts, hemoglobin, and hematocrit initially, but increased them on subsequent lag days ($P < 0.05$). There was a positive effect of $PM_{2.5}$ × THI on eye score, but a negative effect on cough scores (both $P < 0.05$). Greater $PM_{2.5}$ and THI increased TUS on multiple lag days ($P < 0.05$). TTW macrophage proportions were greater during wildlfire-$PM_{2.5}$ exposure compared with baseline (22.9 ± 7.8% vs. 5.7 ± 0.7%; $P < 0.001$). Leukocyte alterations and greater extent of lung consolidation indicate a local inflammatory response in the respiratory tract. Wildfire-$PM_{2.5}$ may predispose calves to a compromised pulmonary system, leading to manifestation of respiratory pathology.

Key Words: air quality, inflammation, health

2180 Programming effects of in utero hyperthermia on adrenal gland development.

A. R. Guadagnin*[1], F. Peñagaricano[1], G. E. Dahl[2], and J. Laporta[1], [1]*University of Wisconsin, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

Maternal stress during gestation can modify the fetal hypothalamic-pituitary adrenal axis, disrupting postnatal adrenal gland development and function. Herein, we characterize the impact of in-utero heat stress on adrenal gland histology and transcriptomic profile. Pregnant cows were exposed to environmental heat stress for the last 54 ± 5 d before calving. Adrenal glands were harvested from a subset of in utero heat-stressed (IUHT, dams exposed to THI >68) and in utero cooled (IUCL, dams exposed to THI >68 with active cooling) daughters euthanized at birth or one week after weaning (0 and 63 d of age, respectively, n = 8 per group and euthanasia) for RNA-Sequencing and histological analysis. Histological data were analyzed using PROC MIXED of SAS, and RNA-Seq data were analyzed using the R package edgeR and Ingenuity Pathways Analysis Software. At weaning, adrenal glands of IUHT were heavier and had greater total tissue area, greater thickness of zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR) relative to IUCL ($P < 0.01$). The ZG and ZF of IUHT had larger cell area ($P = 0.03$), and the ZF tended to have fewer cells relative to IUCL ($P = 0.10$). At birth, one gene was differentially expressed (FDR 20%). At weaning, 48 genes were differentially expressed in adrenal glands of IUHT vs. IUCL calves (FDR 20%). The top downregulated gene in IUHT was ribosomal protein 15 (*RPL15*, log FC = −2.9) and the top upregulated gene was PR/SET domain zinc finger protein 9 (*PRDM9*, log FC = 2.2). Out of 30 enriched pathways identified at birth (primarily upregulated genes), 14 were involved in immune function and inflammatory response, and 7 in cell function and proliferation. The prolactin signaling pathway was also enriched at birth. Out of 30 enriched pathways identified at weaning (primarily downregulated genes), 20 were involved in immune function and inflammatory responses. Calcium transport pathway was also enriched at weaning. In conclusion, late gestation intrauterine heat stress increases postnatal adrenal gland size by inducing ZG and ZF cell hypertrophy and impacts gene expression. These changes might lead to altered stress responses later in life.

Key Words: HPA-axis, RNA-sequencing, heat stress

2181 Effects of heat stress and supplementation of rumen-protected methionine during the transition period on immune function and liver functionality index of Holstein cows.

A. R. Guadagnin*[1], B. D. Davidson[1], D. N. Sherlock[2], D. Luchini[2], S. I. Arriola Apelo[1], and J. Laporta[1], [1]*University of Wisconsin, Madison, WI,* [2]*Adisseo, Alpharetta, GA.*

Exposure to heat stress during the transition period is associated with impairment of immune function. Feeding rumen-protected Met (RPM) during the transition period improves immune function. Therefore, we aimed to evaluate the effects of supplementing RPM to Holstein cows under heat stress during the transition period on immune function and liver functionality index (LFI), which assesses transition cow's metabolic health. Cows blocked by parity and ME305 were assigned to thermoneutral conditions (TN, avg THI 63, n = 19), heat stress induced by electric heat blankets (HS, n = 17), and HS with inclusion of RPM in the total mixed ration (MHS, 0.1% of DM of SmartamineM Adisseo Inc., Antony, France, n = 17) for 4 weeks pre- and 4 weeks post-calving. Blood samples were collected at 1, 8, and 28 d in milk (DIM) for hemogram, leukogram, albumin, cholesterol, and total bilirubin quantification, and granulocyte's phagocytosis and oxidative burst analyses. Data were analyzed using generalized linear mixed models in SAS, considering the fixed effects of treatment (TN, HS, MHS), DIM, and block. Contrasts included C1 (TN vs. average of HS and MHS) and C2 (HS vs. MHS). Cows in TN had greater hematocrit and lower neutrophil count (C1 $P = 0.04$; 31.3 ± 0.69% and $2.27 \pm 0.30 \times 10^9$ cells/L) than cows in HS and MHS (29.8 ± 0.69% and $2.83 \pm 0.30 \times 10^9$ cells/L). Cows in TN had greater granulocyte's phagocytosis percentage (C1 $P = 0.01$; 47.1 ± 9.67%) than HS and MHS cows (17.4 ± 9.67%). Cows in HS had lower albumin concentration at 28 DIM (C2 $P = 0.03$; 26.3 ± 2.04 g/dL) than cows in MHS (32.3 ± 2.04 g/dL). There was a lower ($P = 0.01$) proportion of cows classified as high LFI in HS (18%, 2/11) than in TN (75%, 12/16) and MHS (60%, 9/15). Finally, the odds of being classified as high LFI were lower in HS than in MHS (OR = 0.15, $P = 0.06$) and TN cows (OR = 0.07, $P = 0.01$). In conclusion, HS induced with heat blankets alters immune function mainly related to neutrophils' count and phagocytosis activity. Feeding RPM to transition cows under HS improved LFI, but effects on immune cells and granulocyte's function are limited.

Key Words: heat stress, Met, phagocytosis

2182 Rumen-protected methionine supplementation during the transition period under heat stress: Impact on cow-calf performance.

B. D. Davidson*[1], A. Zambon[1], G. A. Larsen[1], A. R. Guadagnin[1], D. N. Sherlock[2], D. Luchini[2], S. I. Arriola Apelo[1], and J. Laporta[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Adisseo USA Inc., Alpharetta, GA.*

Dairy cows exposed to heat stress (HS) during the dry period produce less milk and milk protein in the next lactation and give birth to smaller calves. Rumen-protected methionine (RPM) has been shown to modulate protein, energy, and placenta metabolism, suggesting RPM may be a nutritional intervention to mitigate adverse HS effects. We examined the effects of RPM supplementation to transition cows under HS induced by electric heat blankets (EHB) on cow-calf performance. Fifty-three Holstein cows were housed in a tie-stall barn 6 weeks before expected calving and fed a control diet (CN, 2.2% Met of MP) or a CN diet with SmartamineM (MT, 2.6% Met of MP, Adisseo Inc., France). Four weeks pre-calving all MT and half CN cows received an EHB. The other half of the CN cows were left at thermoneutrality (TN) resulting in: CNTN, CNHS, MTHS (n = 17–19). Respiration rate (RR) and skin temperature (ST) were measured thrice weekly. Post-calving body weights (BW) and BCS were recorded weekly. Daily milk yield was recorded, and components were analyzed every third day. Calf birth weight and stature were measured, and apparent efficiency of absorption (AEA) of immunoglobulins was calculated. Data were analyzed using the MIXED procedure of SAS with 2 orthogonal contrasts: CNTN vs. the average of CNHS and MTHS (C1) and CNHS vs. MTHS (C2). The use of EHB increased RR (C1: 39.0 vs. 51.9 ± 1.4 bpm) and ST (C1: 31.2 vs. 34.4 ± 0.1°C), relative to TN ($P < 0.0001$). Post-calving BW, BCS, and milk yield were not impacted by the EHB ($P \geq 0.36$), however, protein % was reduced by EHB (C1: 3.3 vs. 3.2 ± 0.04%, $P = 0.07$) but SNF was not (C1: 9.2 vs. 9.2 ± 0.06%, $P = 0.6$). Protein % (C2: 3.1 vs. 3.3 ± 0.05%) and SNF (C2: 8.9 vs. 9.4 ± 0.07%) were significantly lower in CNHS, relative to MTHS ($P \leq 0.005$). Calf birthweight (C1: 42.7 vs. 39.5 ± 0.96 kg) and AEA (C1: 53.8 vs. 38.9 ± 5.2%) were reduced by HS ($P \leq 0.02$), and wither heights tended to be shorter in CNHS (C1: 78.6 vs. 77.4 ± 0.6 cm; $P = 0.13$; C2: 76.5 vs. 78.2 ± 0.7 cm; $P = 0.09$), compared with MTHS heifers. Overall, RPM supplementation to transition cows reverts the negative impact of HS on milk protein and calf wither heights.

Key Words: hyperthermia, heat blanket

2183 The effect of limited outdoor access frequencies on gait score, hoof lesions and hoof surface temperature of non-clinically lame cows housed in a movement-restricted environment. S. Mokhtarnazif*[1], E. Shepley[2], A. Nejati[1], G. M. Dallago[3], and E. Vasseur[1], [1]*McGill University, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*University of Minnesota, Minneapolis, MN,* [3]*Université du Québec à Montréal, Montreal, QC, Canada.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).
Providing outdoor access is beneficial to the welfare of cows housed in restricted movement environments, but its daily implementation is challenging. The study objective was to evaluate the effect of low outdoor access frequency on gait and hoof health of non-clinically lame cows. Thirty-six lactating, tie-stall housed Holstein cows were blocked by parity and days in milk (6 blocks) and provided 1h of outdoor access in 2 treatments (1 d/wk or 3 d/wk) for 5 weeks (Nov-Dec 2022). Data were collected before (pre-trial), after (post-trial) and 8 weeks after (follow-up) outdoor provision. We assessed gait score using a 5-point visual scoring system and clinical claw lesion incidence in the trimming chute. For subclinical signs of lesions, the average hoof and claw surface temperatures were measured from the dorsal view of the coronary bands using an infrared thermography camera. Data were analyzed using mixed effect models with block, period, treatment, and the period × treatment interaction as fixed effects ($\alpha < 0.05$), and either cow or claw nested within cow as random effect. The ambient and cow's eye maximum temperature were added as covariates for analyzing thermography data. Average gait scores at pre-trial, post-trial, and follow-up were 2.13 ± 0.55, 1.98 ± 0.61 and 2.02 ± 0.51, respectively. Only non-severe lesions (sole, n = 39, and white line hemorrhages, n = 2) were observed during the study. No treatment or period effects were found for gait score or hoof lesions ($P > 0.05$). Average hoof temperature was lower ($P < 0.05$) in the post-trial (27.7 ± 0.25°C) and follow-up (27.6 ± 0.27°C) compared with pre-trial (29 ± 0.26°C). Similar trends were observed in the lateral and medial claws ($P < 0.05$). No treatment effect on hoof thermography was observed ($P > 0.05$). Providing outdoor access did not impair gait or the incidence of claw lesions in non-clinically lame cows. While further studies are needed with a larger sample size, the observed reduction in hoof surface temperatures suggests that as little as 1 h/week of outdoor access may benefit cow hoof health.

Key Words: animal welfare, hoof thermography, lameness

2184 Withdrawn.

2185 Total nitrogen in excreta from dairy and beef cows and its relationship to production parameters in central China. Z. Shi*[1,2], L. Xi[1], and X. Zhao[2], [1]*Henan University of Animal Husbandry and Economy, Zhengzhou, Henan, China,* [2]*McGill University, Ste. Anne de Bellevue, Quebec, Canada.*

Accurately measuring total nitrogen (TN) output in excreta from dairy and beef cows is crucial for accurate assessment of the environmental footprint of dairy and beef production and development of sustainable TN mitigation strategies. In this work, we surveyed 6 dairy and 5 beef farms in central China. In each farm, 5 calves, 5 heifers, and 5 lactating dairy cows or 5 beef cows were selected. The total feces and urine of

Table 1 (Abstr. 2185). Total nitrogen in excreta from dairy and beef cows at different stages in 4 seasons (g/animal per day)

		Seasons			
Animals	Stages	Spring	Summer	Autumn	Winter
Dairy cows, mean (SEM)	Calves	14.99 ± 1.01	17.99 ± 1.26	22.33 ± 0.56	36.67 ± 1.67
	Heifers	114.24 ± 0.20	101.09 ± 1.34	95.60 ± 1.53	99.06 ± 1.04
	Lactating dairy cows	171.34 ± 0.56	158.34 ± 2.78	210.05 ± 1.40	232.57 ± 2.39
Beef cows, mean (SEM)	Calves	15.87 ± 2.29	19.61 ± 0.98	29.84 ± 1.99	40.32 ± 2.52
	Heifers	135.07 ± 1.75	102.95 ± 0.15	94.57 ± 0.73	116.72 ± 1.84
	Beef cows	149.82 ± 3.57	124.78 ± 0.42	170.21 ± 0.51	212.69 ± 0.63
Significance (*P*), Dairy cows vs beef cows	Calves	0.313	0.627	0.038	0.395
	Heifers	0.030	0.796	0.822	0.011
	Lactating dairy cows vs beef cows	<0.001	0.009	<0.001	0.026

each animal were collected for 10 d in each of 4 seasons and measured for TN. Data for milk production for lactating dairy cows, dry matter intake (DMI), and gross energy intake (GEI) for both lactating dairy cows and beef cows were collected to calculate their relationships with TN in excreta, using a regression analysis by the MIXED procedure in SAS. Weight and temperature were used as random effects. One-way ANOVA with a paired *t*-test was used to compare significant differences among the groups. As shown in Table 1, lactating dairy cows excreted more TN than beef cows in all 4 seasons and the increase of TN in calves reflected the growth of these animals. The correlation analyses showed a positive correlation ($P < 0.05$) between TN in excreta and milk production ($R^2 = 0.64$), DMI ($R^2 = 0.66$), and GEI ($R^2 = 0.71$) for lactating dairy cows, and between TN in excreta and DMI ($R^2 = 0.61$) and GEI ($R^2 = 0.58$) in beef cows. Our results contribute to calculation of nitrogen excretion from both beef and dairy cows.

Key Words: total nitrogen, excreta, cow

Ruminant Nutrition 1: Gut Physiology, Fermentation, and Digestion

2186 Comparing toxicity of lipopolysaccharide endotoxin (LPS) obtained from pathogenic versus commensal gram-negative bacteria. A. A. Alizadeh, P. A. Azevedo, H. Derakhshani, and J. C. Plaizier*, *University of Manitoba, Winnipeg, MB, Canada.*

Free lipopolysaccharide (LPS) from gram-negative bacteria in the digestive tract of cattle have been implicated in the inflammatory response resulting from subacute ruminal acidosis (SARA). Studies on this response have used LPS from *E. coli*. However, it has been suggested that LPS from *E. coli* could be a more potent endotoxin than LPS of common gram-negative bacteria in the digestive tract of cattle, such as *Prevotella* spp. Methods to quantify LPS include the Limulus amebocyte lysate (LAL) and ELISA assays. The principle of the LAL test is a reaction between LPS and a clottable protein obtained from the blood of horseshoe crabs, whereas the principle of ELISA is based on the interaction between LPS antigen and antibodies. Hence, the results of the ELISA may be more representative of amounts of LPS and the results of the LAL may be more representative of the toxicity of LPS. We, therefore, hypothesize that the ratio between the results obtained from LAL and ELISA differ between LPS extracted from *E. coli* compared with those extracted from commensal bacteria such as *Prevotella* spp. To address this, bacteria were cultured either aerobically (*E. coli* F4, and *Shigella flexneri*) or anaerobically inside a Bactron Anaerobic chamber (*Prevotella copri*) using BHI media without or with supplementation with 0.5 g/L L-cysteine and 10 mg/L Hemin, respectively. LPS was extracted by using a phenol-water method. Extracted LPS was assayed using LAL assay (Chromogenic Endotoxin Quan Kit, Thermo Scientifc, MA, USA) yielding endotoxin units (EU)/mL and the LPS Sandwich ELISA (LSBio Seattle, WA, USA) yielding ng/mL. Data were analyzed using ANOVA and Tukey-HSD. Our results indicated that the ratio of ng over EU for LPS extracted from *E. coli* F4 was similar to *S. flexneri* (0.28 ng/EU, SD = 0.021; and 0.30 ng/EU, SD = 0.015, respectively), while the LPS of both pathogens had lower (*P*-value <0.05) ng/EU ratios compared with *P. copri* LPS (0.39 ng/EU, SD = 0.041). These data suggest a higher toxicity of LPS obtained from pathogenic *Enterobacteriaceae* versus those obtained from commensal gram-negative bacteria. Our results warrant further in vitro and in vivo evaluations of cytotoxic and inflammatory effects of LPS from different lineages of rumen bacteria on epithelial integrity and immune homeostasis.

Key Words: endotoxin, toxicity, gram-negative bacteria

2187 Analysis of dual-flow continuous culture fermenter contents at termination may be a poor proxy for digestibility estimated by effluent sampling. B. A. Wenner*, *Department of Animal Sciences, The Ohio State University, Columbus, OH.*

In dual-flow continuous culture (DFCC) experiments, cumulative daily effluent is commonly used to determine nutrient digestibility. However, non-steady state conditions could result in nutrient accumulation, especially fiber, within the DFCC jar that obscures treatment effects on nutrient digestibility. The objective of this study was to evaluate the potential use of accumulated dry matter in a DFCC jar to determine nutrient digestibility effects. The hypothesis was that decreased fiber digestibility would increase DM, including ADF, in the jars. Samples were taken from a previously conducted experiment randomizing DFCC fermenters (n = 8) to 1 of 8 treatments for 5 periods: negative control (CON, 94 g DM/d, 50:50 orchardgrass:concentrate pellets, 36% NDF, 25% starch, 11.1% CP), CON with supplemental urea (UL, UM, and UH at +1.5%, +3.0%, and +4.5% CP), CON with supplemental peptides (PL, PM, and PH at +1.5%, +3.1%, and +4.6% CP), and positive control (SOY, +8.4% CP from soy protein powder). Experimental periods lasted 11 d whereupon fermentation was terminated and samples analyzed for CP, NDF, ADF, and ash. Statistical analysis included treatment (fixed), and random effects of fermenter and period, while contrasting CP source, level, and source × level effects. Supplemental urea linearly decreased (*P* = 0.03) CP recovered from jars whereas peptide supplementation increased CP recovery from jars (*P* = 0.047). Urea linearly (*P* = 0.046) decreased NDF within the jars by 3.06 g, whereas urea only tended (*P* = 0.08) to decrease ADF accumulation by 1.26 g. There were no other effects of treatment nutrient accumulation. An F-test of variance between effluent digestibility and nutrient accumulation indicated the variance of NDF accumulation was 3-fold greater than NDF digestibility estimated from effluent (*P* < 0.01) whereas there was no difference (*P* = 0.18) between variances for ADF estimates. These data indicate that while accumulation of nutrients in the fermenter jar occurs, especially with fiber or degradation-resistant protein, effluent measurements are likely more sensitive and useful for determining treatment differences.

Key Words: in vitro, method

2188 Exhalomics as a non-invasive method for assessing rumen fermentation in dairy cows: Can exhaled breath metabolomics replace rumen sampling? M. Z. Islam[1], S. E. Räisänen*[1], A. Schudel[1], K. Wang[1], F. Wahl[2], R. Zenobi[3], S. Giannoukos[3], and M. Niu[1], *[1]Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland, [2]Food Microbial Systems Research Division, Agroscope, Bern, Switzerland, [3]Department of Chemistry and Applied Biosciences, Analytical Chemistry, ETH Zürich, Zürich, Switzerland.*

Previously, we characterized the intensity and daily patterns of exhaled volatile fatty acids (eVFA) using a secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) platform. The aim of this study was to further validate the potential of the exhalomics approach to assess rumen fermentation. Four rumen-cannulated Original Brown-Swiss cows were used in a switchback design with three 9-d periods (7-d adaptation, 2-d sampling). Cows were randomly assigned to 1 of 2 diet sequences (ABA/BAB): (A) low-starch (LS; 6.3% starch of DM), and (B) high-starch (HS; 16.2% starch of DM). Feeding was 1×/d at 0800 h. Exhalome (with GreenFeed System), and rumen samples were collected 8× to represent every 3-h of a day, and eVFA and ruminal VFA (rVFA) were analyzed using SESI-HRMS and HPLC, respectively. Pearson correlations for acetate, propionate, and butyrate (as % of the total VFA) between the 2 methods were 0.44, 0.39, and 0.40, respectively (*P* < 0.01). Data were analyzed in a mixed model with fixed effect of period, method, diet, and method × diet interactions, and random effect of time (repeated measures) and cow nested in sequence. Diet × method interactions were not observed. A reduced model was fitted on method-specific subset of data to test diet effect. The VFA molar proportions differed between HS vs. LS regardless of method: acetate was 64.1 vs. 60.1 for exhalome (*P* = 0.01) and 67.0 vs. 64.7 for rumen (*P* = 0.01), propionate 28.1 vs. 30.5 (*P* = 0.09) and 22.9 vs. 24.7 (*P* = 0.04), butyrate 7.87 vs. 9.53 (*P* = 0.04) and 10.1 vs. 10.7 (*P* = 0.11); and A:P ratio 2.49 vs. 2.14 (*P* = 0.05) and 3.13 vs. 2.84 (*P* = 0.04). For VFA daily patterns, a similar model was fitted for diet-specific subset of data but with method × time interactions. Regardless of diet, interactions were not observed (*P* > 0.10). Despite moderate correlation, eVFA and rVFA responded similarly to feeding time and dietary treatments, indicating the potential of eVFA as a proxy to characterize rVFA molar proportions in response to feeding and dietary treatments. Future studies should further explore the potential of exhalomics in ruminant research.

Key Words: exhaled volatile fatty acids, ruminal VFA, SESI-HRMS

2189 Interaction of supplemental branched-chain volatile fatty acids (BCVFA) on neutral detergent fiber digestibility (NDFD) and rumen kinetics in Jersey cows.

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We previously documented 2-methylbutyrate (2MB) improved NDFD in vitro most followed by isobutyrate (Ibu) and isovalerate (Ival). Because Ibu can substitute for Ival in bacterial lipids, more Ival was hypothesized to increase Ibu needs by cellulolytic bacteria. Primiparous rumen-cannulated Jerseys (70 ± 16 DIM) were enrolled in a 6x6 Latin square design with low (15.0%; LCP) or high (16.5%; HCP) CP controls without BCVFA or 4 LCP diets all with 1X 2MB but with 0X or 1X Ival and either 1X or 2X Ibu arranged in a 2x2 factorial; 1X was 14.4 g/d of 2MB or Ival and 12.4 g/d of Ibu (equimolar). Dietary BCVFA were within 7% of target, but CP averaged 14.9 and 16.2% for LCP and HCP. Production data were collected the last 7 d of each 25-d period, and total-tract digestibility was determined on d 18 to 22 using Cr2O3. Yb-marked corn silage, Cr-EDTA, and valerate were dosed to estimate liquid and solids passage rates (kp) and valerate absorption rate (ka; Cr kp minus valerate disappearance rate) on d 23 before rumen evacuations 2 h before and 2 h after feeding on d 24 and 25. Data were analyzed with a mixed model with random effects of period and cow and fixed effects of diet. Orthogonal contrasts were LCP or HCP vs. the 4 BCVFA treatments and main effects and interaction of Ival and Ibu within the 4 BCVFA treatments. Supplementation of BCVFA did not change DMI or ECM vs. controls, but ECM efficiency was lower ($P = 0.02$) compared with HCP. Increasing Ibu increased ($P < 0.10$) propionate molar % and ADG, suggesting Ibu fermentation and insulin effects. Rumen NH_3 was lower ($P < 0.01$) for BCVFA vs. HCP but not vs. LCP. Total-tract NDFD was not affected, but BCVFA diets had lesser ($P = 0.09$) rumen pools of undegradable NDF even though cows fed BCVFA apparently sorted less ($P < 0.05$; smaller orts' mean particle size, MPS). Rumen MPS (from wet sieving) was smaller ($P = 0.05$) with Ival, although Yb kp decreased ($P = 0.06$) and Val ka increased ($P = 0.07$) by Ival with 2X Ibu. NDFD residuals suggest low NH_3 (LCP diets <5 mg/dL) limited NDFD and must be adequate before optimizing benefits of BCVFA.

Key Words: isoacids, lactating dairy cow, rumen volatile fatty acid (VFA) turnover

2190 RNA-sequencing analysis of biopsied rumen papillae revealed that key rumen epithelium functions change in relation to short-chain fatty acids and rumen epithelium-attached microbiota during the weaning transition in dairy calves.

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This study aims to characterize the functional changes of the rumen epithelium in relation to rumen epithelium-attached microbes and ruminal short-chain fatty acids (SCFA) concentration during the weaning transition in 6 Holstein dairy bull calves weaned at wk 6. Ruminal SCFA concentrations were measured, and transcriptome and microbiota profiles of biopsied rumen papillae at wk 5 (W5), wk 7 (W7), and wk 12 (W12) of age were obtained using RNA-sequencing and amplicon-sequencing. False discovery rate (FDR) and *P*-value were adjusted using Benjamini-Hochberg method. The number of differentially expressed (DE) genes between weaning transition periods was 87 (W5 vs. W7), 56 (W7 vs. W12), and 457 (W5 vs. W12), respectively (FDR <0.05 and $|\log_2$ fold change$| > 1.3$). Metabolic pathway analysis using the list of DE genes showed that pathways related to SCFA metabolism and cell apoptosis were up- and downregulated postweaning, respectively ($P < 0.05$). The weighted correlation network analysis and functional analysis showed that a gene module, consisting of 498 genes related to SCFA absorption, metabolism, and protective roles against oxidative stress, was positively correlated to ruminal total SCFA concentration and rumen papillae surface area ($P < 0.05$ and $r \geq 0.6$). The relative abundance of epithelium-attached *Rikenellaceae* RC9 gut group and *Campylobacter* was positively correlated with genes involved in SCFA absorption and metabolism. These findings suggest that SCFA absorption, metabolism and protective roles against oxidative stress-induced apoptosis are the key responses to SCFA concentration changes during the weaning transition, and some epithelium-attached bacteria can cooperatively affect host functions with genes involved in SCFA absorption and metabolism. Future research should examine the contribution of attenuated apoptosis on rumen epithelial functional shifts during the weaning transition in dairy calves.

Key Words: rumen papillae biopsy, RNA-sequencing, rumen epithelium-attached microbiota

2191 Reduction in the colon mucosa thickness during the weaning transition is associated with molecular changes involved in immune function in dairy calves.

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The objective of this study was to characterize the structural and molecular changes in the colon mucosa of calves during the weaning transition. Six Holstein bull calves were weaned at wk 6 of age. Colon mucosa thickness, fecal DM, fecal starch, and blood inflammatory markers were determined from wk 4 to wk 12 and were analyzed using GLIMMIX procedure. Transcriptome profiles of biopsied colon mucosa were obtained at wk 5, wk 7, and wk 12 using RNA-sequencing, and false discovery rate (FDR) was adjusted using Benjamini-Hochberg method. Colon mucosa thickness was lower during wk 7 and 8 compared with wk 5 ($P < 0.05$). Fecal starch was higher in wk 9 than in wk 4, 5 and 6 ($P < 0.05$). There was no correlation between colon mucosa thickness and fecal starch, fecal DM, or blood inflammatory markers. The number of differentially expressed (DE) genes between weaning transition periods were 439 (wk 5 vs. wk 7), 430 (wk 7 vs. wk 12), and 757 (wk 5 vs. wk 12), respectively (FDR <0.05 and $|\log_2$ fold change$| > 2.0$). Metabolic pathway analysis of DE genes showed that pathways related to immune function were upregulated postweaning. Further weighted correlation network analysis and functional analysis showed that a gene module consisting of genes involved in the regulation of a transcription factor inducing the expression of various pro-inflammatory genes, was correlated with colon mucosa thickness ($P < 0.05$ and $r = 0.5$). Seventeen

immune-related genes were identified in this gene module, and their expressions were upregulated at wk 7 or wk 12 compared with wk 5. These results suggest that inflammation in the colon mucosa was not severe enough to induce systemic inflammation, and 17 genes may be related to the inflammation in colon mucosa and recovery from mucosa thickness decrement. Future studies should aim to determine immune cells' function and population in the colon mucosa during the weaning transition in dairy calves.

Key Words: colon biopsy, RNA-sequencing, dairy calf

2192 Withdrawn.

2193 The relationship of gut permeability with inflammation, performance, and colon gene expression in fresh cows.

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The objective of this study was to investigate the relationship of gut permeability in the fresh period with dry matter intake, plasma energy metabolites, milk yield, inflammation, and colon gene expression. To assess this, 20 multiparous cows were fed a closeup TMR (21.5% starch, 39.1% NDF) from 21 d before expected calving date, until calving. For 5 d immediately after calving, cows were fed a lactation diet (26.8% starch, 33.0% NDF) in addition to free choice timothy hay (61.6% NDF, 9.6% CP). At 3 DIM, cows were fitted with a urinary catheter and ruminally dosed with chromium (Li-Cr-EDTA) as a marker of gut permeability. Total urine was collected for 48 h and representative urine samples were analyzed with atomic absorption spectrophotometry. At 5 DIM, colon biopsies were collected for subsequent gene expression analysis using RT-qPCR. The relationship of gut permeability with each parameter was assessed using bivariate regression analysis, and Pearson Correlation coefficients were determined using the MULTIVARIATE procedure (JMP 16.1.0). There was no relationship detected between gut permeability and postpartum DMI, plasma glucose or BHB concentration, or serum inflammatory marker concentration. However, cows with lower prepartum intake tended to have greater gut permeability ($P = 0.06$; r = −0.49). Conversely, cows with greater plasma fatty acid concentration on d 1 and 3 had greater gut permeability ($P < 0.01$, r = 0.68 and $P = 0.04$, r = 0.43, for d 1 and 3, respectively). Cows that produced more milk in the first 5 DIM tended to have greater gut permeability ($P = 0.09$; r = 0.40). Gut permeability was positively correlated with expression Claudin 1 ($P < 0.01$, r = 0.40), a tight junction gene that decreases gut permeability. These findings suggest that cows with lower prepartum intake and greater postpartum fat mobilization and milk production experience greater gut permeability in the fresh period. Additionally, cows with greater gut permeability may upregulate tight junction gene expression in the colon.

Key Words: gut permeability, colon gene expression, fatty acids

2194 Effects of heat stress conditions and dietary organic acid and pure botanical supplementation on milk fatty acid composition in relation to gut permeability.

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To evaluate the effects of heat stress (HS) and dietary organic acid and pure botanical (OA/PB) supplementation on milk fatty acids (MFA), we enrolled 48 Holstein cows (208 ± 4.65 d in milk; 3.0 ± 0.42 lactations) in a completely randomized design. Following a 7-d acclimation in thermoneutral conditions (temperature-humidity index [THI] 68), cows were assigned to 1 of 4 groups: thermoneutral conditions (TN-Con), HS conditions (HS-Con; diurnal THI 74 to 82), thermoneutral conditions pair-fed to match HS-Con (TN-PF), or HS fed OA/PB (HS-OAPB; 75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlusR, Vetagro, Italy) for 14 d. Cows were fed a total mixed ration top-dressed without (triglyceride only) or with OA/PB. Cows were milked twice daily. Milk samples were collected on d −2, 3 and 13. Cr-EDTA (gut permeability [GP] marker) was drenched on d 3 and 13. Data were analyzed using a mixed model including fixed effects of treatment, time, and their interaction. Planned contrasts included HS-Con versus (vs.) TN-Con, HS-Con vs. TN-PF and HS-Con vs. HS-OAPB. Pearson correlation was used to define relationship between MFA and GP (area-under-the-curve) on d 3 and 13. Milk concentrations and yields of de novo and mixed MFA were greater while preformed, total monounsaturated, and C18:0 concentrations were lower in TN-Con, relative to HS-Con ($P < 0.01$). Milk concentrations and yields of *cis*-9,*trans*-11-C18:2 and de novo MFA were increased while C18:0 concentrations were decreased in TN-PF, relative to HS-Con ($P < 0.05$). MFA concentrations and yields were not modified by HS-OAPB, relative to HS-Con. Concentrations of mixed MFA on d 3 ($r = -0.43$; $P < 0.01$) and yields of *cis*-9,*trans*-11-C18:2, total polyunsaturated, *trans*-11-C18:1 and *cis*-9,*cis*-12-C18:2 on d 13 ($r = -0.30$ to -0.33; $P \leq 0.05$) were negatively correlated with GP. We conclude that MFA production is modified by HS but OAPB feeding does not restore these responses. Moreover, the concentrations of select MFA are weakly correlated with gut permeability in dairy cattle.

Key Words: gut permeability, heat stress, milk fatty acid

2195 Description of gastrointestinal tract mycobiota of dairy cows. A. Sadek*[1,2], B. Taminiau[1,3], G. Daube[1,3], F. Coucheney[1], A. Bach[4], F. Chaucheyras-Durand[2,5], M. Castex[2], and D. Drider[1], [1]*Unité Mixte de Recherche (UMR) Transfrontalière BioEcoAgro 1158, Univ. Lille, INRAE, Univ. Liège, UPJV, YNCREA, Univ. Artois, Univ. Littoral Côte D'Opale, ICV—Institut Charles Viollette, Lille, France,* [2]*Lallemand SAS, Blagnac, France,* [3]*Fundamental and Applied Research for Animal & Health (FARAH), Veterinary Medicine Faculty, Liège, Belgium,* [4]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain,* [5]*Université Clermont Auvergne, INRAE, UMR 0454 MEDIS, Clermont-Ferrand, France.*

Gastrointestinal (GI) microbiota is key in digestive processes, gut health, and homeostasis in dairy cows. Whereas bacterial communities have been well described in ruminants, few data exist on fungal communities. This study aimed to characterize the mycobiota associated with dairy cows in samples collected from 3 GI compartments: (rumen, colon, rectum); we also compared results using 3 DNA extraction kits. Ruminal and colonic samples were taken using an endoscope, whereas feces were obtained by manual sampling in the rectum from 5 multiparous dairy cows (milk yield 39.6 ± 3.8 kg/d; 172 ± 6 DIM) fed the same lactation diet (15.2% CP, 28.5% NDF, 1.64 Mcal of NE_L/kg). Total DNA was extracted using commercial kits from Zymo Research (ZQ is commonly used for this type of sample, and ZM is more suitable for microbiome analysis) and Macherey Nagel (MN is advertised to obtain high DNA yield). Only the ZM kit was used for colon extraction. DNA extracts were analyzed by ITS2 amplicon sequencing (Illumina MiSeq platform). Mycobiota composition was different in the rumen compared with colon and feces (Bray-Curtis dissimilarity, AMOVA, *P*-values: 1e-05 and 3e-05 respectively) with no significant difference between the 2 latter compartments. Alpha diversity parameters did not differ between ruminal and fecal samples when using the same kit, except for a greater evenness value for rumen with ZQ (Two-way ANOVA, *P*-value: 0.02); Differences were found depending on the extraction method. For instance, ZM yielded greater diversity than ZQ and MN in ruminal samples (Two-way ANOVA, *P*-values: 5e-03 and 0.01 respectively) and greater evenness similarly (Two-way ANOVA, *P*-values: 0.02 and 4.6e-02 respectively). An appraisal of the core mycobiota in the rumen and feces showed that only one OTU belonging to the genus *Geotrichum* was found in all samples at a relative abundance greater than 1% (average 32%). This study paves the way for a deeper investigation to increase our understanding of the characteristics and roles of the mycobiota across the GI tract of ruminants. Results herein support that feces can be a good non-invasive alternative to studying the colonic mycobiota.

Key Words: mycobiota, colon, *Geotrichum*

2196 Grazing diet promotes mycobiota richness and diversity in dairy cows' hindgut. A. Sadek*[1,2], B. Taminiau[1,3], G. Daube[1,3], F. Coucheney[1], P. Sapountzis[4], F. Chaucheyras-Durand[2,4], M. Castex[2], and D. Drider[1], [1]*Unité Mixte de Recherche (UMR) Transfrontalière BioEcoAgro 1158, Univ. Lille, INRAE, Univ. Liège, UPJV, YNCREA, Univ. Artois, Univ. Littoral Côte D'Opale, ICV—Institut Charles Viollette, Lille, France,* [2]*Lallemand SAS, Blagnac, France,* [3]*Fundamental and Applied Research for Animal & Health (FARAH), Veterinary Medicine Faculty, Liège, Belgium,* [4]*Université Clermont Auvergne, INRAE, UMR 0454 MEDIS, Clermont-Ferrand, France.*

Hindgut microbiota plays a key role in animal health by maintaining the integrity of the gut mucosa, thus acting as a natural barrier against the colonization of intestinal pathogens and serving as a site of nutrient absorption. While its bacterial taxonomic composition and functions have been broadly studied, less is known about the fungal population, called mycobiota, in the large intestine. In this work, mycobiota diversity and composition changes in dairy cows were characterized through feces, as they can serve as a proxy for this compartment. Samples collected in summer and winter from a previous study on bacterial changes in the intestinal microbiota came from the same animals with 2 different diets (21 animals sampled at both periods and 26 more only in summer). During summer, their diet consisted of grazing in grassland and was complemented by 3 to 5 kg/d of corn, grass silage, and hay. In winter the diet was composed of 30% corn silage, 25% grass silage, 15% hay, and 30% production concentrate. The fungal diversity and composition were analyzed by a metataxonomic approach through ITS2 amplicon sequencing (Illumina MiSeq platform). Results showed that fungal α diversity indices (inverse Simpson, chao1, and richness) were significantly decreased during winter compared with the summer period (Unpaired *t*-test for Chao1, Mann-Whitney test for others, *P*-value < 1e-04 for all). Beta diversity analysis showed clearly different mycobiota profiles (Bray-Curtis dissimilarity, AMOVA, *P*-value < 1e-05). With the winter diet, an Operational Taxonomic Unit (OTU) identified to the *Geotrichum*genus, accounted for up to 88% of the total fungal relative abundance (RA). This same OTU was also observed in the summer group but not in the same proportion. Indeed, the mean RA of *Geotrichum* was 5% and 65%, respectively, in the summer and winter groups. This OTU was the only one found in all samples of the winter group with a RA >1%. The grazing diet seems to favor OTU diversity in feces in contrast to winter feeding. To our knowledge, this is the first study to highlight the fungal diversity of ruminant microbiota hindgut according to their diet.

Key Words: mycobiota, diet, *Geotrichum*

2197 Cows with induced ketosis early postpartum have a different immunometabolic profile than healthy cows or cows with inflammatory disorders. J. K. Drackley*[1], H. M. Dann[1], G. Bertoni[2], and E. Trevisi[2], [1]*University of Illinois, Urbana, IL,* [2]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Our objective was to compare immunometabolic profiles of healthy cows, healthy cows with induced ketosis, and cows with inflammatory disorders early postpartum. Cows (n = 32) from a previous study (Dann et al., 2005; JDS 88:3249) underwent a physical examination at 4 DIM and were classified as healthy or having at least one periparturient inflammatory disorder (PD; mastitis, metritis, retained placenta, feet/leg; n = 16). Healthy cows were assigned to control (CON; n = 6) or ketosis induction (KI; n = 10) groups. Cows in KI were fed 50% of their intake on 4 DIM from 5 to 14 DIM or until signs of clinical ketosis, then returned to ad libitum intake. Cows in control and PD were fed for ad libitum intake. Contrasts were healthy vs. disorder (CON vs. KI + PD) and ketosis vs. sick (KI vs. PD). Mean DMI was 18.9, 9.6, and 14.1 kg/d for CON, KI, and PD (lower for disorders than CON and lower for KI than PD). Milk yield (34.2, 26.8, 24.0 kg/d) was lower for cows with disorders than CON. At d 7, KI had lower ($P = 0.001$) serum glucose

(53, 41, 52 mg/dL) and higher NEFA (793, 1438, 775 µEq/L) and BHB (7.2, 16.7, 9.5 mg/dL) than PD. Cholesterol was lower ($P = 0.02$) for PD than KI (2.32, 2.30, 1.91 m*M*). Serum Ca ($P = 0.04$) and Mg ($P = 0.09$) were lower for cows with disorders than for CON. Albumin and albumin:globulin (0.76, 0.84, 0.69) were lower ($P < 0.01$) for PD than KI. Haptoglobin was greater ($P = 0.09$) for PD than KI (0.80, 0.77, 1.57 m*M*). Ceruloplasmin was lower for cows with disorders than CON and lower for PD than KI. Paraoxonase was lower ($P = 0.02$) for cows with disorders than CON. Serum AST was greater ($P = 0.06$) for PD than KI. Bilirubin (1.93, 4.12, 6.01 m*M*) and GGT were elevated ($P < 0.10$) for cows with disorders. Serum retinol and β-carotene were lower ($P < 0.01$) for PD than KI. Total protein, globulin P, Zn, and tocopherol did not differ ($P > 0.10$). Liver total lipid at d 14 was greater ($P = 0.001$) for KI than PD (5.46, 13.54, 6.15% wet weight). Few signs of inflammation were present in uncomplicated ketosis compared with inflammatory disorders; cows with inflammatory disorders did not have altered lipid metabolism.

Key Words: inflammation, ketosis, periparturient

Ruminant Nutrition 2: Protein and Amino Acids

2198 Nutrient dynamics of dairy cattle milk protein concentration under the first limiting theory: A meta-analytical approach. T. Danese*[1], M. Van Amburgh[2], P. A. LaPierre[2], F. Righi[1], and A. Foskolos[3], *[1]Department of Veterinary Sciences, Parma University, Parma, Italy, [2]Department of Animal Science, Cornell University, Ithaca, NY, [3]Department of Animal Science, University of Thessaly, Larissa, Greece.*

The objective of the study was to investigate the relationship between dietary characteristics and milk crude protein concentration (mCP) in dairy cattle with diets differing in first limiting nutrients, either metabolizable energy or protein (ME and MP). A previously developed data set including lactation studies from 2001 to 2015 was updated with relevant studies up to 2021, resulting in 108 lactation studies with 291 nutritional treatments. Statistical analysis was conducted using JMP Pro v16. A regression of mCP and each dietary component (% DM) was evaluated, namely CP, RUP, RDP, NDF, ADF, EE, sugar, and starch; furthermore, the best 3 parameters were paired (1st × 2nd, 1st × 3rd, 2nd × 3rd) and further analyzed (Table 1 in order of decreasing performance). To do that, a mixed effects model (random: study) was used and the best fit was selected based on the highest R^2 adjusted and the lowest root mean square error (RMSE). Analysis took place for 3 data sets: (A) all treatments, (B) MP limiting treatments, and (C) ME limiting treatments. When all diets were included, the EE, starch and ADF were the principal nutrients explaining the greatest variation, respectively. With MP limited diets NDF, EE and RDP were dietary factors explaining the greatest variation and when ME was first limiting, starch, EE and ADF explained the most variation. Multicollinearity was tested, showing high correlation between the variables considered. The current results suggest that different dietary characteristics are influencing mCP depending on the first limiting nutrient.

Key Words: dairy cattle nutrition, limiting nutrient theory

2199 Lysine, methionine, and histidine deficiency affect milk protein synthesis and mRNA expression of transcription factors by primary bovine mammary epithelial cells. B. Li*[1], D. Innes[1], M. Madison[1], J. Kim[1], C. Rodriguez[1], J. Doelman[1,2], and J. Cant[1], *[1]University of Guelph, Guelph, Ontario, Canada, [2]Trouw Nutrition, Putten, the Netherlands.*

Essential amino acids (EAA) may affect milk protein synthesis through a combination of transcriptional and translational activation, and stimulation of cell proliferation and biogenesis of endoplasmic reticulum (ER). The objective was to determine how much an in vitro, single EAA deficiency would alter rates of protein, fat, DNA and ER synthesis and to measure the mRNA expression of hypothetically relevant transcription factors. Mammary epithelial cells were collected from the mammary glands of 3 dairy cows and cultured in a medium with equal parts DMEM/F12 and Medium 170. Cells were differentiated for 5 d and then cultured in triplicate for 60 h in control medium (CTL) containing all EAA at normal physiological concentrations, or media with 20 μM Lys (LK), 5 μM Met (LM), or 10 μM His (LH), each at 25% of CTL concentrations. EasyTag Express 35S, ^{14}C-acetate, and 3H-thymidine were added at 1 mCi/mL into media 2 h before cell harvest to estimate rates of protein, fat, and DNA synthesis, respectively. Cells were also harvested for qRT-PCR and for ER formation analysis. Linear contrasts were estimated by ANOVA assuming fixed effects of treatment. Rates of protein synthesis ($P < 0.01$), DNA synthesis ($P < 0.01$) and ER formation ($P < 0.05$) were significantly lower in all treatments relative to CTL. Fat synthesis rate was not affected by treatment ($P = 0.22$). Expression of ATF6 ($P = 0.06$) and JUN ($P = 0.07$) tended to be higher in LK relative to CTL. Expression of FOS tended to be lower in LK ($P = 0.06$), was lower in LM ($P < 0.01$) and higher in LH ($P = 0.02$), relative to CTL. These findings indicate that single EAA deficiencies inhibited mammary protein synthesis, cell proliferation and ER formation, but did not affect the rate of milk fat synthesis. The transcription factors ATF6 and JUN were upregulated by deficiencies of Lys while FOS was downregulated by Lys and Met.

Key Words: amino acids, transcription factors, mammary epithelial cells

2200 Source and frequency of rumen-protected protein supplementation affects mammary gland amino acid metabolism. K. Nichols*[1], N. Wever[1], C. Cirot[2], M. Rolland[2], and J. Dijkstra[1], *[1]Animal Nutrition Group, Wageningen University and Research, Wageningen, the Netherlands, [2]Ajinomoto Animal Nutrition Europe, Paris, France.*

We investigated mammary gland metabolism in response to protein source and oscillating AA supplementation. Twenty-eight Holstein-Friesian cows (93 ± 27 DIM) were blocked by DIM and parity and fed a base ration (15.5% CP; formulated to 95 and 100% of MP and NE_L requirements) in tie stalls for 16 d at a fixed amount according to the mean ad libitum intake of each block during a 7-d diet adaptation period. Cows within block were randomly assigned to the base ration (CON) or 1 of 3 rumen-protected (RP) supplements mixed into the base ration. Supplements were 1) 384-g mixture of RP His (Ajinomoto Co, Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition, USA), and Met (Smartamine; Adisseo, France) fed daily (RPAA), 2) 768-g mixture of RP His, Lys, and Met fed every-other day (OS-RPAA), and 3) 315-g mixture of RP soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily (RPSR). The base ration + RP supplements were designed to deliver 100% of required MP per 48-h period. The MP supply of RP supplements was determined based on manufacturer

Table 1 (Abstr. 2198). Results of study

ME and MP limiting			MP limiting			ME limiting		
Parameter	R^2 Adj	RMSE	Parameter	R^2 Adj	RMSE	Parameter	R^2 Adj	RMSE
EE	0.929	0.081	NDF	0.959	0.072	Starch	0.919	0.092
Starch	9.928	0.082	EE	0.953	0.070	EE	0.918	0.090
ADF	0.925	0.083	RDP	0.952	0.070	ADF	0.916	0.093
EE × starch	0.933	0.079	NDF × EE	0.954	0.069	Starch × EE	0.922	0.090
EE × ADF	0.933	0.079	NDF × RDP	0.952	0.071	Starch × ADF	0.920	0.090
ADF × starch	0.928	0.082	EE × RDP	0.956	0.068	EE × ADF	0.921	0.090

specifications. The RPAA mixture was formulated to deliver His, Lys, and Met in amounts corresponding to their content in casein. Mammary gland metabolism was estimated based on arterial (coccygeal vessels) and venous (subcutaneous abdominal vein) blood samples collected at 5 time points between the morning and afternoon milking on the last 2 d of feeding, capturing a supplemented and non-supplemented day for OS-RPAA. Data were subjected to ANOVA with treatment, day, and their interaction as fixed effects and block as a random effect. Total arterial concentration of His, Lys, and Met (HLM) increased with RPAA compared with CON and RPSR ($P \leq 0.01$). On the non-supplemented day compared with the supplemented day for OS-RPAA, arterial concentration of HLM was higher ($P < 0.01$) and net uptake and the uptake to milk output ratio of HLM tended to be higher ($P < 0.10$). Mammary plasma flow was not affected. Preliminary results suggest that the mammary gland responded to 24-h oscillating supply of His, Lys, and Met by increasing sequestration of these AA during the non-supplemented day.

Key Words: oscillation

2201 Abomasal infusions of essential and non-essential amino acids to evaluate energy and amino acid utilization, productive efficiencies, and metabolism in lactating dairy cattle. A. F. Ortega*[1], A. Zanotti[2], A. B. P. Fontoura[1], J. L. Marumo[1], P. A. LaPierre[1], D. M. Barbano[1], and M. E. Van Amburgh[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Parma, Parma, Italy.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

The use of metabolizable protein (MP) in diet formulation incorporates the essential amino acids (EAA) and non-essential AA (NEAA). Further, NEAA synthesis can be energetically unfavorable and might be a limiting step if other substrates are limiting. The objective of this study was to evaluate production outcomes and metabolism of cows fed a diet meeting metabolizable energy (ME) requirements but MP-limited using CNCPS v7 and abomasally infused with NEAA and EAA. Twelve multiparous Holstein cows were randomly assigned to one of 5 treatments in a replicated 6x5 balanced incomplete block design. 1) Water to meet 90% of EAA and NEAA (90AA; 0 g/d); 2) EAA to meet 100% of EAA and 90% of NEAA (100EAA; 188 g/d); 3) 90% of EAA and 100% of NEAA (100NEAA; 158 g/d); 4) 100% of EAA and NEAA (100AA; 346 g/d); 5) 100% of EAA and 110% of NEAA (110NEAA; 504 g/d). Continuous infusions were conducted for 18 d, and sampling occurred on d 15–18 for milk, blood, diet, refusals, and feces. Dry matter intake (DMI) and milk yield were recorded daily. Data were analyzed by fitting a mixed-effects model and Tukey pairwise comparison using R. DMI, body weight, milk yield and components (protein, fat, and lactose) were not different among treatments ($P > 0.05$). Milk total fatty acid (FA; $P = 0.01$) was lowest in the 110NEAA treatment, 4.15%. Preformed FA ($P = 0.03$) were lower for the 110NEAA treatment (30.0 g/100 g FA) compared with the 100EAA (31.4 g/100 g FA), while the opposite was observed for mixed FA (0.04; 42.3 vs. 41.4 g/100 g FA, respectively). Plasma insulin concentration tended to be lower ($P = 0.08$) for the 100EAA and 100NEAA compared with the 110NEAA treatment (0.79 and 0.77 vs. 0.95 ng/mL). While total plasma AA did not differ among treatments ($P = 0.47$), plasma NEAA ($P = 0.11$) and EAA ($P = 0.01$) responded to the infused treatment. Overall, infusing more NEAA caused higher insulin concentration, which is related to lower lipid turnover in adipose tissue. This is supported by a significant drop in preformed milk FA.

Key Words: amino acids, energy, milk fatty acids

2202 Ile, Leu, and Met effect on milk production is independent of energy source. M. Killerby*[1], G. M. de Souza[2], K. Ruh[1], V. Pszczolkowski[1], L. A. C. Ribeiro[1], E. Cohan[1], M. A. C. Danes[1], and S. I. Arriola Apelo[1], [1]*Animal and Dairy Science, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Science, Federal University of Lavras, Lavras, MG, Brazil.*

The objective of this study was to compare the response of lactating cows to AA supplementation along with 2 energy sources (ES). Sixteen ruminally-cannulated Holstein cows (70 ± 26 DIM), blocked in 2 groups by calving date, were enrolled in a replicated 4 × 4 Latin square study with 21-d periods, and treatments arranged as a 2 × 2 factorial. The 2 factors were ES: isoenergetic (2.86 Mcal/d) amounts of glucose (GLU) or buffered acetate (ACE); and AA level (AAL): ILM (12 g Ile, 50 g Leu, and 20 g Met) or water as control (CON). Treatments were continuously infused into the abomasum, except during milking. Cows were housed in tie stalls, milked twice daily and fed a diet formulated to meet 96% of the energy and 84% of metabolizable protein requirements. Body weight was recorded weekly, DMI and milk yield was recorded daily, milk samples were collected on d 15–19 of each period and 10 coccygeal blood samples were collected between AM and PM milkings on d 17–19 staggered to represent hourly sampling. Results were averaged by cow-period and analyzed with a mixed model containing fixed effects of ES, AAL, ES × AAL interaction, period, and square, and the random effect of block and cow within square. No effects of ES × AAL interaction were detected for any analyte. Relative to ACE, GLU increased DMI (+0.8 kg/d; $P = 0.05$), plasma glucose (+2.4 mg/dL; $P < 0.01$) and insulin (+0.123 µg/L; $P < 0.01$). Plasma insulin was also increased by ILM compared with CON (+0.065 µg/L; $P = 0.01$). Average daily gain tended to be greater with GLU (254 g/d) vs. ACE (53.7 g/d; $P = 0.09$). Milk yield was greater with GLU (+1.7 kg/d; $P < 0.01$) and tended to decrease with ILM ($P = 0.06$). Protein yield was greater with GLU (+71 g/d; $P < 0.01$) but fat yield was greater with ACE (+110 g/d; $P < 0.01$). Fat yield tended to increase (+42 g/d; $P = 0.05$) and percent increased with ILM ($P < 0.01$). Meanwhile, protein yield was not affected by AAL ($P = 0.41$), but percent was increased by ILM ($P < 0.01$). Nitrogen efficiency tended to be greater for GLU relative to ACE (27.2 vs. 26.3%; $P = 0.09$). Overall, abomasal glucose infusion improved milk yield, protein yield, and N efficiency, while acetate and AA independently stimulated fat yield.

Key Words: amino acids, energy, milk

2203 Production responses of dairy cows receiving jugular infusion of methionine and lysine or leucine and isoleucine. Y. T. Taguti[1,2], T. Fernandes*[2], A. Hruby-Weston[2], A. Pennington[2], M. Meador[2], D. Luchini[3], M. D. Hanigan[2], and I. A. M. A. Teixeira[1,4], [1]*Universidade Estadual Paulista, Jaboticabal, Sãoa Paulo, Brazil,* [2]*Virginia Tech, Blacksburg, VA,* [3]*Adisseo North America, Naperville, IL,* [4]*University of Idaho, Twin Falls, ID.*

Previous studies showed that essential amino acids (EAA) affect de novo fatty acid (FA) synthesis, especially Lys, Met, Leu, and Ile. We aimed to assess production responses in cows supplemented with 2 groups of EAA. Twelve Holstein cows (117 ± 29 DIM and 41 ± 5.3 kg/d of milk production) were randomly assigned to Lys/Met or Ile/Leu groups and subsequently assigned to treatment sequences within 2 orthogonally replicated 4 × 3 Youden squares (6 repetitions per treatment). Treatments were with or without Lys (37.6 g/d) or Met (12.0 g/d), and with or without Ile (28.6 g/d) or Leu (41.0 g/d) arranged as 2 × 2 factorials. Treatments were administered through jugular infusion for 10 d. Cows were fed a basal diet (1.77 Mcal of NE_L/kg and 9.55% of MP). On the last day of each period, 2-[^{13}C]-acetate was infused for 24 h as a tracer for de novo FA synthesis. Data were analyzed at R software, using EAA as fixed effects and period, cow, and square as random effects. Tukey test was used for multiple comparisons ($P < 0.05$). Leucine or Ile infusion does not affect DMI (18.7 kg/d SEM = 0.42). Leucine infusion reduced ($P = 0.04$ and $P = 0.05$) energy-corrected milk (2.6 kg/d, SEM = 0.92) and milk protein production (40 g/d, SEM = 0.03). Isoleucine infusion did not change milk production, but it reduced ($P = 0.01$) milk protein concentration (0.16 percentual units, SEM = 0.04) and increased ($P = 0.01$) 0.08 percentual units milk lactose (SEM = 0.02). No effect on milk protein was observed from the combination of Ile+Leu. Milk fat concentration, milk fat production (g/d), and milk mixed FA (palmitic and palmitoleic) proportion tended to increase ($P = 0.06$, $P = 0.09$, and $P = 0.07$) for Ile+Leu infusion. On the other hand, Ile+Leu tended to reduce ($P = 0.06$) de novo milk FA proportion. Methionine infusion tends to decrease ($P = 0.08$) DMI (from 19.7 to 18.6 kg/d, SEM = 0.42). However, infusion of Lys/Met did not affect milk production and composition responses, except by a tendency to decrease ($P = 0.07$) milk lactose concentration by infused Met. Isoleucine and Leu additively affected milk fat synthesis and independently affected ECM, milk protein, and milk lactose. Under the conditions of the study, Met and Lys did not independently or additively affect milk production and composition responses in dairy cows.

Key Words: amino acids, milk fat synthesis

2204 Supplemental methionine effects on plasma amino acid concentrations. G. I. Zanton*, *USDA-Agricultural Research Service, U. S. Dairy Forage Research Center, Madison, WI.*

Balancing dietary AA composition for lactating dairy cows is an important nutritional practice to optimize production, nitrogen use efficiency, and feed costs. Feeding rumen protected Met (RPM) is a common component of balancing for AA, but this practice may affect the concentration of other plasma AA (pAA). Therefore, the objective of this meta-analysis was to evaluate the changes in pAA when cows were provided supplemental Met. Literature studies were identified that fed cows a control diet and the control diet supplemented with Met as either RPM or through post-ruminal infusion. These studies also had to report multiple pAA (i.e., Met and ≥ 1 additional AA). This resulted in 40 publications feeding 60 control diets with 715 control cows and 78 Met treatments with 861 cows. Not all studies reported all AA with the number of treatment means reported for controls ranging from 8 for Hcy to 56 for several EAA. Treatment responses were calculated as Met supplemented pAA concentration – control pAA. Since several pAA and responses to Met had skewed distributions, summary statistical analyses were conducted as weighted medians where the weighting term was the $\sqrt{n}$. Regression was also conducted where pAA was regressed against pMet weighted by $\sqrt{n}$ and including the random effects of study. Significance was declared when $P < 0.05$ or trending toward significance when $0.10 > P \geq 0.05$. Median pMet in control cows was 20 µ*M* and pMet increased with Met supplementation, but the responses varied widely ranging from −2.1 to 62.3 µ*M*. Regression analysis resulted in 11 AA associated with changes in pMet. Sulfur-containing AA Tau, Cys, and Hcy were increased and closely related ($R^2 = 0.881$, 0.586, and 0.956, respectively) with increasing pMet whereas Tyr, Phe, His, Ile, Leu, Ser, and Val decreased with increasing pMet but did so less consistently ($0.181 \leq R^2 \leq 0.397$). The largest reduction in pAA to increases in pMet was in Gly which declined 0.78 µ*M*/µ*M* pMet, although this relationship was somewhat inconsistent ($R^2 = 0.282$). In conclusion, when formulating diets with supplemental RPM, the effects on the plasma concentration of other EAA and NEAA should also be considered.

Key Words: methionine, plasma amino acids, meta-analysis

2205 Effects of supplementing rumen-protected arginine (RPA) on production performance of transition cows. B. S. Simoes*[1], R. Lobo[1], T. Adeoti[1], M. N. Marinho[1], M. Perdomo[1], L. Sekito[1], F. Saputra[1], M. Bari[1], U. Arshad[1], A. Husnain[1], Y. Sugimoto[2], C. Nelson[1], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*Ajinomoto Co., Inc., Tokyo, Japan.*

Objectives were to study the effects of supplying 30 g/d of metabolizable arginine (Arg) as RPA from 250 d of gestation to 21 d in milk (DIM) on production in dairy cows. Forty-two nulliparous (Null) and 60 parous Holstein cows were blocked by parity and predicted transmitting ability of energy-corrected milk (ECM) in Null or 305-d ECM yield in parous cows. Within block, cows were assigned to control (CON), 30 g/d of metabolizable protein (MP) from heat-treated soybean meal, or RPA, 30 g/d of metabolizable Arg (Ajinomoto Co., Inc., Japan), which increased the dietary Arg from 5.7 to 7.5% of the MP (NASEM Dairy, 2021). Diets contained 74% forage prepartum and 51% forage postpartum. Data were analyzed by mixed-effects models using the MIXED procedure

Table 1 (Abstr. 2205). Effects of supplemental RPA on performance of Holstein cows

	CON		RPA			
Item	Null	Parous	Null	Parous	SEM	*P*-value[1]
Prepartum						
DM intake, kg/d	10.4	13.4	9.7	13.5	0.4	0.35
BW change, kg/d	1.07	1.00	1.19	1.04	0.27	0.76
Days 1 to 21 postpartum						
Colostrum yield, kg	5.38	5.16	8.52	7.19	1.23	0.02
DM intake, kg/d	14.7	17.4	14.8	17.9	0.5	0.47
ECM, kg/d	34.1	41.5	36.6	45.3	1.4	0.02
BW change, kg/d	−1.62	−2.26	−2.15	−2.23	0.29	0.28
Days 22 to 84 postpartum						
DM intake, kg/d	20.9	23.6	20.3	23.8	0.6	0.56
ECM,* kg/d	39.3^{c}	44.2^{b}	39.6^{c}	48.5^{a}	1.5	0.05
BW change, kg/d	0.04	0.03	−0.01	−0.09	0.13	0.39

[a–c] Different superscripts differ ($P < 0.05$).

[1] Effect of treatment (CON vs. RPA).

*Interaction between treatment and parity ($P < 0.05$).

of SAS. Prepartum, treatment did not affect intake, body weight (BW), body condition score (BCS), or energy balance (Table 1). Cows fed RPA produced an additional 2.5 kg of colostrum and secreted 220 g more IgG than CON cows (526 vs. 746 ± 93 g). Intake of dry matter (DM) did not differ in the first 84 DIM, but supplementing RPA increased urea N in milk in the first 21 DIM (11.0 vs. 12.0 ± 0.3 mg/dL), and the yields of ECM and total solids in milk in the first 21 and between 22 and 84 DIM without affecting BW or BCS. The carryover effect on ECM yield was observed only in parous cows. Supplementing RPA benefited productive performance in dairy cows and the increased yield of ECM extended beyond the period of supplementation.

Key Words: arginine, milk yield, transition

2206 Effect of peripartum metabolizable protein supply on performance and metabolic indicators.

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Our objective was to examine the effect of altering peripartum metabolizable protein (MP) supply on performance and metabolic indicators. Parous Holstein cows (n = 96) were assigned to 1 of 4 treatment groups (trt) at 28 d before expected calving in a randomized block design. All prepartum cows were fed a TMR formulated in AMTS.Farm(Pro) (v. 4.17) to contain methionine (Met) and lysine (Lys) at 1.24 and 3.86 g/Mcal of metabolizable energy (ME), and either 87 or 115 g of estimated MP/kg DM in groups control (C) and high (H), respectively. A postpartum TMR was fed from 1 to 21 DIM and formulated to contain Met and Lys at 1.15 and 3.20 g/Mcal ME in both groups and either 107 or 133 g of estimated MP/kg DM in groups C and H, respectively. Combination of pre- and postpartum diets resulted in 4 trt: 1) CC, 2) CH, 3) HC, and 4) HH. Pre- and postpartum DMI and milk yield (MY) were recorded daily. Milk components were analyzed weekly. Blood was collected twice weekly and analyzed for concentrations of fatty acids (NEFA) and β-hydroxybutyrate (BHB). Repeated-measures ANOVA were conducted with PROC MIXED (SAS v. 9.4) with the fixed effects of trt, time, parity, and trt × time interaction and a random effect of block. Tukey's test-corrected results are presented as LSM ± SEM. Dry matter intake as a percent of body weight did not differ by trt in the pre- ($P = 0.29$) or postpartum period ($P = 0.71$). Milk yield in HH (44.7 ± 1.0 kg/d) was greater than CC (39.2 ± 1.0 kg/d; $P < 0.01$) and HC (38.0 ± 1.0 kg/d; $P < 0.01$) and MY of CH (42.4 ± 0.9 kg/d) was greater than HC ($P < 0.01$), respectively. Lactose concentration was lower in CC vs. CH (4.75 ± 0.02 vs. 4.81 ± 0.02%; $P = 0.04$), respectively, but content of milk protein ($P = 0.15$), fat ($P = 0.79$), or total solids ($P = 0.90$) did not differ by trt. From 1 to 21 DIM, trt did not affect NEFA ($P = 0.58$), yet the proportion of samplings with hyperketonemia (BHB ≥1.2 mmol/L) was lower in HC vs. HH (11 vs. 32%; $P < 0.01$), respectively. In summary, MP supply in the transition period affected MY and postpartum hyperketonemia events. Feeding CH or HH is a strategy to increase early lactation MY.

Key Words: metabolizable protein, transition cow, metabolism

2208 Amino acid supplementation as a potential strategy to mitigate milk fat depression.

K. E. Ruh*[1], M. Killerby[1], G. M. de Souza[1,2], E. M. Cohan[1], A. D. Benn[1], L. A. Coelho Ribeiro[1], V. L. Pszczolkowski[1], D. N. Sherlock[3], and S. I. Arriola Apelo[1], [1]*Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Animal Science, Federal University of Lavras, Lavras, MG, Brazil,* [3]*Adisseo USA Inc., Alpharetta, GA.*

In dairy cows, a shift in rumen biohydrogenation of dietary polyunsaturated fatty acids (PUFA) leads to milk fat depression (MFD). The objective of this study was to determine the effect of increasing metabolizable Met and Leu supply on milk fat production in control (high-palmitate fat source) and MFD-induced (soy oil) cows. The study was designed as a replicated 4×4 Latin square with 4 28-d periods and 4 treatments arranged as a 2×2 factorial. All diets included high starch (28%). Factors were fat source (FS): soybean oil (SBO; 1.83% DM) or a 80% palmitate fat supplement (CTL; 1.89% DM); and AA level (AAL), either 5% metabolizable protein deficient (AAD), or sufficient, balanced for Leu and Met with corn gluten meal and rumen protected Met (AAS). Fifty-six Holstein cows (71 DIM), housed in tie stalls and fed 1x/d and milked 2×/d were enrolled as 2 cohorts and grouped within cohort by DIM into 14 squares. Cows were weighed weekly, and DMI and milk yield and composition were measured on d 23–26 of each period. Data were analyzed with a mixed model containing FS, AAL, FS×AAL, period, and square within cohort as fixed effects and cow within square as random. There was no significant interaction between factors for any reported variables. Body weight change was not affected by either factor ($P > 0.10$). Dry matter intake was not affected by FS ($P = 0.52$) but was decreased by AAS (−1.6 kg/d, $P < 0.001$). Milk and lactose yield were not affected by either factor ($P > 0.21$). As expected, compared with CTL, SBO decreased ($P < 0.001$) fat yield (−150 g/d) and percent (3.32, 3.69 ± 0.06%), and AAS tended to increase fat yield compared with AAD (+43 g/d, $P = 0.09$), but not percent (3.54, 3.48 ± 0.06%; $P = 0.30$). Protein yield was not affected by fat source ($P = 0.44$) but was increased by AAS (+69 g/d, $P < 0.001$). Milk urea nitrogen decreased with SBO vs. CTL (13.0 vs. 13.7 mg/dL, $P = 0.01$) and tended to increase with AAS vs. AAD (13.6 vs. 13.1 mg/dL, $P = 0.08$). Overall, AA supplementation tended to increase milk fat yield; however, the effect of AA supplementation was not sufficient to overcome the differences in milk fat production between dietary fat sources.

Key Words: milk fat depression, amino acids

2209 Effects of individual and additive amino acids on intracellular concentrations in bovine mammary epithelial cells.

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Amino acid (AA) uptake by mammary cells is affected by AA concentrations, interactions among AA and transporter expression. Previous work has focused on uptake responses to 1 or 2 AA or groups of AA, without capacity to untangle interactions. Thus, we conducted an experiment to examine the effects of extracellular concentrations of the 10 essential AA (EAA); Ala, Gln, and Gly (NEAAG1); and the remaining NEAA (NEAAG2) on intracellular free AA concentrations of mammary cells isolated from 3 lactating Jersey cows within 3 passages. The experiment was a replicated, central composite, fold-over design containing fractional factorial (n = 38) and radial run (n = 30) sub-blocks. The fold-over allowed estimation of all 2-way interactions among AA. Treatments (n = 136) were centered on mean AA plasma concentrations of lactating dairy cows (Yoder et al., 2020) with treatments of 16%, 76.3%, 100%, 123.7%, and 184% of the mean. After cells reached confluency, cells were plated on collagen, randomly assigned to treatments, and cultured for 2 d in serum-free treatment media (DMEM/F12, no AA) containing U-[^{15}N]-labeled AA replaced every 12 h for 2 d, followed by serum-free, treatment media containing U-[^{13}C]-labeled AA. Media and cells were

collected at 0, 5, 30, and 120 min following the [^{13}C]-label introduction. AA concentrations were determined by isotope dilution using an external tracer. Only data from t = 120 was evaluated and reported. Statistical analyses were conducted using R studio with treatment AA level as a fixed effect (−3.55, −1, 0, 1, 3.55) and cow (n = 3) as a random effect. The following slopes reported were declared significant at $P < 0.05$. Intracellular Val concentrations were positively affected by Val (slope = 2.06), negatively affected by Ile x Val (slope = −2.58) and Met x Val (slope = −0.61). Intracellular Leu was positively affected by Leu (slope = 0.48), and negatively by the interaction of His x NEAAG1 (slope = −5.02). The negative interaction for Ile x Val is indicative of competitive inhibition of Val transport, and the Met x Val interaction may also reflect competitive inhibition. The positive interaction of His x NEAAG1 on Leu concentrations is indicative of AA exchange effects for the NEAAG1.

Key Words: central composite design, competition, transport

2316 Impact of EcoFeed on feed consumption and performance of group-housed commercial Holstein heifers. J. Johnson*, N. Deeb, P. Khanal, and P. Ross, *STgenetics, Navasota, TX.*

The EcoFeed program implemented by STgenetics is one of the leading commercial genomic programs aiming to improve feed efficiency in dairy cattle. The program is based on progeny testing of over 7,000 Holstein heifers for residual feed intake with High EcoFeed females being those who consume less feed while maintaining production compared with their herd mates. EcoFeed score is moderately heritable and uncorrelated with traits currently selected for in dairy cows. The objective of this study was to evaluate the impact of genomic EcoFeed score on feed consumption and performance of group-housed Holstein heifers (n = 1,304; initial BW = 245 ± 38 kg) in a commercial heifer lot. Heifers with genotypes were group-housed in dry lot pens according to their EcoFeed scores starting at 6 to 8 mo of age. Given the continuous flow of the commercial farm, 1,000 heifers reached the 6 to 8 mo age classification every 60 d and from that group, the top, middle, and bottom 10% of EcoFeed females were assigned to a trial and placed in 1 of 3 pens to create Low-, Med-, and High- EcoFeed groups (100 head/pen). For each trial, daily feed supply and refusals were recorded for at least 85 d following a 14 d adaptation. Daily feed calls targeted 5 to 10% refusals. Body weights were recorded at 14 d intervals and pen DMI was calculated daily from feed supplied and refused. Results represented data from 4 iterations of an ongoing study. EcoFeed-group effect on DMI was evaluated using a model with trial and EcoFeed group as fixed effects and initial BW was fitted as a covariate. The DMI and ADG ranged from 6.41 to 9.38 and 0.48 to 1.17 kg/d, averaging 8.13 (±0.99) and 0.94 (±0.20) kg/d, respectively. High EcoFeed heifers had lower DMI (7.92 vs. 8.39 kg/d; $P < 0.05$) and numerically higher ADG (1.00 vs. 0.89 kg/d) than low EcoFeed heifers, resulting in a 16% improvement in their feed conversion efficiency. These results indicate EcoFeed as a useful tool to improve the feed conversion efficiency of commercial dairy heifers. Detailed results and expected impact of selection for EcoFeed will be discussed at the upcoming meeting.

Key Words: feed efficiency, residual feed intake, EcoFeed

Small Ruminants 1

2210 A good start for a good productive life as a dairy goat. N. Gafsi*[1,2], F. Bidan[2], B. Grimard[3], M. Legris[2], O. Martin[1], and L. Puillet[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR MoSAR, Palaiseau, France,* [2]*Institut de l'Elevage, Paris, France,* [3]*Université Paris-Saclay, INRAE, ENVA UMR BREED, Jouy-en-Josas, France.*

Increasing longevity in dairy goats enables to reduce replacement costs and increase individual milk yield. How to early identify candidate goats for a long productive lifetime? Primiparous goats have an increased risk of being culled due to supplementary needs for growth. They also must produce milk and safeguard body reserves for health and reproduction, but energy can't be maximized for each biological function. Assessing diversity of phenotypic trajectories (e.g., milk yield (MY), body weight (BW)) is an interesting way to understand interactions and priorities among biological functions. The objective of this work was to evaluate associations between MY and BW trajectories in primiparous goats and seek for unfavorable associations that lead to culling. Routine data from an experimental station in South France (Le Pradel, French Livestock Institute) were used. The data set included 655 Alpine primiparous goats from 1996 to 2021. Culling dates and data of the next lactation were provided. Weekly MY records were fitted using the Wood model and monthly BW records were fitted using the Grossman multiphasic model. After fitting procedure, level, and speed indicators for MY and BW were calculated to summarize each individual trajectory. Principal component analysis was performed on MY and BW indicators separately to generate clusters of dynamic profiles. Chi^2 test was performed on MY and BW clusters. Three clusters were identified for both MY ("low peak yield-high persistency" (7%), "middle peak yield-low persistency" (33%), "high peak yield-low persistency" (60%)) and BW ("growth" (14%), "intense depletion-longer repletion" (30%), "intense depletion-fast repletion" (56%)). Nine profiles, characterized by combination of one MY and one BW cluster, were established and 76% of goats were mainly in 4 profiles suggesting a typology of energy partitioning among these functions. The highest milking goats with intense depletion and fast repletion have 49% risk of being culled at the end of their first parity. These results open the perspective to identify animal profiles that are economically interesting for breeders because they will be able to go through several lactations.

Key Words: milk yield, body weight, dairy goats

2211 Microbial enterotypes shape the divergence in gut fermentation, host metabolism and growth rate of young goats. D. Wang, G. Tang, J. Zhang, L. Wang, J. Yao, and Y. Cao*, *Northwest A&F University, Xianyang, Shaanxi, China.*

Enterotype can be a useful tool for studying gut microbial community landscape, which is thought to play a crucial role in animal health and performance. However, few studies have been carried out to identify enterotypes and their influences on growth performance in young goats. In this study, 76 young goats were fed the same diet and raised under the same condition. The relationships of the enterotypes with gut fermentation, serum biochemistry, and the growth rate of goats were further investigated. Two enterotypes were identified in all goats: cluster 1 (n = 39) and cluster 2 (n = 37). Compared with cluster 2, cluster 1 had greater growth rates, the concentrations of acetate, propionate, valerate, and total volatile fatty acids (VFA) in the gut ($P < 0.05$). Several serum glycolipid metabolism parameters, including glucose, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, were also increased in cluster 1, while serum IgG was decreased in cluster 1 ($P < 0.05$). Using α-diversity analysis, a microbiome with lower richness and diversity was found in cluster 1. And some gut bacteria, including *Succinivibrio*, several members of the *Prevotellaceae* family, were enriched in cluster 1 ($P < 0.05$). While *Christensenellaceae R-7 group*, *Romboutsia*, and *Clostridium sensu stricto 1*, were enriched in cluster 2 ($P < 0.05$). Co-occurrence network analysis revealed that the differential interaction patterns exist in 2 enterotypes, and microbial function prediction suggested that some nutrient metabolism-related pathways, including amino acids biosynthesis and starch and sucrose metabolism, are enriched in cluster 1 ($P < 0.05$). Furthermore, correlation analysis reveals that enterotype-related bacteria are closely correlated with gut fermentation, serum biochemistry, and growth rate ($P < 0.05$). Overall, our data provide a new perspective for understanding enterotype characteristics in goats, offering insights into important microbial interaction mechanisms for improving the growth performance of ruminant animals.

Key Words: enterotype, gut microbiome, co-occurrence network

2212 Potential roles of the rectum keystone microbiota in modulating the microbial community and growth performance in goat model. D. Wang, G. Tang, J. Zhang, L. Wang, J. Yao, and Y. Cao*, *Northwest A&F University, Xianyang, Shaanxi, China.*

Ruminal microbiota in early life plays critical roles in the life-time health and productivity of ruminant animals. However, understanding of the relationship between gut microbiota and ruminants' phenotypes is very limited. Here, the relationship between the rectum microbiota, their primary metabolites, and growth rate of a total of 76 young dairy goats (6-mo-old) were analyzed, and then 10 goats with the highest or lowest growth rates respectively were further compared for the differences in the rectum microbiota, metabolites, and animal's immune parameters. The analysis of Spearman correlation and microbial co-occurrence network indicated that some keystone rectum microbiota, including unclassified *Prevotellaceae*, *Faecalibacterium* and *Succinivibrio*, were the key modulators to shape the rectum microbiota and closely correlated with the rectum SCFA production and serum IgG ($P < 0.05$), which contribute to the health and growth rate of young goats. In addition, random forest machine learning analysis suggested that 6 bacterial taxa in feces could be used as potential biomarkers for differentiating high or low growth rate goats, with 98.3% accuracy of prediction. Moreover, the differences in rectum SCFA, microbiota features were analyzed between young goats (6-mo-old) and adult goats (19-mo-old). The concentrations of rectum total SCFA, propionate, and butyrate were significantly decreased ($P < 0.05$), the rectum microbiota richness and diversity were significantly greater in adult goats, compared with these in young goats ($P < 0.05$). Some SCFA-related bacteria, such as *Succinivibrio* was significantly decreased in the adult goats ($P < 0.05$). The rectum microbiota played more important roles in gut fermentation in early life (6 mo old) than in adulthood stage (19 mo old) of goats. We concluded that the rectum microbiota was associated with the health and growth rate of young goats, and can be a focus on the design of the early-life gut microbial intervention.

Key Words: early life, growth performance, rectum microbiota

2213 Copper metabolism in growing lambs as affected by copper source. J. B. Daniel*[1], N. R. Kendall[2], and J. Martin-Tereso[1], *[1]Trouw Nutrition R&D, Amersfoort, the Netherlands, [2]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, UK.*

The effect of different forms of supplemental Cu was evaluated in their ability to prevent unregulated Cu retention at moderately high dietary Cu levels in growing lambs fed straw and concentrate ad libitum. A total of 400 lambs (body weight = 23.4 ± 0.1 kg) were enrolled and blocked based on body weight in 10 blocks of 4 pens each. Pens within a block were randomly assigned one of the following treatments: no Cu supplementation (CTR) (basal Cu was 9 ppm) or 11 ppm of supplemental Cu, either fed as Cu sulfate (ING), Cu hydroxy chloride (HYD, IntelliBond, Selko) or Cu proteinate (ORG, Optimin, Selko). Gastrointestinal tissues (rumen, abomasum, duodenum, pancreas, jejunum, ileum, cecum and colon), and whole organs (heart, liver and kidney) were collected at slaughterhouse from 40 lambs for baseline (1 per pen), and from 120 lambs after 5 weeks of dietary treatment (3 per pen). All samples were dried, milled, and analyzed for trace minerals. Data were analyzed using block as random effect and data from baseline as covariate (Proc Mixed, SAS 9.4). Concentrate intake and average daily gain did not differ between treatments, with an average of 1.14 kg/d and 283 g/d, respectively ($P > 0.20$). Among gut tissues, Cu concentration was unaffected by treatment except for the duodenum, in which HYD had higher Cu than CTR and ORG ($P = 0.02$). Dried liver size decreased with Cu supplementation in all forms by about 10% as compared with CTR ($P = 0.03$). Within 5 weeks, a greater increase in total Cu in liver was observed for lambs supplemented Cu (from ×2.0 to ×2.3) as compared with control (×1.2, $P < 0.01$) with no significant differences between Cu sources. Expressed as concentration, liver Cu was highest with ORG (379 mg/kg DM) as compared with HYD (310 mg/kg DM), and all supplemental Cu treatments resulted in higher concentration than CTR (173 mg/kg DM). Whereas total Cu in heart was unaffected, kidney of lambs fed HYD had the highest concentration of Cu (+27%, $P < 0.01$), as well as the highest total Cu (+34%, $P < 0.01$), as compared with all other treatments. Adequate Cu status was achieved for lambs fed control treatment. When Cu was supplemented at 11 ppm for 5 weeks, total Cu in liver doubled.

Key Words: copper metabolism, lamb

2214 Assessment of nutrient digestibility and ruminal protozoa count in lambs fed increasing levels of faveira pod.

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Faveira pod (*Parkia platycephala* Benth.) is a leguminosae tree found in Northeast of Brazil with great content of crude protein (CP) and nonfiber carbohydrate (NFC). Because of this, it has potential to replace ground corn in diet of lambs. The objectives in this research were to evaluate the effects of replacement of dietary ground corn by faveira pods (FP) on nutrient digestibility and rumen protozoa count of lambs. Twenty 8 lambs (26.3 ± 5.78 kg BW) were used in a complete randomized block design. Control diet (CONT) consisted of 70% concentrate and 30% Tifton 85 hay. In the remaining 3 treatments, FP replaced ground corn (30%, DM basis) at 0 (0FP), 33 (33FP) and, 100% (100FP). Lambs (7 per group) were penned individually during 55 d in feedlot. The intake was registered and total feces collected during the last 5 d of feedlot for nutrient digestibility determination. Then, lambs were slaughtered and ruminal content was collected for pH measurements and protozoa count by microscope examination. The effects of increasing levels of FP content added in the diets on nutrient digestibility and rumen protozoa count were evaluated using linear and quadratic orthogonal contrasts ($P < 0.05$). The intake of dry matter (DM), CP, neutral detergent fiber (NDF), NFC and total carbohydrate (TC) did not change ($P > 0.05$) with experimental diets (1.3 ± 0,04; 0,19 ± 0.006; 0.39 ± 0.015; 0.62 ± 0.019 and 1.0 ± 0.03 kg/day, respectively). However, the ether extract (EE) intake (0.031, 0.031, 0.027 and 0.024 kg/day), CP digestibility (80.5, 72.5, 69.0 and 65.0% for 0, 33, 66 and 100FV, respectively), EE digestibility (81.9, 85.6, 79.4 and, 78.5%) and NDF digestibility (84.5, 83.2, 78.7 and 74.6% for 0, 33, 66 and 100FV, respectively) reduced linearly, while the NFC digestibility increased linearly with FP inclusion (93.8, 95.7, 96.9 and 98.8% for 0, 33, 66 and 100FP). The rumen pH decreased linearly with FP addition (6.6, 6.3, 6.3 and 6.4), but did not change the rumen protozoa count (5.6, 5.4, 6.9 and, 7.8×10^5 protozoa/mL of ruminal fluid). In conclusion, replacing ground corn with faveira pod modified nutrient digestibility without negative effects on ruminal pH and protozoa count.

Key Words: non-fibrous carbohydrate, *Parkia platycephala* Benth., rumen pH

2215 Effect of suckling method (natural vs. artificial) on the growth of lambs and their metabolic response to weaning stress.

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Farm animals face different kinds of stress throughout their productive lives (e.g., weaning). The objective was to investigate how the suckling method (natural vs. artificial) would affect the growth of lambs and their metabolic response to weaning stress. Lacaune lambs (n = 80; 3.7 ± 0.1 kg BW) were divided into 2 balanced groups according to birth weight, sex, and birth type (single or multiple). The treatments were: 1) natural suckling (NAT; n = 40), in which the lambs remained with their mothers until weaning (35 d), and 2) artificial suckling (ART; n = 40), in which the lambs were fed milk replacer ad libitum (200 g/L). NAT lambs were kept in pens with their mothers, whereas ART lambs were separated from their mothers at birth and bottle-fed colostrum. Lambs were weighed weekly, and blood was collected at weaning (d 0), and at 24 (d 1) and 48 h (d 2) after weaning for the analyses of cortisol (CORT), haptoglobin (Hp), lactate dehydrogenase (LDH), creatine kinase (CK), glucose (G), and free fatty acids (NEFA). Statistical analysis was performed using PROC MIXED (SAS Inst. v.9.4) for repeated measures. Although there were no differences in the birth weight of NAT (3.8 kg) and ART (3.7 kg), NAT lambs tended ($P < 0.10$) to weigh more at weaning (14.5 vs. 13.8 kg). During the 2 d post-weaning, ART lambs had lower serum values of CORT (–27%; $P < 0.10$), LDH (–43%; $P < 0.05$), CK (–97%; $P < 0.001$), and NEFA (–26%; $P < 0.01$) than NAT lambs, indicating weaker weaning stress in ART lambs. A treatment × day interaction ($P < 0.05$) was detected for CK, where between d 0 and 2 of weaning there was a 136% increase in NAT, while the increase was only 44% in the ART. Weaning increased ($P < 0.01$) Hp at d 0 (0.30 mg/mL), d 1 (0.45 mg/mL), and d 2 (0.66 mg/dL) in both NAT and ART lambs. However, G levels decreased ($P < 0.01$) from 102 to 69 mg/dL at d 0 to d 1 and 2 of weaning. In conclusion, NAT lambs tended to grow more rapidly than ART lambs. However, weaning at 35 d caused stronger stress in NAT than in ART lambs. Acknowledgments: Project RTA #PID2020–113913RR (Ministry of Science and Innovation) and TechCare (Contract # 862050) H2020 UE Program.

Key Words: weaning, stress, lambs

2216 Fat deposition in the mammary gland of ewe lambs fed stearic acid (C18:0). C. G. Padilha[1], L. M. A. Pereira[2], T. R. Wiggers[1], R. Larsen*[1], R. Horstmann[1], and D. E. Oliveira[1], [1]*Universidade do Estado de Santa Catarina, Lages, Santa Catarina, Brazil,* [2]*Universidade do Oeste de Santa Catarina, Campos Novos, Santa Catarina, Brazil.*

Fat supplements could be used to meet the energy requirements of growing ewe lambs. However, the excess energy in the diet could modify the development of mammary tissues, causing greater deposition of adipose tissue in relation to the parenchyma, impairing milk production. The objective of this study was to evaluate, through ultrasonography (US), the accumulation of fat in the mammary gland (MG) of lambs supplemented with stearic acid (C18:0). Eight ewe lambs (Lacaune × Texel), aging ± 5 mo, were used in a completely randomized design, submitted to the following treatments: 1) Control and 2) 1.5% of dry matter intake (DMI) of C18:0 (87% of C18:0). The experiment lasted 63 d and the animals were submitted to US of MG, once a week, by the same trained person. In each evaluation, at least 3 images were obtained on each side of the MG, selected by definition of the structures and then compared, using the ImageJ (2013) software, calculating the area and the number of "pixels" corresponding to the echogenicity of the structures in a scale of 255 levels of gray, being the echogenicity of the fat considered medium. A total of 176 images over time were selected and evaluated. Data were analyzed by SAS University (2017) through PROC MIXED as repeated measures over time, with treatment and period considered fixed effects, animals considered random effects and interactions tested. No treatment effects were observed on fat deposition in the MG of ewe lambs supplemented with C18:0 ($P = 0.54$), which from the point of view of MG development and milk production in the future can be positive, because a greater fat deposition in MG could occur and limit the development of the parenchyma.

Key Words: dairy sheep, animal nutrition, lactation

2217 An ultrasound image-guided technique for liver biopsying in dairy small ruminants. S. González-Luna*[1,2], X. Moll[3], S. Serhan[2], B. Chaalia[2], A. A. K. Salama[2], X. Such[2], and G. Caja[2], [1]*Departmento de Ciencias Pecuarias, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Mexico,* [2]*Group of Research in Ruminants (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra, Spain,* [3]*Department of Animals, Medicine and Surgery, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

Liver biopsies help to understand metabolic processes, but they are risky chirurgical interventions that could severely affect animals. There is no a reference protocol for small ruminants. With this aim, we developed a liver biopsy procedure using an ultrasound image-guided technique for dairy ewes and goats. A total of 20 Murciano-Granadina goats (42.4 ± 1.3 kg BW) in mid lactation (141 ± 4 DIM) and 10 Manchega ewes (68.6 ± 1.2 kg BW) in late lactation (199 ± 8 DIM) were used. Liver biopsies were obtained twice from each animal, with a 6-wk interval between biopsies. For biopsying, right flank was shaved from 7th to 12th intercostal spaces. Animals were sedated and placed in left lateral recumbency for the antisepsis and local anesthesia at the site of biopsy. Semiautomatic VI Trucut type SuperCore 14G × 9 cm biopsy needles (Argon Medical Devices, Athens, TX) were used. Visualization of the liver and insertion path of the needle was achieved by real time B-mode ultrasonography with a convex C60/5–2 MHz transducer (SonoSite Ultrasound System, Vet180 Plus, Bothell, WA). Liver biopsies were washed by cold saline, snap frozen in liquid N, and stored at −80°C. Surgical staples were applied after the biopsy and removed 10 d later. On average, time from antisepsis to stapling was 13 ± 2 min in goats and ewes. Milk yield before biopsy averaged 1.78 ± 0.08 and 0.59 ± 0.05 kg/d in goats and ewes, respectively. Milk yield drop was 52% (–0.92 ± 0.10 kg/d) the day after biopsy in goats, and they recovered after 4 d (1.75 ± 0.09 kg/d). The drop was 57% (–0.34 ± 0.05 kg/d) in ewes and milk yield recovered after 7 d (0.56 ± 0.09 kg/d). Additionally, 1 ewe died 1 h after the biopsy. The RNA concentration in the obtained biopsies averaged 1,313 ± 102 and 246 ± 52 ng/μL in goats and ewes respectively. The obtained liver biopsies proved to be suitable for RNA extraction and RNA-seq analyses. In conclusion, we recommend the use of an ultrasound-guided technique for liver biopsying in dairy small ruminants, which allowed a full recovery of milk production.

Key Words: liver biopsy, ultrasound image-guided, small ruminant

2218 Differences in essential and toxic mineral content and in fatty acid profile between colostrum and mature milk in Sarda dairy sheep. A. Nudda*[1], G. Sanna[2], G. Battacone[1], S. Carta[1], F. Correddu[1], and G. Pulina[1], [1]*Department of Agricultural Science, University of Sassari, Sassari, Italy,* [2]*Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy.*

Colostrum is a major source of immunity in ruminants. It provides the transport of antibodies from mother to fetus and is the exclusive source of nutrients for the newborn. Currently, there is little data available on the nutritional components of sheep colostrum, such as the content of mineral trace elements and the nutritional quality of fats. This study aimed to characterize the fatty acid profile and essential and toxic mineral content of colostrum and to compare it with that of mature milk. Colostrum and milk samples were collected from 12 ewes per farm, randomly selected in 8 dairy sheep farms in northern Sardinia (Italy), for a total of 96 animals. The statistical model to compare colostrum with mature milk included the fixed effects of type of secretion and farm, their interaction, and the day of parturition as a random factor. The mean concentrations of fat and protein in colostrum were 7.8% and 16%, respectively. In addition, an average amount of 40.5 mg/L of immunoglobulin G (IgG) was measured. High levels of selenium (Se), zinc (Zn), and copper (Cu) were found in the colostrum (200 ppb, 25,000 ppb, and 1,200 ppb, respectively). A strong positive correlation was observed between TP and IgG ($r = 0.91$). In colostrum, the amount of IgG correlated positively with Se and Zn as essential minerals for the immune system. The mean concentrations of heavy metals, such as Pb and Cd, in colostrum, are markedly lower than the maximum limits indicated by the European Union for raw drinking milk and baby foods (i.e., 20 ppb and 10 ppb, respectively). The FA profile presented greater levels of medium-chain FA and long-chain FA in colostrum than in mature milk, especially EPA (0.07 vs. 0.05 g/100 g FAME), DPA (0.17 vs. 0.09 g/100 g FAME) and DHA (0.06 vs.0.03 g/100 g FAME). This study provided new information on colostrum quality in Sarda dairy sheep and showed the difference in fatty acid profile and mineral content between colostrum and mature milk.

Key Words: dairy sheep colostrum, essential and toxic minerals, fatty acids

2219 Investigating gross composition and microbial quality of Ontario goat milk. O. Graydon* and C. Bauman, *University of Guelph, Guelph, Ontario, Canada.*

The objective of the study was to establish benchmark values for milk quality factors such as protein, fat, and lactose, as well as identify an

Table 1 (Abstr. 2219). For the 3173 samples collected to date. A correlation coefficient between the bacterial count and the other parameters was also calculated.

Quality factor being assessed	Mean value observed (range)	Correlation coefficient
BactoScan (ibc/mL) × 1,000	250.93 (0.0–117,238.0)	—
SCC (SCC/mL) × 1,000	1835 (0.0–23,921)	0.61
Fat (kg/hL)	4.008 (0.860–13.270)	0.13
Protein (kg/hL)	3.287 (2.200–8.290)	0.25
Lactose (kg/hL)	4.184 (0.670–5.100)	−0.22

association between somatic cell count (SCC) and bacterial count (BactoScan) in lactating does. Traditionally in the dairy industry SCC is an indicator measure for mastitis, however, SCC is naturally high in goats due to influences like parity, season, diet, or stage in lactation. In caprine lactogenesis, epithelial cells are released during milking and are often mistakenly counted as SCCs in flow cytometry tests. Therefore, routine tests for SCC are unreliable predictors of subclinical mastitis in dairy goats. Identifying the association between SCC and the milk composition parameters to bacterial counts (Table 1) will help to predict samples that should undergo further testing for subclinical mastitis and future surveillance studies. Province-wide sampling was conducted on 20 licensed Ontario dairy goat farms. For the animal to be sampled, the producer had to be actively milking one or both halves and the animal was excluded if they were not contributing to the bulk tank for a variety of reasons. A composite milk sample (half from each half) from each eligible doe was collected into 3 40mL vials and stored in a chilled container until it could be refrigerated at 2°C at the University of Guelph. The vials remained refrigerated until testing was performed at the Agriculture & Food Laboratory (University of Guelph). The samples were analyzed using a MilkoScan, Fossomatic, and BactoScan for milk composition, SCC analysis, and bacterial count, respectively. Each machine utilized in the milk analysis process use a flow cytometry technique. Preliminary results were computed using R/RStudio 4.1.1 (2021–08–10) “Kick Things” [R Core Team].

Key Words: somatic cell count, milk composition, small ruminant

2220 Life Green Sheep: A European project to reduce the carbon footprint of sheep farming by 12%. L. Lanzoni*[1], A. S. Atzori[2], J. Dollé[3], M. Acciaro[4], C. Buckley[5], T. W. J. Keady[5], L. Bragina[5], O. Del Hierro[6], R. Ruiz[6], C. Dragomir[7], Giorgio Vignola[1], D. Usai[8], and S. Throude[3], *[1]Department of Veterinary Medicine, University of Teramo, Teramo, Italy, [2]Department of Agriculture, University of Sassari, Sassari, Italy, [3]Division on Livestock Techniques and Environmental issues, Institut de L'Elevage, Lyon, France, [4]AGRIS Sardegna, Sassari, Italy, [5]Animal & Grassland Research and Innovation Centre, Teagasc, Athenry, Co. Galway, Ireland, [6]Animal Production Department, Neiker-Basque Institute for Agricultural Research and Development, Arkaute, Spain, [7]National Research-Development Institute for Animal Biology and Nutrition, Balotesti, Romania, [8]LAORE Sardegna, Cagliari, Italy.*

Small ruminants account for 55% of the world's domestic ruminant population and play an important social, economic, and environmental role in many countries. However, their farming represents 6.5% of GHG emissions of livestock origin but with high relative mitigation potential due to the low efficiency and production levels. The LIFE Green Sheep project (LIFE19 CCM/FR/001245), articulated in 5 actions, aims to reduce the carbon footprint of sheep meat and milk production by 12% while ensuring the social and economic sustainability of the entire production chain in 5 European countries (France, Ireland, Italy, Spain, and Romania). The first action developed a common, harmonized methodology to assess the carbon footprint reaching comparable predictions from different tools available (CAP'2ER, ArdiCarbon, SheepLCA and CarbonSheep) tested on 24 meat and milk farms surveyed in different countries and production systems. Social and economic indicators were also defined to enlarge the sustainability framework of sheep farms. In the second action, 143 advisors were trained on the methodology, harmonized in the first action, to carry out on-farm data collection for environmental impact assessment and covering >30% of the areas in those countries. The third action aims to create a European observatory of the environmental sustainability of 1355 “demonstrative” farms (885 in France, 185 in Ireland, 100 in Italy, 100 in Romania, and 90 in Spain). The trained advisors are currently carrying out a simplified environmental, economic, and social assessment on these farms. In the fourth action, 282 so-called “innovative” farms are enrolled to test the effectiveness of mitigation practices through the development of a carbon plan. The fifth action focuses on promoting the knowledge gained through a policy formulation on a national, European, and global scale modeling proper mitigation scenarios in the medium term.

Key Words: sustainability, carbon footprint, sheep farming

Teaching/Undergraduate and Graduate Education Symposium and Workshop: Novel Teaching Strategies in Dairy Science

2221 Case-based teaching in undergraduate animal and dairy science courses. C. Cramer*, *Colorado State University, Fort Collins, CO.*

Higher education courses often rely on instructor-centered methods, like lecture, which can be a beneficial method for facilitating lower-order cognitive skills such as remembering and understanding. However, graduates of animal and dairy science programs need skills such as problem-solving, self-reliance, and decision-making to be successful in their careers. These skills often require higher-order cognitive skills such as applying, analyzing, evaluating, and creating. Case-based teaching, which is an active learning strategy that simulates real-life scenarios via case studies, can facilitate higher-order cognitive skills in students. Data regarding case-based teaching in animal or dairy science is limited. However, other disciplines, such as nursing, have utilized case-based teaching and have reported increased student motivation, understanding, performance, and problem-solving skills. Thus, case-based teaching can be a useful tool to implement in animal and dairy science courses to facilitate student learning. The objectives of this presentation are to 1) summarize previous research regarding case-based teaching in higher education, 2) describe the presenter's experience with and students' perceptions toward case-based teaching in both small (n = 30 students) and large (n = 150 students) 300- and 400-level animal and dairy science courses, and 3) identify important considerations for implementing case-based teaching into courses. Workshop participants will briefly outline the design for a case study for a topic of their choosing and receive feedback from peers. By the end of this presentation, participants should be able to design and implement a case study in their course.

Key Words: active learning, case study, critical thinking

2222 Teaching with the narrative and model in NASEM (2021) Nutrient Requirements of Dairy Cattle. M. G. Erickson*[1], M. D. Hanigan[2], and M. A. Wattiaux[1], [1]*Department of Animal & Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, VA.*

The consensus report "Nutrient Requirements of Dairy Cattle, 8th edition" (NASEM, 2021) summarized decades of dairy nutrition research into narrative text and a mathematical model available for public use. There is potential for instructors to use these resources in undergraduate and graduate courses on topics such as nutrition, physiology, management, and sustainability. For example, reading and discussing the narrative may promote understanding of the scientific process and core nutritional physiology concepts. Working with the mathematical model may allow learners to apply quantitative skills and practice systems thinking. However, the NASEM (2021) resources were designed for advanced dairy nutrition practitioners. As such, postsecondary instructors likely need to adapt the NASEM resources to make them accessible to less-experienced learners and enhance their compatibility with classroom settings. Our workshop will share newly-developed instructional resources for several chapters of the consensus report (Requirements; Energy; Protein; Nutrition and the Environment) and guidance on using the open-source NASEM-8 software in university classrooms. These instructional aids were developed based on theories of active learning and scaffolding and intended to function across a range of instructional situations. For each chapter, we listed learning objectives, highlighted core concepts, prepared optional background learning materials, formulated simple examples and visualizations, suggested in-class and independent activities, and proposed assessments. By sharing this starting point, we aim to foster discussion among dairy science instructors about the challenges and opportunities associated with using materials from NASEM (2021) in university classrooms.

Key Words: NASEM, teaching, instructional resources

2223 Experiences in teaching applied dairy cattle welfare to senior veterinary students. D. B. Haley*[1,2], L. J. Levison[1,2], and T. D. Duffield[1,2], [1]*Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, ON, Canada.*

The Ontario Veterinary College offers a one-week intensive rotation focused on the welfare of dairy cattle to final-year Doctor of Veterinary Medicine (DVM) students. The rotation is unique in its focus on the well-being of dairy cattle specifically and is, to our knowledge, one of only 2 animal welfare-focused rotations offered to veterinary students, in North America. The rotation is 5 full days of interactive and experiential learning that involves multiple modes of pedagogy. This includes hands-on learning during farm visits, and evaluation of students' knowledge and comprehension of the material taught, through various qualitative methods including both individual and group work. Throughout the week, we discuss the predominant dairy cattle care and welfare issues and approaches to dealing with them, including lameness, pain management, cull cow management, and various calf management practices. During the week, students typically visit one dairy farm for animal-based measures training, 2 additional dairy farms to carry out animal-based assessments, a male calf management facility (red veal or dairy beef), and a stockyard/auction market facility. While the attending students are all enrolled in DVM programs, we believe the principles, concepts, and teaching techniques used are relevant and of benefit to the instruction of students in a wide array of other programs – including those who may be pursuing careers in other sectors of animal agriculture. In this workshop attendees will participate in an exercise designed to help students practice addressing the often complex and multi-layered issues that are commonly presented in dairy cattle welfare exposé videos that appear across various forms of media.

Key Words: dairy cattle, education, animal welfare

2224 The Dairy Consortium: 15 years of educating and training the next generation of dairy industry professionals. M. Tomaszewski[1], H. Czerminski[1], and R. Hagevoort*[2], [1]*Texas A&M University, College Station, TX,* [2]*New Mexico State University, Clovis, NM.*

The US Dairy Education and Training Consortium (USDETC), established in 2008 to provide a unique educational opportunity through both classroom and experiential learning experiences, will be celebrating its 15th year of educating and training the next generation of dairy professionals. Since its inception, 610 students have completed the 6-week program. A 2022 survey of the former students was conducted (34% response rate) to determine the impact the consortium had on their future and the impact the classes and hands-on experiences had on their professional career. Of the 206 respondents, 47 are currently still in school, while 159 are employed. Of the former students which have entered the job market, 81% are employed in agriculture and 28% have found employment on a dairy, and 24% in the dairy allied industry, while 15% have continued to become veterinarians. Only 19% are not employed in agriculture, which means that 4 out 5 students attending the Consortium receive valuable guidance and instruction for their future careers in agriculture. The importance of the program is clearly shown in the following responses from former students. When asked “To what extent did the Consortium help you decide which direction to go in your career,” over 61% said, “opened a new door,” “definitely made a change” or “made me completely change gears.” When asked “What impact did the consortium have on other courses you have taken,” 60% gave a score 8 or higher (out of 10), indicating many students revisited their degree programs as a result of attending the Consortium. When asked “Did attending the Consortium assist you in your career development, 81% scored 8 or higher. When the 2022 class was asked if the program met their expectations, 96% scored an 8 or higher. When asked “Would you recommend this program to another student, 96% gave an 8 or higher. However, more importantly, a total 51% of the students have moved into a dairy or dairy related profession. The Dairy Consortium is attracting students that are continuing into the dairy profession.

Key Words: dairy education, dairy training, experiential learning

ADSA Southern Branch Symposium: Incorporating Beef in Dairy Systems

2225 Characterizing postpartum resumption of cyclicity on a biological and genetic basis in Jersey cattle. S. Johnson*, J. Lourenco, C. Welch, J. Clark, and J. Bohlen, *University of Georgia, Athens, GA.*

Although often masked by management and breeding programs, selection intensity for production traits has led to a decline in many aspects of reproductive efficiency. Genetic markers for reproductive recovery are available, and heritable and biological markers for fertility such as anti-Müllerian hormone (AMH) are well documented. However, other variables such as the uterine microbiome are yet to be fully described. Therefore, the objective of this study was to characterize the genetic and biological factors influencing postpartum resumption of cyclicity in Jersey cattle. Jersey cows (n = 25) of varying lactations (1st–5th) were enrolled in the study at 14 (±3) days in milk (DIM) with weekly blood samples collected for progesterone analysis. Blood was collected at d 45 was analyzed for AMH and ear tissue samples were genetically analyzed for daughter pregnancy rate (DPR). A subset of animals (n = 20) were sampled using a double guarded culture swab in duplicate for uterine microbiome analysis at 45–60 DIM. All animals were fitted with a DeLaval activity collar and monitored via DelPro. Estrous events and estrous intensities (EI) were recorded for ++ and +++ reproductive attentions and/or a minimum threshold of 120% EI. Animals were removed from the study after an estrous event occurred or at 100 DIM if no estrous event was recorded. Animals were considered to have normal (N) resumption of cyclicity if an estrous event was identified on or before 45 DIM or delayed (D) resumption beyond 45 DIM. The DNA extracted from the microbiota was abundant enough for sequencing with the sampling technique described. There was no difference in AMH or EI between animals with D resumption (305.8 ± 61.9 pg/mL and 128.9% ± 2.9, respectively) and N resumption (319.3 ± 91.7 pg/mL and 139.0% ± 4.9, respectively). AMH increased with lactation number ($P = 0.001$; $R^2 = 0.6$) while 1st estrous EI increased with DPR ($P < 0.05$; $R^2 = 0.45$). Tendencies in the data additionally indicate that as DPR increases, DIM at first estrous decreases ($P = 0.094$). As a preliminary trial, these conclusions provide avenues of further investigation.

Key Words: estrus, daughter pregnancy rate, AMH

2226 Can light affect microbial fermentation in in vitro rumen fermentation techniques? R. R. Lobo* and A. P. Faciola, *University of Florida, Gainesville, FL.*

Most in vitro assays used to evaluate ruminal fermentation, such as gas production, roll tube, batch cultures, and dual-flow continuous culture, are carried out using clear glass apparatus that allows light penetration; however, the rumen is a dark environment, where no light is present. Some bacteria species are light-sensitive, and any light stimuli may impact their survival, growth, and proliferation. Thus, this study's objective was to evaluate light's effects on ruminal fermentation in a dual-flow continuous culture. Using 4 fermenters and 3 experimental periods, the ruminal fluid of 2 lactating dairy cows was collected, pooled, and incubated for 10 d. Laboratory lights (234 ± 15 lx) were turned on 24h per d for the entire experiment duration. Two of the fermenters were covered with aluminum foil to avoid light penetration within the jar. The basal diet was formulated to meet the requirements of a 680 kg Holstein dairy cow producing 45 kg/d of milk and all fermenters were fed 106 g/d split into 2 feedings of the same diet that had 16% CP; 35% NDF; 31% starch, DM basis. After 7 d of adaptation, samples were collected for 3 d of each period for analyses of ruminal fermentation at 0, 1, 2, 4, 6, and 8 h post-morning feeding. Statistical analysis was performed with MIXED procedure of SAS, and significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. When compared with uncovered, covered fermenters had a greater dry matter and organic matter degradability (48 vs. 51% and 44 vs. 47%, respectively). Also, covered fermenters tended to improve NDF degradability (44 vs. 48%) and molar proportion of acetate (48 vs. 50%) and tended to reduce non-ammonia nitrogen flow (2.40 vs. 2.28 g/d). There were no major effects on the molar concentration of volatile fatty acids and nitrogen metabolism. In summary, this pilot study demonstrated that light might affect ruminal fermentation. Factors such as light intensity, exposure duration, and affected populations should be further investigated. Those results indicate that some ruminal microorganisms may be light-sensitive, and whenever possible, darker conditions should be practiced.

Key Words: degradability, dual-flow, glass

2227 A new look on beef: The end product value of beef × dairy. D. R. Woerner*, J. L. Frink, and B. A. Foraker, *Texas Tech University, Lubbock, TX.*

Dairy-origin cattle comprise 15 to 20% of the approximately 25 million fed cattle harvested annually in the US. In recent years, crossbreeding beef sires to dairy cows, commonly termed beef-on-dairy, has greatly altered the type of dairy-origin cattle entering the US beef supply chain. Beef × dairy crossbreds have largely replaced the once black-and-white landscape at many feedlots that historically fed Holsteins. The beef-on-dairy model offers a sustainable alternative to producing straightbred dairy fed steers and heifers for beef production. This model produces high quality beef and has potential to do so in a high-yielding manner; however, dairy producers should seek out beef genetics that complement the diary females on the dairy farm by selecting beef sires that add muscularity and red meat yield to progeny, while maintaining marbling potential. Producers should continually seek solutions to the liver abscess problem that persists in this population, as this continually serves as the leading obstacle to achieving premiums for these cattle.

Key Words: beef on dairy, quality, yield

2228 Genetic selection considerations when using beef sires on dairy cows. C. D. Dechow*, B. L. Basiel, and T. L. Felix, *Penn State University, University Park, PA.*

Improved fertility coupled with sexed semen has resulted in a stable supply of dairy replacements which, when combined with a price premium for beef × dairy (BxD) calves, has driven a rapid rise in beef semen use by dairy herds. Factors that drive beef semen sales include semen fertility, incidence of dystocia and stillbirths, the ability to accurately separate BxD females from dairy replacements based on coat color in Jersey crossbred herds, and production of a BxD calf with a high market value. Selection indexes have been developed to promote the use of beef bulls that prevent overlarge frame size when mated to Holstein and with additional emphasis on proper muscle development. Most beef semen used in dairy remains Angus or Angus crossbreds due to familiarity and a market for calves with black hides. We have evaluated steer performance (n = 124) from Angus, SimAngus, Charolais, and Wagyu sires crossed with Holsteins and 9508 calving records from dairy cows mated to either Holstein or beef bulls. Gestation length was 2 d longer ($P < 0.001$) for

BxD calves than for pure dairy calves with no difference in calving ease or stillbirth rates. There are differences among BxD breed groups ($P < 0.05$) for average daily gain, days on feed, hot carcass weight, and other performance traits with Angus sired calves generally having the highest performance and Wagyu the lowest. About 84% of Angus BxD calves graded Choice versus 31% of SimAngus ($P = 0.03$). The use of beef semen on low genetic merit and sexed dairy semen on high genetic merit cows can reduce the genetic lag between elite and commercial dairy herds. Genomic testing to select females for beef semen reduces genetic lag most because more accurate selection decisions are made; however, generating dairy replacements from virgin and first lactation heifers is also effective because genetic trends favor young animals and epigenetic programming of developing fetuses may be superior in nulliparous dams. The use of beef semen in dairy herds has accelerated genetic improvement of dairy heifers but high-quality beef genetics will be required to maintain a robust market for BxD calves.

Key Words: beef, dairy, selection

2229 Management of beef-on-dairy calves: Should we raise them differently?

V. S. Machado* and M. A. Ballou, *Texas Tech University, Lubbock, TX.*

Although the practice of raising dairy calves for beef purposes is not new, this is a growing trend in the American dairy industry. Factors intrinsic to the dairy industry such as increasing reproductive success, use of sexed-semen, and increased cow longevity led to a surplus of dairy calves. To increase the value of surplus calves, dairy farmers have been increasing the output of dairy × beef crosses calves, also known as beef-on-dairy calves, which are meeting the high demand for animals from the beef sector. Although some dairy farmers hold ownership of these animals for most of their productive life, most beef on dairy calves are transported, often for long distance, to calf raising facilities at a very young age, usually within the first week of life. Recent findings suggest that calves transported for 16 h are more likely to experience scours than calves transported for 12 or 6 h. Also, animals transported within the first week of life were more likely to experience bovine respiratory disease (BRD) than calves transported when older than 7 d of age. Findings from our group suggest that metaphylaxis does not reduce BRD incidence on calves transported to a calf raising facility, but it may reduce mortality. Recent research efforts have suggested that factors such as body weight at arrival, or variables from blood analysis may be used to identify animals at high risk of morbidity, mortality, and low growth performance. Beef on dairy calves are being managed in the same manner as replacement dairy heifers, but anecdotal data suggests that their feeding behavior and resilience to common health events such as BRD is different from the traditional dairy calf. Also, high prevalence of liver abscesses at slaughter poses as a major health challenge for dairy × beef crosses. Therefore, more research is needed to understand whether management and diet should be adjusted to improve the short- and long-term performance and health of beef on dairy animals.

Key Words: beef-on-dairy, calves health, management

2230 Improving mating decisions for beef-on-dairy production profitability.

A. De Vries*[1], P. Pinedo[2], N. Bliznyuk[1], F. Fourdraine[3], and J. Clay[3], [1]*University of Florida, Gainesville, FL,* [2]*Colorado State University, Fort Collins, CO,* [3]*Dairy Records Management Systems, Raleigh, NC.*

Mating decisions for beef-on-dairy production are often based on parity and breeding number, or genetic merit of the heifer or cow. Factors such as female and male fertility, semen cost, and risk of culling and dystocia should also play a role. Our objectives were to develop a system that maximizes expected profitability from beef-on-dairy mating decisions subject to a limit on the number of dairy heifer calves that are produced. The system should also be practical to implement on farms and be applied to the farm's heifers and cows eligible for insemination. We calculated insemination values for each potential sire mated to every eligible heifer and cow using a dynamic programming model that maximizes future cash flows. We also calculated the probability of a live heifer calf born for each possible mating and the probability the female is mated next week. Inputs to the model are animal records, herd parameters and external variables, such as prices. Herd profitability is maximized by choosing per eligible animal the sire with the greatest insemination value, subject to generating a target number of dairy heifer calves. Therefore, a sire with a lower insemination value may need to be chosen for some females to meet the constraint for number of heifer calves. This combinatorial problem was solved with a mixed integer linear program. To illustrate, we used a 1000-cow herd with the constraint to produce the expected number of dairy heifer calves to replace 35% of the cows annually. The optimal average insemination value was $88 per mating, whereas a user-defined strategy based purely on the genetic merit of the female had an average insemination of value of $72. This finding implies the system delivered an average increase in profit of $16 per mating over the user-defined strategy. Testing of this system has started on dairy farms. In conclusion, our mating decision support system may improve profitability compared with commonly used mating strategies in beef-on-dairy production.

Key Words: economics, mating decisions genetics, beef-on-dairy

Animal Health 2

2231 Feeding different iodine sources to transition dairy cows: Effects on production performance and thyroid hormones. D. C. Reyes*[1], K. R. Johnston[1], K. V. Almeida[1], A. Konopka[1], M. A. Rahman[1], E. A. Cruz[1], N. Price[2], P. E. Erickson[1], and A. F. Brito[1], *[1]Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH, [2]Bigelow Laboratory for Ocean Sciences, East Boothbay, ME.*

The brown seaweed *Ascophyllum nodosum* (ASCO) bioaccumulates iodine (I) and has bioactive compounds that may improve transition cow's health and performance. We aimed to evaluate the effects of feeding incremental amounts of ASCO meal, and to compare ASCO vs. ethylenediamine dihydroiodide (EDDI), on production performance, metabolic parameters, and thyroid hormones in transition dairy cows. Forty Holstein cows (3.2 ± 1.1 lactations) were blocked by parity and expected calving date, and assigned to 1 of 4 treatments: (1) Control [EDDI supplemented at 0.5 mg I/kg of DMI (CON)], (2) CON plus 57 g/d of ASCO meal (LOAM), (3) CON plus 113 g/d of ASCO meal (HIAM), and (4) CON plus 124.8 mg/d EDDI (HIEDDI) to match the amount of I provided by HIAM. Diets were fed as TMR, and I sources were top-dressed and fed from wk −4 before expected calving through wk +4 after parturition. Dry matter intake and milk yield were measured throughout the experiment, and blood was collected weekly and analyzed for plasma NEFA, BHB, and free and total T_3 and T_4. Data were analyzed using the PROC MIXED of SAS as repeated measures. Preplanned contrasts were used to determine linear and quadratic effects of ASCO and to compare HIAM vs. HIEDDI. Prepartum DMI was not affected by treatments (mean = 16.9 kg/d). However, HIAM cows tended ($P = 0.07$) to have lower postpartum DMI than HIEDDI cows (20.6 vs. 22.3 kg/d). Milk yield and concentrations and yields of milk fat and protein were similar among treatments. In contrast, MUN increased ($P < 0.01$) with feeding HIAM (11.5 mg/dL) vs. HIEDDI (9.11 mg/dL). No differences were observed for prepartum BHB or free and total T_3 and T_4 in cows fed ASCO. Plasma NEFA increased linearly ($P = 0.05$) with ASCO prepartum, but not changes in NEFA, BHB, or free and total T_3 and T_4 were found postpartum. Cows fed HIAM had greater postpartum free T_3 (2.46 vs. 2.38 pg/mL; $P = 0.05$) and lower total T_4 (37.7 vs. 43.0 ng/mL; $P = 0.05$) than those fed HIEDDI. In brief, feeding different I sources and amounts of ASCO meal had minor effects on production performance and I metabolism in transition dairy cows.

Key Words: brown seaweed, iodine, transition period

1258M Prepartum feed intake level is associated with transition metabolism and subsequent milk production in dairy cows. M. G. S. Santos*, B. Mion, B. Van Winters, and E. S. Ribeiro, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objectives were to identify factors associated with the level of prepartum DMI and its consequences on transition metabolism and performance. Cows (n = 273) were assigned to automatic feeding bins to measure feed intake from 45 d prepartum to 98 DIM. Rumination time (RT) was monitored by sensors. Within parity, cows were ranked based on average DMI (% of BW) in the 5 wk preceding calving and classified into terciles (T1, T2, and T3). Metabolites were measured in blood sampled on d −21, −10, −3, 0, 3, 7, 10, 14, and 23 relative to calving. Data were analyzed using GLIMMIX of SAS considering group, parity, diet, time, their interactions, season, and the random effect of cow. Prepartum DMI (T1 = 1.47; T2 = 1.72; T3 = 1.91 ± 0.1% of BW; $P < 0.01$), BW (T1 = 798; T2 = 769; T3 = 733 ± 9 kg; $P < 0.01$), EBAL (T1 = 1.5; T2 = 4.3; T3 = 5.8 ± 0.2 Mcal/d; $P < 0.01$), and BCS (T1 = 3.73; T2 = 3.62; T3 = 3.54 ± 0.03; $P < 0.01$) differed among all groups. During transition, T1 cows had shorter RT (T1 = 445; T2 = 464; T3 = 476 ± 7 min/d, $P < 0.01$), greater concentration of NEFA (T1 = 0.51; T2 = 0.45; T3 = 0.41 ± 0.02 mmol/L, $P < 0.01$) and BHB (T1 = 0.65; T2 = 0.57; T3 = 0.56 ± 0.02 mmol/L, $P < 0.01$) than T2 and T3; and lower cholesterol (T1 = 2.25; T2 = 2.37; T3 = 2.44 ± 0.05 mmol/L, $P = 0.04$), albumin (T1 = 36.05; T2 = 36.25; T3 = 36.63 ± 0.1 mmol/L, $P = 0.02$), and SOD (T1 = 2.08; T2 = 2.14; T3 = 2.37 ± 0.08 U/mL; $P < 0.01$) than T3. An interaction between group and time was detected ($P < 0.01$) for glucose. On d 0, T1 cows had lower glucose than T2 and T3. After calving, DMI (T1 = 3.08; T2 = 3.26; T3 = 3.40 ± 0.03% of BW, $P < 0.01$) and EBAL (T1 = −2.76; T2 = −1.63; T3 = −0.53 ± 0.3 Mcal/d; $P < 0.01$) differed among all groups, and cows in T3 were lighter (T1 = 680; T2 = 672; T3 = 649 ± 6 kg; $P < 0.01$) than T1 and T2. There was an interaction between group and wk for milk yield ($P = 0.04$) and ECM ($P = 0.03$). T1 cows produced less milk than T3 on wk 3 and 13, and less milk than T2 on wk 4 and 6. T1 tended ($P \leq 0.07$) to produce less ECM than T2 from wk 12–14. In conclusion, lower prepartum DMI was associated with important changes in energy metabolism and antioxidant status during transition, and moderate reductions in milk production during the subsequent lactation.

Key Words: ingestive behavior, transition health

2233 Effects of supplementing native rumen microbes on lactation performances and blood biomarkers in transition and mid-lactation Holstein cows. M. Bulnes*[1], J. Lefler[2], C. Marotz[2], E. Trevisi[4], M. Embree[2], J. Osorio[1,3], and M. E. Uddin[1], *[1]Dairy and Food Science Department, South Dakota State University, Brookings, SD, [2]Native Microbials Inc., San Diego, CA, [3]School of Animal Sciences, Virginia Tech, Blacksburg, VA, [4]Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Our objective was to evaluate the effects of a rumen derived microbial product [Galaxis Frontier (GF); Native Microbials, Inc., CA, USA] on lactation performances and welfare during the transition until 100 d in milk (DIM). Fifty-six cows were enrolled in a randomized complete block design study from −22 to 100 DIM. Cows were blocked by calving date, parity, and previous lactation milk yield or genetic merit. At −21 DIM, cows were randomly assigned to a control (CON, basal diet+150 g/d of ground corn; n = 29) or treatment group (GF, basal diet+150 g/d of ground corn+5 g/d of GF; n = 27), top-dressed once a day. All cows received the same basal close-up diet until calving (1.29 Mcal/kg DM and 10.8% CP) and lactation diet from calving to 100 DIM (1.67 Mcal/kg DM and 15.3% CP). Blood plasma samples were collected for energy metabolism, inflammation, liver function, and oxidative stress biomarkers from a subset of multiparous cows (n = 12/trt) at −22, −14, −7, 7, 14, and 30 DIM. Data were analyzed using the MIXED procedure of SAS. Compared with CON, GF cows tended to produce greater milk (39.71 vs. 37.07 kg/d, $P = 0.08$) during mid-lactation (31 to 100 DIM). While DMI was not affected by treatment, GF cows tended ($P = 0.10$) to have greater feed efficiency (+0.11, milk/DMI) from 0 to 30 DIM. Compared with CON, GF cows had lower blood plasma glucose and greater BHB (0.49 vs. 0.37 mmol/L; $P = 0.03$). Liver function biomarkers including paraoxonase was lower ($P = 0.03$) in GF than CON cows, suggesting increased oxidative stress. We observed greater ($P = 0.01$) reactive oxygen metabolites for GF than CON cows. Inflammation biomarkers showed greater concentrations of ceruloplasmin ($P = 0.01$) and haptoglobin ($P = 0.02$) for GF than CON. Though our data indicate a greater degree of inflammation (peak at 7 DIM) in GF than CON cows; however, values are within the normal physiological levels associated with parturition. Overall, our results suggest a potential benefit of supplementing GF in the transition period on lactation performances, but the increased inflammation after calving deserves further investigation.

Key Words: feed additive, blood metabolites, transition cows

2234 Validation of an on-farm dry chemical analyzer as a tool for quantifying blood analytes in transition Holstein dairy cows.

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Availability of a user friendly, on-farm quantification tool of blood analytes would expedite on-farm diagnosis and could improve health status monitoring in transition Holstein dairy cows. The objective of this study was to validate an on-farm dry chemical analyzer (DCA; BoviLab, I.B. Co., Tokyo, Japan) by comparing the whole blood metabolite concentrations obtained via DCA analysis with lyophilized reagents to laboratory colorimetric assay concentrations in heparinized plasma. This study occurred in 2 phases (Ph) using samples collected at −14, +3, or +14 (±1 d) d relative to calving in transition Holstein cows. In Ph 1, samples (n = 225) were collected at Farm 1 for quantification of albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), β-hydroxybutyrate (BHB), blood urea N (BUN), total calcium (tCa), creatine kinase (CK), γ-glutamyl transferase (GGT), glucose (Glc), magnesium (Mg), nonesterified fatty acid (NEFA), and phosphorous (Phos). In Ph 2, samples (n = 105) were collected at Farm 2 for quantification of Alb, Ca, cholesterol (Chol), Mg, and NEFA. Repeat metabolites in Ph 2 that were analyzed in Ph 1 had changes in lyophilized reagent to test performance enhancement. Plasma ALT, AST, BHB, BUN, CK, Chol, GGT, Glc, Mg, and Phos were quantified by colorimetric assay (Catachem Inc., Oxford, CT) on the ChemWell-T Autoanalyzer. Quantification of Alb, tCa, and NEFA were by colorimetric assay on 96-well plate (Catachem Inc., Cayman Chemical, and Catachem Inc., respectively). Data were analyzed within Ph via PROC CORR (SAS 9.4) after outliers were removed via Bland-Altman plots for each metabolite. In both Ph, all metabolite concentration correlations were significant ($P < 0.0001$). In Ph 1, ALT, BHB, BUN, CK, GGT, Glc, and Phos had a correlation coefficient (r) ≥ 0.91; AST, tCa, Mg, and NEFA were $0.89 \geq r \geq 0.84$; and Alb was r = 0.79. In Ph 2, Chol was r = 0.99; tCa, Mg, and NEFA were $0.89 \geq r \geq 0.85$; and Alb was r = 0.81 indicating similar performance for Alb, tCa, and NEFA. Overall, the DCA device performs comparably well to laboratory assays and would be a viable on-farm tool to monitor health status in transition Holstein dairy cows.

Key Words: transition cow, health status, metabolic diseases

2235 Effects of dietary rumen-protected choline supplementation on the plasma metabolome during an intramammary lipopolysaccharide challenge.

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The objective of this study was to examine the effects of supplementation and dose of rumen-protected choline (RPC) on the plasma metabolome during an intramammary lipopolysaccharide (LPS) challenge. Parous Holstein cows were blocked by calving month and randomly assigned within block to receive 45 g/d of RPC (20.4 g/d of choline ions; CHOL45, n = 18), 30 g/d of RPC (13.6 g/d of choline ions; CHOL30, n = 21), or no RPC (CON, n = 19) as a top-dress starting 24 d before expected calving until 21 d postpartum. Cows were alternately assigned within treatment group to either receive an intramammary LPS challenge (200 μg in each rear quarter) or not at 17 DIM. Blood samples were taken at 0, 4, 8, 24, and 48 h relative to LPS challenge. Metabolites were quantified using LC-MS/MS. Data were analyzed using linear mixed models. Treatment false discovery rates (FDR) were calculated using the Benjamini-Hochberg method. When FDR ≤0.10, pairwise comparisons were investigated using the Tukey procedure. Aside from trimethylamine *N*-oxide, which was increased in CHOL45-LPS as compared with CON-LPS (14.8 vs. 9.8 μmol/L, SE = 1.3, $P < 0.01$), most plasma choline metabolite concentrations were unaffected by treatment. Both CHOL45-LPS and CHOL30-LPS groups had greater acetylcarnitine concentrations than CON-LPS (0.36, 0.19, and −0.12 μmol/L [natural log], respectively, SE = 0.11, both $P \leq 0.04$), potentially reflecting greater fatty acid transport into the mitochondria. Plasma lactic acid concentrations were decreased in CHOL45-LPS and CHOL30-LPS compared with CON-LPS (606, 616, and 761 μmol/L, respectively, SE = 40, both $P < 0.001$), suggesting reduced glycolysis or enhanced lactic acid cycle flux to support gluconeogenesis. Plasma concentrations of fumaric acid, a byproduct of amino acid catabolism and the urea cycle, were increased in CHOL45-LPS and CHOL30-LPS as compared with CON-LPS (1.4, 1.4, and 1.0 μmol/L, respectively, SE = 0.12, both $P < 0.01$). Cows in the CHOL45-LPS group had reduced plasma methionine sulfoxide concentrations compared with CON-LPS (1.1 vs. 1.4 μmol/L, SE = 0.079, $P = 0.01$), potentially reflecting an improvement in oxidant status. Our data suggest that the effects of dietary RPC supplementation on periparturient dairy cattle performance could be mediated through numerous metabolic pathways.

Key Words: methyl donor, metabolomics, mastitis

2236 Decreased prepartal intake induces clinical ketosis postpartum: A retrospective analysis on dry matter intake from clinical and subclinical ketotic transition dairy cows.

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The aim of this retrospective study was to compare the pre- and postpartum dry matter intake (DMI), milk production, and efficiency of dairy cows undergoing subclinical (SK) and clinical ketosis (CK). Data from 97 Holstein dairy cows from 3 transition cow experiments were used. Cows were monitored during the peripartum period and precision Xtra was used to measure blood BHB at 1, 3, 5, 7, and 9 d postpartum. Cows were then classified as subclinical ketosis (SK; ≥ 1.4 mmol/L, n = 29) clinical ketosis (CK; ≥ 2.1 mmol/L, n = 16) or nonketotic (NONKET; < 1.4 mmol/L, n = 52). Dry matter intake and milk yield were recorded daily. Data were analyzed using the MIXED procedure of SAS. Prepartal DMI tended ($P = 0.07$) to be 1.4 kg/d lower in CK cows than in NONKET, while the SK group did not differ between groups ($P \geq 0.13$). Postpartal DMI in CK cows was 3.3 kg/d lower ($P < 0.01$; 14.5 vs. 17.8) than SK and 6.1 kg/d lower ($P < 0.01$; 14.5 vs. 20.6) than in NONKET cows, while SK cows had 2.78 kg/d less ($P < 0.01$; 17.8 vs. 20.6) DMI than NONKET cows. These effects on postpartal DMI were reflected on a Group × DIM interaction ($P < 0.01$) with distinctive trends for each group maintained throughout 30 DIM. Clinically ketotic cows produced 4.9 kg/d less ($P = 0.02$; 32.5 vs. 37.4) milk than SK and 8.29 kg/d less ($P = 0.01$; 32.5 vs. 40.8) than NONKET cows, while SK cows produced 3.38 kg/d ($P = 0.03$; 37.4 vs. 40.8) less milk than NONKET. Even though NONKET cows had greater milk yield, when calculating feed efficiency as milk/DMI, it resulted in lower efficiency for NONKET cows compared with SK and CK cows ($P < 0.01$; 0.9, 1.12, 1.28, respectively). However, this high efficiency cannot be maintained through the whole lactation; thus, an extended evaluation of feed efficiency will be necessary. These results suggest that a metabolic deregulation occurs in CK cows during late gestation that causes a low DMI that is maintained throughout early lactation.

Key Words: clinical ketosis, intake, subclinical ketosis

2237 Postpartal inflammation affects dry matter intake but not milk production: A meta-analysis. J. Halfen*[1], N. Carpinelli[2], M. Bulnes[2], E. Trevisi[3], J. J. Loor[4], and J. S. Osorio[1], *[1]School of Animal Science, Virginia Tech University, Blacksburg, VA, [2]Dairy and Food Science Department, South Dakota State University, Brookings, SD, [3]Department of Animal Science, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Piacenza, Italy, [4]Department of Animal Sciences, University of Illinois, Champaign-Urbana, IL.*

This study aimed to associate the postpartum inflammatory status of lactating dairy cows with dry matter intake (DMI), milk production, and blood biomarkers. Data from 114 Holstein dairy cows from 4 transition cow experiments were used. Haptoglobin (HP) concentrations from 3, 7, 14, and 15 d postpartum across studies were averaged and then used to classify cows into HIGH (HP ≥0.70 g/L, n = 41) or LOW (HP <0.70 g/L, n = 73) inflammatory status. Blood time points were grouped into −2, −1, +1, and +2 periods corresponding to data collected between −4 to −3 wk, −2 to −1 wk, 1 to 2 wk, and 3 to 4 wk relative to calving, respectively. Data were analyzed using the MIXED procedure of SAS. Daily milk yield and DMI data during the transition period were analyzed There was no interaction between Group × DIM ($P > 0.05$) for DMI; however, an overall group effect was observed, resulting in 1.40 kg/d lower ($P = 0.01$; 17.1 vs. 18.6) DMI in HIGH cows compared with LOW. A trend for Group × DIM ($P = 0.06$) was observed for milk yield kg/d, which was associated with lower ($P \leq 0.03$) milk yield at 3 and 4 DIM in HIGH cows compared with LOW. However, no overall group effect ($P = 0.35$) was observed for milk yield. As expected, greater HP was observed in the HIGH compared with LOW group at periods 1 ($P < 0.01$; 0.81 vs. 0.26 g/L) and 2 ($P < 0.01$; 0.28 vs. 0.16 g/L), which was reflected in an overall greater HP in HIGH cows compared with LOW ($P < 0.01$; 0.39 vs. 0.26 g/L). Albumin, a liver function biomarker, was lower in HIGH cows at periods 1 ($P < 0.01$; 33.43 vs. 34.49 g/L) and 2 ($P < 0.01$; 34.60 vs. 35.76 g/L). Paraoxonase concentration was greater for HIGH cows at period −2 ($P = 0.03$; 94.78 vs. 86.41 U/mL) but decreased at period 1 ($P = 0.04$; 70.90 vs. 78.96 U/mL) when compared with the LOW group. While NEFA and BHB were similar between groups, there was a trend for greater ($P = 0.09$) ROM in the HIGH group compared with LOW. These results suggested that postpartal inflammation may be detrimental to feed intake, but not to milk production. Our results confirm the negative association between inflammation and liver function, but a lesser connection with lipid mobilization and oxidative stress was observed.

Key Words: inflammation, postpartum, dairy cow

2238 Associations of heat stress during the close-up period with body condition loss in early-lactation dairy cows. C. Wagemann Fluxá*[1], S. J. Leblanc[2], E. S. Ribeiro[1], and T. J. DeVries[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

This study aimed to identify associations between temperature-humidity index (THI) during the close-up period and body condition loss in early-lactation cows. From April to October 2022, 40 commercial dairy farms in Ontario, Canada (190 ± 140 milking cows, mean ± SD) were visited once every 4 wk for a total of 6 visits. During the first visit, a logger that recorded ambient temperature and relative humidity every 10 min was placed in each close-up pen in each farm. The daily average THI (THIa; 65.1 ± 3.5, range = 54.8 to 70.9) and daily maximum THI (THIm; 70.5 ± 3.2, range = 60.7 to 75.3) were calculated for each farm for the 2 wk before visits 2–6. The body condition score (BCS) of up to 10 close-up cows (n = 1,484) within 14 d of expected calving was recorded on each visit; at a herd level these BCS averaged 3.5 ± 0.2 (range = 3.1–3.7) and were taken at 7.3 ± 1.1 (range = 9.3–4.0) days before calving. BCS of those cows was measured at each of the 2 subsequent visits. For the first measurement after calving BCS averaged 3.0 ± 0.2 (range = 2.5–3.3) and were taken at 21.5 ± 1.6 (range = 18.0–26.3) DIM. For the second measurement after calving BCS averaged 2.8 ± 0.2 (range = 2.4–3.3) and were taken at 49.9 ± 1.9 (range = 46.0–56.0) DIM. The BCS loss (BCL) of these cows was calculated between: 1) the close-up period and the first BCS measurement after calving (BCL1; average = −0.5 ± 0.2, range = 0 to −1.3), and 2) the close-up period and the second BCS measurement after calving (BCL2; average = −0.7 ± 0.2, range = 0 to −1.4). Mixed-effect linear regression models, using visit as a repeated measure, were used to identify associations of THIa and THIm with herd-level mean BCL. THIa and THIm were associated ($P < 0.001$) with greater herd-average BCL1; each 1-unit increase in these measures was associated with −0.02-point greater BCL1. Similarly, each 1-unit increase in THIa tended to be associated ($P = 0.1$) with a −0.01-point greater BCL2. In conclusion, these associations suggest that implementing heat stress mitigation practices before calving may help to reduce the BCL of fresh cows in herds in temperate regions.

Key Words: heat stress, transition cows, body condition loss

2239 Targeted lipidomics reveals depot-specific effects of subclinical ketosis in adipose tissue oxylipid profile of dairy cows. B. B. Sparks*[1], T. C. Michelotti[1], A. P. Tegeler[1], J. F. Fiallo[1], L. S. Flores[1], and C. Strieder-Barboza[1,2], *[1]Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, [2]School of Veterinary Medicine, Texas Tech University, Amarillo, TX.*

Ketosis develops in 40–60% of high producing, early lactation dairy cows, driven by dysfunctional adipose tissue (AT) lipid mobilization. Visceral (VAT) and subcutaneous (SAT) AT differ in function and cellular composition, however how ketosis affects depot-specific lipidome remains unstudied. We evaluated the effects of subclinical ketosis (SCK) on SAT and VAT PUFA-derived lipidome (oxylipids) in dairy cows. Serum, omental VAT, and flank SAT were collected from 10 multiparous Holstein cows (DIM 8 ± 2) assigned to a case control study based on SCK diagnosis (blood BHB in mmol/L): SCK (n = 3, BHB ≤ 1.4 and ≤ 2.6) and control, non-ketotic (NK, n = 3, BHB ≤ 0.8). AT PUFA metabolites were extracted and fatty acyllipidomics was analyzed by HPLC in a Prominence XR system. Statistical analysis was performed using PROC MIXED of SAS with depot, group, and their interaction as fixed effects. Serum NEFA was higher in SCK vs. NK (1.2 vs. 0.9 ± 0.07 mEq/L), yet both groups had increased lipolysis. Independent of ketosis, pro-inflammatory AA-derived prostaglandins FA20:3;O3 (PGE1), FA20:5;O3 (PGE3, 15-keto-PGE2), FA20:5;O2 (PGJ2), FA20:4;O3 (13,14dh-15k-PGE2, 15-Keto-PGF2a), and FA20:4;O (11- and 15-HETE) were higher in SAT, while FA18:1;O2 (12–13-DiHOME) and FA18:1;O2 (9–10-DiHOME), oxylipids associated with immune dysfunction, were greater in VAT. FA20:5;O2 (PGA2), an anti-proliferative factor, tended to be greater across VAT and SAT in SCK. In SCK FA18:3;O (13(S)-HOTrE) and FA18:4;O (9-KOT) were higher in VAT than SAT. Also, FA18:3;O (9(S)HOTrE) was the highest in SCK VAT relative to all other comparisons. Depot-ketosis interaction affected mainly ALA-derived oxylipids associated with reduced lipogenesis and anti-inflammatory effects. FA20:3;O4 (TXB2) and FA20:4;O (5-HETE) were uniquely increased in NK SAT, indicating that pro-inflammatory responses and immune cell activation are not unique of SCK AT. FA18:2;O (9-HODE) and FA18:2;O (13-HODE) did not differ between depots or upon ketosis diagnosis. Overall, postpartum dairy cows have a pro-inflammatory, depot-specific oxylipid profile that is differentially affected by SCK.

Key Words: lipid mediators, ketosis, lipidome

2240 The effect of lipolysis inhibitors on adipose tissue function during clinical ketosis in dairy cows.

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Dairy cows with clinical ketosis (CK) exhibit excessive adipose tissue (AT) lipolysis due to loss of insulin sensitivity and inflammation. The current treatment for CK is oral propylene glycol (PG). However, PG does not reduce lipolysis. Niacin (NIA) and flunixin meglumine (FM) can inhibit lipolysis, however their effects on AT are unknown. We aimed to determine the impact of PG, NIA and FM on AT function during CK. Multiparous Jersey cows [n = 18; 7.1 (SD = 3.8) DIM] were selected from a commercial dairy. Inclusion criteria: CK symptoms (lethargy, depressed appetite, and milk yield) and hyperketonemia [β-hydroxybutyrate; BHB ≥ 1.2 mmol/L]. Cows with CK (BHB:1.77 ± 1.01) were randomly assigned to one of 3 treatments: T1) PG: 310g oral once per d for 5 d, T2) PG+NIA: 24g oral once per d for 3 d, T3) PG+NIA+FM: 1.1 mg/kg IV once per day for 3 d. Healthy cows (HC n = 6; BHB:0.61 ± 0.21) matched by lactation and DIM (±2 d) were sampled. Subcutaneous AT (right flank) biopsies were collected at d 0 (d0) and 7 (d7) relative to enrollment. The AT explants were treated with insulin (INS = 1 μg/L) during ex-vivo lipolysis stimulation with isoproterenol (β-adrenergic). Lipolysis was assessed by glycerol release in the media. Protein biomarkers of lipolysis, insulin signaling, and AT inflammation including pHSL(Ser563):HSL, pAKT:AKT, and pERK1/2:ERK1/2 were quantified by capillary western immunoassay. Flow cytometry of AT cellular components was used to characterize macrophage (CD45+, MHCII+) inflammatory phenotypes (M1:CD14+; M2:CD11b+). Statistical analysis included a *t*-test and ANOVA test. At d0, INS reduced lipolysis by 41 ± 8% in AT from HC, while CK cows were unresponsive (−2.9 ± 4%). CK cows presented higher pHSL and pERK1/2 content while reduced pAKT compared with HC. AT from CK showed increased M1 and M2 macrophage populations compared with HC. By d7, the AT from T3 cows had a more robust response to INS reducing glycerol release by 36.5 ± 8% compared with T1 (26.9 ± 7%) and T2 (7.4 ± 8%). By d7, T3 cows presented lower pERK and higher pAKT, and reduced M1 and M2 populations compared with T1 and T2. These data suggest that including NIA and FM in CK treatment reduced AT inflammation and macrophage infiltration while improving AT insulin sensitivity

Key Words: ketosis, lipolysis

2241 Supplementation of omega-3 fatty acids as a strategy to regulate postpartum inflammation.

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Our objective was to investigate the effects of dietary supplementation of omega-3 (n3) fatty acids (FA) on physiological responses to lipopolysaccharide (LPS) challenge in early postpartum cows. Healthy cows were blocked by parity and BCS and randomly assigned to 1 of 3 top-dress treatments at 2 d in milk (DIM): 1) unsupplemented control received basal diet top-dressed with high moisture corn at 0.8% of dry matter (DM) (UNSUP; n = 16); 2) isocaloric control received UNSUP diet plus calcium salts (CaS) of palm oil at 0.8% of DM (CaPO; n = 8); or 3) n3 supplemented group received UNSUP diet plus CaS of fish oil at 0.8% of DM (CaFO; n = 8). At 14 DIM, UNSUP were assigned to 1 of 2 IV infusions: 1) LPS at 0.0625 μg/kg of BW (UNSUP-LPS; n = 8) or 2) saline (UNSUP-CON; n = 8). All fat-supplemented cows received LPS IV at 14 DIM. Blood, milk and peripheral blood leukocytes (PBL) were collected from 2 to 21 DIM and hourly for 12 h after challenge for analyses of FA composition, metabolites and markers of immune response. Data were analyzed using mixed models considering treatment, time, parity, BCS, their interactions and random effect of cow. Compared with UNSUP, fat supplementation decreased DM intake but did not impact energy-corrected milk yield. Fat increased concentration of monocytes in whole blood at 14 DIM (0.27 vs. $0.38 \pm 0.04 \times 10^9$ cells/L). CaFO resulted in greater ($P < 0.01$) % composition of EPA and DHA in plasma (1.8 vs. 1.9 vs. 3.4 ± 0.1%) and milk (0.096 vs. 0.089 vs. 0.173 ± 0.01%), as early as 7 DIM, and increased abundance of EPA in phospholipids of PBL at 14 DIM (1.47 vs. 1.48 vs. 1.8 ± 0.1%; $P = 0.05$). Body temperature increased in all LPS IV cows and did not change in UNSUP-CON. Compared with UNSUP-LPS and CaPO, CaFO spent less ($P = 0.08$) time above 40°C following infusion (245 vs. 225 vs. 169 ± 23 min) and had a smaller ($P = 0.06$) area under the curve above 40°C (16,164 vs. 1,5301 vs. 1,1396 ± 1,427 arbitrary units). Before and after infusion, concentration of IFNγ was higher ($P = 0.06$) in CaFO and macrophage inflammatory protein-1α was higher ($P < 0.01$) in CaFO than in CaPO. In conclusion, postpartum n3 supplementation via CaFO modestly altered inflammatory responses in early lactation.

Key Words: health, transition

2242 Effects of a dietary immunomodulatory feed additive and different starch contents on the endotoxin response and measures of hepatic health in periparturient dairy cows.

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Differences in dietary starch content have potential to modify the immune response of early lactation cows, which may be influenced by dietary immunomodulatory feed additive (IFA) supplementation. Seventy-four Holstein cows were assigned at −6 wk prepartum to far-off or close-up diets unsupplemented (U; n = 35) or supplemented (S; n = 39) with an IFA (56 g/cow/d; OmniGen AF, Phibro Animal Health; Teaneck, NJ). At parturition, cows continued to be U or S with an IFA but were ad libitum fed a TMR containing a low or high starch content (21 vs. 27% of ration dry matter; LSU, LSS, HSU, or HSS; n = 18–20 cows/treatment) until wk 6 of lactation. Blood samples were collected twice weekly before feeding. An intravenous lipopolysaccharide (LPS; *E. coli* O111:B4) challenge was introduced at 3 wk postpartum with blood sampling at h 0, 8, and 16. Liver biopsies were performed at −2 wk, and 1 and 3 wk, relative to calving. Data were analyzed using a mixed model with fixed effects of treatment and time, and their interaction.

Planned contrasts included: 1) LS (LSU + LSS) vs. HS (HSU + HSS) and 2) U vs. S (LSU + HSU vs. LSS + HSS). Before LPS challenge, fresh cow plasma fatty acid concentrations were greater in LS, relative to HS ($P = 0.02$), while plasma glucose concentrations were not modified. Serum total bilirubin concentrations were lower in HS, relative to LS ($P = 0.03$). Rectal and vaginal temperatures and, respiration rates were increased post LPS challenge (time, $P < 0.05$) but were not modified by treatment. Endotoxin increased serum cortisol concentrations ($P < 0.01$); however, treatment did not modify. By h 8 after LPS administration, blood hemoglobin concentrations by 3%, and erythrocyte and eosinophil counts were greater by 4% and 25% in S cows, relative to U cows, respectively ($P < 0.05$). Liver steatosis scores tended to be greater in LS, relative to HS ($P < 0.07$); however, remain unchanged in U vs. S. Postpartum dietary starch content and IFA supplementation uniquely modify the endotoxin response and measures of hepatic health in periparturient cows.

Key Words: endotoxin, liver, starch

Breeding and Genetics Platform Session: Novel Traits, Novel Technologies

2243 Genetic improvement of calf health: Genomics makes it possible. B. Fessenden*[1,2], A. McNeel[1], D. Weigel[2], D. Gonzalez-Pena[1], N. Vukasinovic[3], and F. Di Croce[3], [1]*Zoetis Precision Animal Health, Kalamazoo, MI,* [2]*Zoetis Outcomes Research, Kalamazoo, MI,* [3]*Zoetis Precision Animal Health R&D, Kalamazoo, MI.*

Heifer disease and death losses represents a financial cost for dairy producers. Genetic improvement programs that incorporate differences in risk of calf disease and livability have potential to improve profitability of dairy production. To partially address this effect on profitability, a genomically enhanced genetic evaluation for calf health was developed using a single-step genomic BLUP and a univariate threshold animal model. The estimated heritabilities are 0.042, 0.045, and 0.060 for calf respiratory disease, calf scours, and calf livability. An animal's relative risk for each of the diseases is reported as a standardized transmitting ability (STA). This study was conducted to demonstrate the ability of calf wellness STA to predict calf disease and death incidence using 10,751 Holstein heifers born in 2021 from 10 US commercial herds that recorded at least 2 of the desired outcomes. Heifers were ranked by their STA within herd and birth season then assigned to genetic quartiles based upon this ranking. Calf health events were collected from on-farm herd management software and heifers were assigned as healthy (0) or diseased/died (1) for each event. Statistical analysis was performed using generalized linear mixed model with genetic quartile as a fixed effect and herd and birth season as random effects. Calf wellness prediction quartiles were significantly different for calf disease incidence. The difference between the worst and best genetic quartiles being 1.81% for calf livability, 3.66% for calf scours, and 13.99% for calf respiratory disease, which represents reductions in incidence of 34%, 52%, and 33%, respectively. The differences in the STA between the worst and best quartiles were 11 STA for each trait with the STA standard deviation being 5 for all 3 traits. This demonstrates that improving calf health and livability through genetic selection presents an opportunity for dairy producers to reduce calf disease incidence, herd replacement costs, and improve herd profitability when combined with comprehensive management practices.

Key Words: calf wellness, genomics, Holstein

2244 Genetic evaluation for stillbirth and pre-weaning mortality. M. M. Axford*[1,2], M. Khansefid[1,2], and J. Pryce[1,2], [1]*Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia,* [2]*School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia,* [3]*DataGene Ltd., Bundoora, Victoria, Australia.*

The welfare of calves is important to both farmers and consumers. Practices that increase the proportion of calves born alive and continue to thrive through to weaning contribute to improved sustainability as they improve animal welfare, improve farm financial performance and reduce environmental waste. Farmers often record whether the calf is alive or dead when noting a calving observation. Stillbirths (SB) are calves recorded as dead at birth or die within 24 h after birth. Pre-weaning mortality (PWM) refers to calves that die after the first day of life but before weaning based on termination data. Both stillbirth and pre-weaning mortality are characterized by low heritability and are binomial in nature. Data collection for these traits is incomplete, compared with traits like milk yield in cows. Despite these challenges, genetic variation can be measured and used to produce breeding tools, such as EBVs, to reduce calf mortality over time. The aim of this study was to compare the performance of various linear models to predict stillbirth and pre-weaning mortality traits in Holstein and Jersey cattle and evaluate their applicability for industry-wide use in the Australian dairy industry. Calving records from 2.78M Holstein and Jersey dams were obtained from DataGene's Central Data Repository from 1990 onwards. About 7% of calves were recorded as stillborn in the period 2000–2021 (n = 1.74M calvings). Pre-weaning mortality is much lower than stillbirth during the same period at 2% (n = 0.89M calves). Genetic parameters were estimated using bivariate models for stillbirth direct, stillbirth maternal and pre-weaning mortality using calving ease as a covariate. The heritability of calf traits was 1–2% depending on the breed, trait and model. Based on this study, it is possible to measure genetic variance in calf traits as a first step to using genetic selection to increase the proportion of calves born alive and calves weaned. Improved calf and calving recording practices and further research is expected to increase the reliability of genetic predictions.

Key Words: calf survival, calf weaning rate, stillbirth

2245 Candidate mutation for calf recumbency in Holsteins. C. D. Dechow*[1], P. M. VanRaden[2], D. J. Null[2], A. Al-Khudhair[2], and M. C. McClure[3], [1]*Pennsylvania State University, University Park, PA,* [2]*USDA Animal Genomics and Improvement Lab, Beltsville, MD,* [3]*ABS Global, DeForest, WI.*

A recessive recumbency haplotype that results in elevated calf mortality but with apparent incomplete penetrance was previously linked to the end of chromosome 16 (78.7 to 80.7 Mbp). The region included 110 unique genes, 13 of which were plausible candidate genes based on association with diseases reported in the Online Mendelian Inheritance in Man database. Genotype analysis indicated that the haplotype was common and traced back to 1952 with a key ancestor born in 1984 (HOUSA1964484, Southwind) identified from chip genotypes as homozygous for the suspect haplotype. Sequence data (30x, Illumina HiSeq pair-ended) from Southwind, an affected calf, and the sire of the affected calf was scanned for candidate mutations. A missense mutation in calcium channel, voltage-dependent, L type, α 1S subunit (CACNA1S) that alters a GGC codon to AGC and facilitates a glycine to serine amino acid substitution at 79,613,592 bp was considered the most promising candidate. The mutation was homozygous in the affected calf and heterozygous in the calf's sire and Southwind. Sequence data available from the Cooperative Dairy DNA for 299 other Holsteins indicated a 97% concordance with the haplotype and an 89% call rate. The Sorting Intolerant From Tolerant score (range 0 to 0.01) indicated that the mutation was deleterious with a moderate projected impact which may explain the incomplete penetrance. CACNA1S is predicted to have multiple transcripts of 39 to 40 exons with the mutation in exon 30 to 32; the exon amino acid sequence appears to be broadly conserved. CACNA1S enables calcium channel voltage gate activity and is highly expressed in skeletal muscle. In humans, heterozygotes for a CACNA1S mutation may experience periodic paralysis whereas homozygotes may have no ability to stand and can be more severely affected than the recumbency. Mice homozygous for CACNA1S knockout mutations show degenerated muscles, edema, failure of myoblast differentiation by d 13 of embryonic development, and perinatal death. A stand-alone test for the mutation is under development and the mutation is expected to be added to a genotyping array.

Key Words: calf recumbency, mutation, CACNA1s

2246 Feasibility study of genetic evaluation for Johne's disease in US Holstein cows.

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Paratuberculosis, or Johne's disease (JD), is an infectious enteric disease in cattle and other ruminants, causing substantial economic loss annually worldwide. This work investigated the feasibility of a national genetic evaluation for Johne's Disease susceptibility in US Holstein cattle. The data, provided by the Council of Dairy Cattle Breeding, comprised 365,980 Holstein cows from 1,048 herds participating in the voluntary control program for JD between 2002 and 2022 diagnosed with 2 protocol kits: Idexx (IDX) and Parachek (PCK). Only test results from the first 5 parities were analyzed. An animal was considered infected if it had at least one positive result or uninfected otherwise, with an overall incidence of 4.72% of animals. Genotypes of 78,964 SNP markers were extracted for 25,000 animals. Variance components were estimated using 3 models, namely, a pedigree-only threshold model (THR), a single-step threshold model (ssTHR), and a single-step linear model (ssLR). For the threshold models, the Markov chain Monte Carlo simulation consisted of 80,000 iterations with a burn-in period of 15,000 and thinned for every 100th. The heritability estimates of JD susceptibility were low to moderate. The 2 threshold models gave higher posterior heritability estimates (0.11–0.16) than the linear model (0.05–0.09). The average reliability of the estimated breeding values of JD susceptibility based on the 3 models varied from 0.18 (THR) to 0.22 (ssLR) for IDX and from 0.14 (THR) to 0.18 (ssTHR and ssLR) for PCK. Despite no prior direct genetic selection on JD, the estimated genetic trends of JD susceptibility were negative and highly significant ($P < 0.0001$). Based on these results, we conclude that a national genetic evaluation of JD susceptibility in the US Holstein cattle using ELISA-based tests is feasible, which will reduce the JD infection rate in the long term.

Key Words: infectious disease, reliabilities, single-step model

2247 Individual measure of body weight on in-house commercial dairy cattle using a 3D camera system for genetic evaluations and improved management decisions.

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In this study data on individual body weight measures from the 3D camera-based system CFIT was analyzed. The data are measured on dairy cows in commercial in-house settings. The system was developed to predict body weight based on the curvature of the back of the cow after each milking. A total of 9142 cows were measured in 19 herds across 3 breeds – Jersey (2513 cows), Red Dairy cattle (2813 cows) and Holstein (3816 cows). Mean body weight was higher for Red Dairy cattle (647.9 kg) and Holstein (683.8 kg) than for Jersey (469.6 kg). Repeatability estimates for body weight as a weekly average was 0.83, 0.85 and 0.88 for Jersey, Red Dairy cattle and Holstein respectively. The perspectives in having such records available are huge both for the farmer and for the dairy industry. The records can both be used for improving management in farms on an individual cow level and herd level, but also for genetic evaluation and selection. When selecting for improved efficiency maintenance is a major part of the resource usage complexity and controlled by body weight. Changes in body weight are an indicator for the cows' wellbeing. The data are currently presented for the farmer for 4 different parameters: 1) dry cow management to know how body weight change has been through the dry period, 2) early lactation body weight change to indicate how well the lactation startup has been, 3) energy balance to maximize probability of a successful insemination and 4) late lactation body weight change to point at cow gaining too much body weight before dry off. The numbers are presented both at an individual cow level as well as means within lactation. These numbers are highly influenced by herd strategy but can still be used to benchmark within breed and across herds.

Key Words: body weight, efficiency, dairy cattle

2248 Estrus expression in dairy cows: Phenotyping, genetic variability, and association with reproductive performance.

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Dairy cow estrus is marked by acute changes in behavior, such as increased activity, decreased feed intake, and decreased rumination. These behavioral changes can be measured in the field using wearable sensors and other monitoring systems. The main goals of this study were to develop phenotypes for estrus expression, evaluate their genetic variability, and assess their associations with pregnancy success. Data consisted of bi-hourly activity and rumination records collected using automated monitoring devices in 10k lactating cows during 2 years on a large commercial farm. We developed an algorithm that quantifies activity and rumination changes to characterize estrus duration and strength. The identified estrus events were assigned to the corresponding insemination events. The association between estrus traits and pregnancy success was evaluated using alternative logistic regression models, including year-season, lactation, sexed or conventional semen, and estrus traits. The statistical analysis showed that the addition of a detected estrus event (binary variable, yes/no) contributes significantly to explaining pregnancy success, relative to insemination in the absence of a detected estrus. In addition, estrus duration, classified as none, low, intermediate, and long, was significantly associated with the probability of pregnancy success. Similar results were found for activity strength and rumination strength. The estimates for heritability and repeatability were 0.104 ± 0.017 and 0.136 ± 0.009 for estrus duration, 0.134 ± 0.021 and 0.214 ± 0.010 for rumination strength, and 0.125 ± 0.021 and 0.218 ± 0.010 for activity strength. Overall, longer, and more intense estruses were significantly associated with pregnancy success. In addition, estrus traits are heritable, and breeding for improved estrus expression has the potential to boost estrus detection and increase insemination and pregnancy rates without relying on hormonal treatments.

Key Words: estrus expression, genetic parameters, pregnancy success

2249 Genome-wide association analysis of white spotting in Montbeliarde-sired crossbred dairy cattle.

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This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).

The Montbeliarde is a red pied dairy breed and white faces are a characteristic of the breed. However, crossbred cattle may exhibit a characteristic of a split face trait that may contain more than one color and not a solid white face. The objective of the study was to investigate the genetic architecture of the white face trait in Montbeliarde-sired crossbred dairy cattle. A genome-wide association study was conducted with 150 Montbeliarde-sired crossbred dairy cattle, and cows and heifers were born from 2010 to 2022 at the University of Minnesota West Central Research and Outreach Center dairy herd in Morris, MN. All animals were Montbeliarde-sired; however, the maternal grandsire breed was either Holstein or Viking Red. The cows and heifers were sired by 18 bulls and ranged from 1 to 28 daughters in the herd. Cattle were genotyped with a 50K, 65K, or 150K SNP chip from Neogen (Lincoln, NE) and imputed to 150K. Face color was scored as a 0 (all white face) or 1 (split face) and 39 of the 150 animals (26%) had a split face of more than one color. Quality control and association analysis was with the SNP and Variation Suite v8.9.1 (Golden Helix, Inc., Bozeman, MT) with a minor allele frequency of 0.05 and SNP call rate of 0.6. For significance testing, a *P*-value of 5×10^{-6} as the significance threshold. The analysis identified 2 SNP on chromosome 22 with a strong signal and 1 SNP on chromosome 1. The top variant (BTA-54082-no-rs; $P = 8.78 \times 10^{-10}$) was mapped to the 32.1404 RSID A>T and the second the top variant (BovineHD2200009163; $P = 8.0647 \times 10^{-10}$) was mapped to the 31.9513 RSID A>G. The BovineHD2200009163 variant was mapped to the melanocyte inducing transcription factor (MITF) downstream of the FERM domain containing 4B (FRMD4B) genes which was linked to BTA-54082-no-rs on BTA22. Most animals with a split face carried the 'G' allele and all cattle except 1 carried the 'A' allele for solid white face. The MITF gene is involved in cell pigmentation and is the causal locus for white spotting in cattle. The results indicate that the white face in Montbeliarde-sired crossbreds may not be dominant, and the split face may occur because of incomplete dominance.

Key Words: Montbeliarde, coat color, crossbreeding

2250 Environmental factors and genetic parameters for milk fatty acids in Japanese Holstein cattle.

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Fatty-acid (FA) content in milk represents the energy balance of milking cows; therefore, the measurement can be used to monitor the animal's health condition. The FA content is now easily predicted by Fourier-transform mid-infrared spectroscopy (FT-MIR) in the dairy herd improvement program in Japan. This study aimed to determine environmental factors for the FA contents and to estimate genetic parameters for the FA traits. Data included 1,448,373 test day records from 211,462 Holstein cows in the 1st lactation, calving in Hokkaido, Japan, between April 2021 and October 2022. Two groups of FA, de novo FA (between C4 and C14) and preformed FA (equal to or greater than C18), were predicted by FT-MIR. The de novo FA percentage (DNF%) and preformed FA percentage (PFF%) was calculated as the ratio of the predicted FA content to the total FA content. The data set was divided by lactation stage, defined as every 30 d in milk (DIM) in each lactation, i.e., DIM 6 to 35 as stage 1, DIM 36 to 65 as stage 2, and so on. Significant environmental factors were determined with the F-test of Type III sum of squares. Variance components were estimated with a simple animal model. For each trait, (co)variances were estimated with a bivariate mixed model for phenotypes between 2 different stages. At all stages, herd-year of calving, year-month of calving, and a regression coefficient on DIM nested within the year-month of calving were significant ($P < 0.01$). Age of calving and regression on DIM within the age of calving were significant only at stages up to DIM 155. Heritability at stage 1 for DNF% was 0.24, for PFF%, 0.30, and the value decreased up to stage 7 (DIM 215). For both traits, stages 1 and 2 showed lower genetic correlations with later stages, e.g., for DNF%, 0.21 between stages 1 and 5. The genetic correlations of stage 3 or later were high (>0.90) up to stage 7. Further research will focus on genetic relationships between health traits and the FA content up to DIM 65, where the negative energy balance is a major concern.

Key Words: de novo fatty acid, milk components, preformed fatty acid

2251 Genetic parameters of milkability traits derived from automatic milking systems in US Holstein cows.

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The number of dairy herds with automatic milking systems (AMS) is increasing rapidly which reduce the need of human involvement in milking process. Study of the genetic architecture of milkability traits are important for dairy producers focusing on precision dairy system. Therefore, the objectives of this study were to estimate the heritabilities and repeatabilities for different milkability traits and to estimate the genetic correlations among different milkability traits from AMS in US Holstein cows. A total of 504,141 daily records of milking speed (MS), maximum milking speed (MMS), harvest rate (HR), box time (BT), number of milkings (NOM) and milking duration (MD) were collected from 2,941 multiparous (n = 1, 2 or 3) lactating cows. Data were recorded by Lely AMS installed at STgenetics research farm at Ohio from 2019 to 2022. The model included the fixed effect of contemporary group, lactation number, lactation stage and milk yield as covariate, as well as random additive genetic effect and permanent environmental effect. Analyses were conducted using LMT software. Univariate and bivariate analyses were implemented to estimate heritabilities and genetic correlation among traits. Pedigree information was used to specify the genetic relationships to estimate the variance components and genetic correlations. The heritability of MS was 0.52 ± 0.05, MMS was 0.56 ± 0.04, HR was 0.40 ± 0.04, BT was 0.44 ± 0.04, NOM was 0.23 ± 0.04 and MD was 0.49 ± 0.05. Repeatabilites were moderate to high for all traits analyzed which ranged from 0.40 ± 0.04 for NOM to 0.88 ± 0.05 for MMS. The genetic correlation between all traits ranged from moderate to high in both directions which ranged from −0.97 ± 0.01 between MS and MD to 0.97 ± 0.01 between MS and MMS. The moderate to high heritabilities of different traits suggested that all the traits studied could be improved by genomic selection. The results from this study could be used to design and refine the selection index for implementation in precision dairy breeding program.

Key Words: automatic milking system, milkability, genetic parameter

2252 Genomic prediction of breeding values for behavior traits measured in automatic milking systems in Holstein cattle using machine learning methods.

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Identifying genomic prediction (GP) models that might provide more accurate breeding values is of great interest when evaluating complex traits such as livestock behavior. In this context, the main objective of this study was to compare the predictive performance of genomic breeding values using deep learning algorithms (DL) and traditional methods for milking refusals (MREF) and milking failures (MFAIL) in Holstein cows measured by automatic milking systems (milking robots). A total of 1,993,509 daily records were collected using 36 milking robot stations for 4,511 genotyped Holstein cows. A total of 57,600 single nucleotide polymorphisms remained after quality control. Four GP methods were considered in this study: 1—Bayesian Lasso (LASSO); 2—Multiple

Layer Perceptron (MLP); 3—Convolutional Neural Network (CNN); and 4—Genomic Best Linear Unbiased Prediction (GBLUP). The first 3 methods were implemented using Python (v.3.9), in which the DL methods were estimated using the Keras and Tensorflow packages. The GBLUP method was implemented using the BLUPF90 family package. The accuracy of prediction (MSE) for MREF and MFAIL was 0.349 (0.079) and 0.275 (0.089) for LASSO; 0.374 (0.098) and 0.325 (0.090) for MLP, 0.379 (0.078) and 0.302 (0.091) for CNN, and 0.352 (0.092) and 0.307 (0.090) for GBLUP, respectively. Furthermore, a lower re-ranking of selection was observed in MLP versus CNN methods compared with the other approaches for both MREF and MFAIL. Promising results showed slightly higher accuracies when DL methods were applied. However, the results observed may not be sufficient to justify the use of DL compared with more traditional methods due to the computational demand required and the difficulty of performing GP for non-genotyped animals using DL procedures.

Key Words: accuracy of prediction, automatic milking systems, deep learning

2253 Exploring opportunities to evaluate genomically mid-infrared spectroscopy (MIR)-predicted residual CH_4 exploiting correlations to multiple across country evaluation (MACE) traits.

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Breeding has been identified as a potentially promising strategy to reduce methane (CH_4) emissions. However, trait definitions and, despite availability of genomic data, setup of such genetic evaluation systems remain important issues. The aim of this study was to demonstrate an optimized computational ssGBLUP setting exploiting correlations to traits evaluated in an international context by INTERBULL through Multiple Across Country Evaluation (MACE). The trait evaluated was Residual CH_4 (RCH_4) defined on a genetic level as the deviation of MIR-predicted CH_4 (MCH_4) from Expected CH_4 (ECH_4) obtained form traits available internationally through MACE. Genetic parameters and EBV for MCH_4 were estimated using test-day MCH_4 records on 229,465 first-, 151,726 second-, and 90,484 third-parity Walloon Holstein cows. Mean (SD) MCH_4 yields were 327 (68), 356 (70), and 358 (72) g/day and mean (SD) h^2 estimates for daily MCH_4 were 0.12 (0.04), 0.14 (0.05), and 0.13 (0.05), for the first 3 lactations, respectively. For 1,129 bulls with at least 30 daughters (727 genotyped), EBV for 305-d MCH_4 for each lactation (and average of first 3 lactations) were regressed on published EBV of MACE evaluated milk, fat and protein yields, derived fat and protein percentages, and linear type traits. EBV of ECH_4 were defined as predictions combing the MACE evaluated traits using the obtained regression equations. Correlations between EBV of 305-d MCH_4 and ECH_4 ranged from 0.57 to 0.61 in the first 3 lactations and was 0.60 for the averaged 3 lactation EBV of 305-d MCH_4. Linear type traits including body depth, chest depth, bone quality, teat placement, udder support and udder texture showed significant association to MCH_4. An innovative evaluation setup is possible considering direct MCH_4 data and, as external information, EBV for ECH_4 combined in a bivariate ssGBLUP exploiting the indirect extra information provided by correlated traits that are available for sires based on daughters found internationally. EBV of RCH_4 can be predicted by subtracting ssGBLUP recomputed 305-d EBV for ECH_4 from EBV for MCH_4.

Key Words: external information, ssGBLUP, evaluation system

Dairy Foods Symposium: Dairy Beverages 2.0—Current Innovations to Fuel Dairy-Based Beverages of the Future

2254 What's driving innovation in dairy beverages—A global perspective. K. Alexander*, *Dairy Management Inc., Rosemont, IL.*

Consumers look to functional food and beverage solutions for a variety of health and wellness reasons. In fact, 66% of Americans now choose products based on personal health needs. And while the functional food and beverage category continues to grow, consumers look to different products and different benefits based on the condition they are looking to treat or prevent. Dairy beverages are in a prime position to be the go-to option for consumers as they look to the functional space—whether it be for energy, digestion or other top conditions. This session will provide a global perspective on what's driving innovation in functional dairy beverages, including consumer needs, global trends, and opportunities to capitalize on this growing category.

Key Words: dairy beverage, functional food

2255 Ingredient/process innovations in high-protein dairy beverages. D. M. Barbano*[1] and M. Drake[2], [1]*Cornell University, Northeast Dairy Foods Research Center, Ithaca, NY,* [2]*North Carolina State University, Southeast Dairy Foods Research Center, Raleigh, NC.*

Sales of high-protein dairy-based beverages produced from liquid or dried ultrafiltered milk have grown in the North American market. These beverages range from 13 to 30 g of protein per serving in both shelf-stable and refrigerated formats. Serving sizes vary but the protein concentration is typically between 5.5 and 9% protein. At lower protein concentration, the beverages often have no added nondairy ingredients (other than added vitamins or lactase) and are available in shelf-stable or refrigerated formats. The primary challenge in these products is cooked-sulfur off-flavors that consumers do not like. However, the thermal stability of protein becomes a challenge in higher protein concentration beverages. Heat induced protein-protein interactions in shelf-stable products causes protein to settle to the bottom of the container. When the protein layer at the bottom of the package comes out in chunks, the consumer concludes the product is spoiled. One common approach to overcome protein aggregation and settling is to add non-dairy ingredients (gums, stabilizers, and emulsifiers). However, push-back by consumers for simple clean label ingredient decks is increasing. To address flavor and protein stability issues, while eliminating the need to add non-dairy ingredients, an understanding of the origin and mechanism of the formation of off-flavor compounds and nature of the interaction of thermal processing conditions that create protein aggregation is required. Innovations in thermal processing technology to minimize thermal damage to milk components combined with other pre-thermal processing treatments of milk to maximize heat stability without adding non-dairy ingredients are needed to make high-protein dairy beverages standout with clean labels, excellent flavor, uniformity and stability.

Key Words: dairy protein beverage

2256 Value-added dairy ingredients for functional beverage development. A. Abbaspourrad*, *Cornell University, Department of Food Science, Ithaca, NY.*

Milk proteins, such as lactoferrin and α-lactalbumin, are great and balanced sources of amino acids and are functional as protein-based ingredients in food such as texture modifiers, and emulsifiers. However, these proteins are instable and can be denatured and lose their functionality during isolation, processing, or storage; moreover, light, heat and pH could also negatively affect their functionality and bioactivity. To address these proteins' instability challenges, we apply strategies including coacervation, complex formation, nano- and microencapsulation to maintain the native structure and functionality. Our approaches provide a platform to co-deliver milk proteins and other sensitive bioactives in food matrix. I will discuss these strategies to develop dairy-based value-added ingredients for use in food products and supplements.

Key Words: encapsulation, lactoferrin, coacervation

2257 Ingredient technologies to address technical challenges with high protein dairy beverages. J. K. Amamcharla*, *Kansas State University, Manhattan, KS.*

Protein enriched beverages are the most sought out commodity among the US consumers according to a recent study. Milk protein-rich ingredients such as milk protein concentrate (MPC) and casein enriched ingredients (milk casein concentrate) are preferred choice of ingredients used in the beverage formulation due to their rapid digestive and absorptive properties. However, complications and mode of failure during thermal processing and storage should be considered when developing a beverage with increased protein content. Increased protein-protein interactions in high-protein beverages can pose challenges during processing and also can lead to defects like sedimentation and age gelation during storage. Recent studies have shown that reducing the calcium ion activity in MPC, either by addition of calcium chelators or by partial demineralization during ultrafiltration can help in improving heat stability and storage stability of high protein dispersions. In a study, beverages formulated using 20% reduced calcium MPC resulted in improved storage stability compared with control MPC and 30% reduced MPC beverages. In addition to milk protein enriched ingredients, other ingredients such as chelating agents and hydrocolloids used in the formulation also influence the heat stability and storage stability of the finished product. Cyclic polyphosphates such as sodium hexametaphosphate (SHMP) can interact with caseins due to its homogeneous distribution of 6 negative charges. In excess SHMP conditions, it can lead to calcium–casein phosphate complexes. The primary role of hydrocolloids in the dairy beverages was to stabilize the proteins in the beverage and delay storage defects like sedimentation. Understanding the role of calcium, caseins, hydrocolloids, and SHMP as a chelator improves our understanding of the changes in the physical and functional properties of high-protein dairy beverages during storage.

Key Words: high protein ingredients, processability, storage stability

2258 Selecting ingredients to perform in dairy beverages—Tools and techniques. H. Zheng*[1] and C. K. Yeung[2], [1]*North Carolina State University, Raleigh, NC,* [2]*California Polytechnic State University, San Luis Obispo, CA.*

Dairy beverage is a product category consisting of a range of diversified food structures and textures. From colloid science perspective, dairy beverages may be categorized into 3 types of systems including solution, colloids, and suspensions. Therefore, materials of interest which determine the physical/phase stability and (sensory) textural attributes of the processed consumer products may include the followings: 1) soluble materials such as dissociated cations/anions, lactose, proteinaceous (monomer) molecules; 2) mesoscopic sized colloidal particles including insoluble minerals, protein complexes (e.g., casein micelles and/or microparticulated whey protein aggregates), and oil droplets; 3) larger insoluble materials with great phase separation potential [e.g., fruit pulp particles and large micro-gel particles (>1 μm). Understanding ingredient functionality is crucial to both ingredient development and applications for consumer foods. Different analytical instruments/techniques have been reported in the literature for characterizing ingredient functionalities and many of those have been widely adopted by beverage manufacturers, however, selecting/utilizing a technique for generating meaningful results for quality control or problem solving purposes is often a challenge. The current presentation outlines a range of useful techniques available to the dairy industry based on related colloidal interaction theories. Rational selections of tools are justified by example-based elaboration of the nature of a functionality or the mechanism of a technical problem. Techniques for evaluating size/structure, colloidal stability (i.e., thermodynamic and kinetic stability), interfacial behavior, and bulk rheology/texture are discussed in details, covering different size scales with a focus on mesoscopic scale. The scope of the presentation focuses on dairy ingredients with relevant side considerations of non-dairy ingredients; the highlights are with recent developments of techniques/methods for mining more novel ingredient functionalities.

Key Words: ingredient functionality, dairy beverage, dairy chemistry

2259 Fermentation 2.0—Novel dairy beverages. S. D. Alcaine*, *Cornell University, Ithaca, NY.*

Dairy by-products, like permeates from milk and cheese and whey streams from acid-set cheeses and strained yogurt, are considered burdens to dairy foods processors, requiring costly disposal. These by-products, however, are rich in nutritionally important carbohydrates, minerals, and vitamins. The use of fermentation, enzyme, and filtration technologies, in solo and unison, can allow us to retain the unique benefits of dairy, like bioavailable electrolytes and vitamins, while resolving traditional hurdles, like lactose intolerance. With these platforms we can transform these undervalued streams into value-added beverages and ingredients that can compete in economically important beverage categories that dairy currently does not have a significant presence in. In this talk we highlight research on the use of yeast-based fermentations, like *Brettanomyces claussenii* and *Saccharomyces cerevisiae*, that can be leveraged to create multiple consumer products such as beer-like alcoholic beverages, non-alcoholic kombucha and sparkling water-like beverages, and specialty powders. Commercial examples of these dairy by-product-based beverages and foods are starting to the hit the market, leading the way in to grow new categories for the dairy industry.

Key Words: fermentation, beverages, dairy by-products

Dairy Foods 1: Cheese

2260 Effect of ultrafiltered milk and calcium reduction on the properties of cream cheese.

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The manufacture of cream cheese using ultrafiltered (UF) milk reduces acid whey production and can increase yield and productivity but less is known about the biochemical, microstructural and functional properties of the resulting cheese and whether calcium reduction can improve cheese properties. UF milk was concentrated 2.5 or 5-fold by volume using skim milk or 2% cation resin-treated skim milk, and then blended with cream to a protein-to-fat ratio of 0.29 ± 0.02, generating 4 cream cheese milk (CCM) preparations: CCM2.5, CCM5, resin-treated CCM2.5 and resin-treated CCM5; the control prepared from skim milk was denoted CCM1. The homogenized milk blend was cultured with lactic acid bacteria to a pH of 4.6–4.7 and further processed into cream cheese. Two independent cheese-making experiments were performed for each treatment. A 2-way ANOVA was used to analyze the effect of milk concentration factor and resin treatment on the chemical composition, textural, rheological and microstructural properties of the cream cheeses. The UF cheeses had a higher calcium content (~146 mg/100 g, ~225 mg/100 g, ~119 mg/100 g, and ~176 mg/100 g for CCM2.5, CCM5, resin-treated CCM2.5 and resin-treated CCM5 cheeses, respectively, vs. ~91 mg/100g for the control) but did not differ in protein (~9%), fat (~33%), moisture (~52%) and pH (4.7–4.8). An increase in concentration factor decreased the cream cheese hardness at 10°C and storage modulus at 40–80°C and led to a more heterogeneous and porous microstructure, providing new insights into the link between the microstructure and functional properties of UF cream cheese. Calcium reduction by resin treatment (~20%) did not significantly affect the texture and rheological properties of the cheese but the size of the corpuscular structures within the microstructure increased. The concentration of free amino acids and peptides increased in the UF cheese but reduced with calcium reduction. Acid whey generation was also reduced from ~70% (wt/wt) of total input mass for CCM1 cheese to ~45% (wt/wt) and ~23% (wt/wt) for cream cheeses made from 2.5- and 5-fold retentate respectively. This study improves our understanding of the structure and properties of cream cheese produced with UF retentate with differing calcium concentration and this knowledge may benefit future industrial production.

Key Words: cream cheese, membrane ultrafiltration, texture

2261 Impact of type of acid used for pre-acidification on calcium balances and functionality of low-moisture part-skim Mozzarella made from high casein milk.

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Pre-acidification (PA) of cheese milk is commonly used during low-moisture part-skim (LMPS) Mozzarella manufacture, especially when using high casein (CN) milk, to dissolve excessive colloidal calcium phosphate (CCP) associated with CN. The amount of CCP dissolved and lost from curds at various steps during cheese make is not precisely known and different types of acids used for PA can influence total (final) and insoluble calcium (ICa) content in cheese. Prior work investigated the impact of PA in unconcentrated milk for LMPS. Four vats of LMPS Mozzarella, with control (CL)-no PA and others PA to pH 6.00, with acetic (AC), citric (CT), carbonic (CB) acids, were manufactured from 4% CN milk (n = 5), with similar pH values at whey drainage and milling. Multiple comparison of means ($\alpha = 0.05$, Tukey test) were used for statistical analyses. All cheeses had similar composition except for CB cheese, which had a lower fat-in-dry matter ($P < 0.05$). During cheese manufacture, CT curd at end of whey drain had ICa of 16.3 mg/g protein compared with CL, AC, CB curds which had 20.7, 20.5, 19.9 mg Ca/g protein, respectively, as measured using a water-soluble calcium method. There were differences ($P < 0.05$) in total and ICa content between cheeses during storage. At 2 wk, total and ICa content (mg/g protein) of CL, AC, CT, CB cheeses were 26.5, 24.1, 20.6, 24.6 and 20.2, 18.1, 14.5, 19.1, respectively. The pH values were similar between all cheeses (5.15–5.20) except for CT cheese which had slightly lower pH value (5.06). During storage, PA cheeses had slightly higher proteolytic breakdown as compared with CL. The performance of cheeses on pizzas were assessed using Sensory Spectrum and quantitative descriptive analysis (8 panelists). At 1 and 3 mo. of storage, CT cheese had more flow-off crust, lower first chew hardness, and higher cohesiveness values due to its lower ICa content compared with other cheeses. Type of acids used for pre-acidifying concentrated cheese milks has a major influence on ICa content and functional performance of LMPS Mozzarella.

Key Words: low-moisture part-skim Mozzarella, high casein milk, pre-acidification acids

2262 Evaluation of plant proteases as rennet alternatives source in cheese development.

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A dairy product that has undergone fermentation, cheese has several health benefits. The cost of making cheese is increased in Pakistan because rennet is imported to cause milk to coagulate, while the cheese sector is still in its infancy. One ethical concern is whether rennet is produced using halal or haram sources. The goal of the current study was to assess plant extracts of *Citrus aurantium flowers extract* (CAFE), bromelain (from pineapple), ficin (from fig latex), and melon juice as a viable halal and cost-effective commercial sources for cheese production, and to compare their activity with rennet. Buffalo milk was used for manufacture of miniature fresh cheese. The starter cultures (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) was added to all cheese samples at rate of 2%. The 2 control cheese samples were made from rennet and acid and compared with cheese made from plant extracts (30 to 50 mL extract for 1 L of milk at 35–55°C). The miniature cheese samples were evaluated for physicochemical parameters and sensory analysis with 3 replicates at 28 d of storage period. The results were analyzed by 2-way ANOVA and Duncan's multiple range test with SAS 9.1 statistical software. Results showed that no significant differences were observed in moisture, fat and salt contents but a slight difference in pH (5.49 ± 0.07 for acid, 5.51 ± 0.13 for rennet and pH range of 5.52 to 5.50 for plant extracts was recorded. The protein content was observed during the ripening period. The higher protein content (25–29%) in cheese made with plant extract at ripening stage contributed to the significant flavor and aroma development due to higher protein contents (20 to 35 mg/mL in floral stage of a plant) of plant extracts. The significant change in protein was due to the presence of higher protein contents in plants which also depends on plant type, protein content, extraction method of extract, and processing conditions which ultimately enhanced the protein contents of the product. The rennet coagulated cheese showed a “creamy,” “salty,” “fatty’ and “slightly sour” taste and acid showed an acidic and less creamy taste with no bitterness. While cheese coagulated with bromelain, CAFE and melon showed “low sour,” “sweet,” ‘buttery” and “high salt” taste. Cheese with fig latex and CAFE showed “bitter “and “umami” tastes. These vegetative extracts have high protein contents and enhanced the nutritional aspects. It provided an economical, easily available, new innovative, and halal source of coagulant to be introduced in cheese production technology.

Key Words: plant protease, vegetative coagulant, rennet replacer

2263 Standardization and characterization of a pressed cheese soaked in wine. D. Olmos*, E. Dormedy, M. Pedroza, and C. Licon, *Fresno State University, Fresno, CA.*

Cheese and wine are 2 iconic US products that can be blended for creating additional value and reinforcing regional identities. Nevertheless, literature characterizing the production process is scarce. The aim of this study was to evaluate the effect of wine soaking on the microbiological, physicochemical, and sensory properties of a Manchego-style cheese. Two batches of cheese were made using Cabernet Sauvignon (W1) and Alicante Bouschet (W2) wines plus 2 control batches (C1 and C2) in duplicate. A uniform wine soaking time of 8 d was adopted and all cheeses were analyzed after 3, 30, and 60 d of ripening. Fat, protein, salt, and moisture content (%) were measured using a Food Scan (Foss Electric), and microbiological analysis included Total Aerobic Counts, *Escherichia coli*, Lactic acid bacteria, Yeast, and Mold. Sensory evaluation included color, aroma, texture, overall flavor, and hedonicity using line scaling, and salt level using a just-about-right scale. Volatile compound analysis was done by SPME-GC-MS only after 60 d. Analysis of variance was used to analyze differences between wine treatments and ripening times. Results showed significant differences in protein and salt content ($P < 0.01$) between wine and control cheeses, while significant differences were observed ($P < 0.002$) for moisture, protein, and fat content ($P < 0.002$) relative to the sampling times. Microbiology analysis found only significant differences ($P < 0.046$) for lactic acid bacteria between d 3 and 30. Sensory evaluation showed significant differences ($P < 0.001$) between wine and control cheeses for all attributes except texture, resulting in W1 sample as the best overall liked, and highest score in flavor and smell. Volatile analysis showed ethyl esters as characteristics in W2 but not in the controls. In conclusion, wine did not affect the ripening of the cheese or microbiology; Cabernet Sauvignon wine is suggested to use as soaking agent.

Key Words: cheese, wine, ripening

2264 Cheese properties during ripening of milk collected from lactating dairy cows fed lipid supplements of varying fatty acid composition. M. Blouin*[1,2], M. Landry[1,2], I. Bennis[3], J. Larouche[1,2], É. Paquet[1], P. Y. Chouinard[1,2], R. Gervais[1,2], G. Brisson[1,2], and J. Chamberland[1,2], [1]*Université Laval, Québec, QC, Canada,* [2]*STELA Dairy Research Center, Institute on Nutrition and Functional Foods (INAF), Québec, QC, Canada,* [3]*Institut Agro Rennes-Angers, France.*

Feeding palmitic acid-enriched supplement to dairy cows helps support the high energy demand of lactation and modifies milk fatty acid (FA) composition. The first animal trial of this study reported that this practice had a limited impact on the cheese-making process when milk is standardized to a constant casein-to-fat ratio. The objective of this second phase was to explore if the FA composition of lipid supplement fed lactating cows (milk FA modification) affects the cheese properties when lipolytic bacteria such as *Propionibacterium freudenreichii* are used for ripening. Five maasdam-type cheese-making trials were performed in which milks were collected from pairs of multiparous Holstein cows randomly allocated to one of 3 dietary treatments: basal diet containing no supplemental fat (CTL), or 2% added as either palmitic acid-enriched supplement (PA) or soybean oil (SO), on a dry matter (DM) basis. For each trial, after 18 d of treatment, milk from 4 consecutive milkings was collected from each cow and pooled in refrigerated tanks. Milk was transferred to a pilot plant, where it was pasteurized, skimmed, and standardized to a constant fat-to-protein ratio of 1.0 before cheese processing (10-L vats). Data were analyzed as a randomized complete block design, with trial as a random effect. Cheese moisture (44.6 ± 1.2%; mean ± SEM), fat in DM (46.4 ± 2.2%), and moisture in nonfat substances (59.5 ± 2.1%) were similar among treatments ($P > 0.15$). Treatments had no effect ($P > 0.39$) on cheese yield (11.2 ± 0.4%), and 45% moisture-adjusted yield (11.3 ± 0.6%). After 10 d at 8°C, no difference was seen ($P > 0.65$) on cheese firmness (texture profile analysis) (374 ± 54 gf) and free FA content (0.78 ± 0.12 mEq/100 g of cheese fat). Results suggest that cheesemaking performance of milk standardized to a constant fat-to-protein ratio and cheese properties at pre-ripening are not affected by the changes in the milk FA profile by lipid supplements. More analysis have to be conducted to understand the effects of treatments on cheese properties and flavor development during ripening.

Key Words: cheese ripening, lipid supplement

2265 Pilot-plant production of natural Cheddar cheese supplemented with dairy-based bioactive peptides: Peptide retention and inhibition of angiotensin converting enzyme. B. V. Iesalnieks*[1], R. A. Ibáñez[2], J. J. Jaeggi[2], B. W. Bolling[1], and J. A. Lucey[1,2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Center for Dairy Research, Madison, WI.*

Dairy-based bioactive peptides (BP) could have positive impacts on human health, including antihypertensive effects by inhibiting angiotensin converting enzyme (ACE) activity. Supplementation of BP in natural cheese may increase bioactive properties, but previous methods have been limited to laboratory-scale, leading to cheeses with varying protein contents. The objective of the current study was to produce natural Cheddar cheese at pilot-plant scale with the addition of a bioactive dairy protein hydrolysate (DPH, 80% crude protein wt/wt) without altering cheese composition. Cheeses were produced in 2 vats of milk (226 kg milk/vat) with different casein (CN) to fat ratios (Vat 1 CN:fat 0.73:1.00; Vat 2 CN:fat 0.69:1.00, n = 4). Fresh curd was equally divided and the DPH was added during curd dry-salting [Vat 1: 0 (Control) and 1.8%; Vat 2: 0 (Control) and 3.6% (wt/wt) curd]. Press whey was collected during the first 5 min of pressing to determine the retention of BP in cheeses. After manufacture, cheeses were ripened at 4°C for 6 mo. All cheeses met the standards of identity for Cheddar (<39% moisture; >50% fat-in-dry matter). ANOVA showed that increasing fat content of milk led to cheeses with similar composition and pH as levels of added DPH increased ($P > 0.05$). The concentration of crude protein in press whey from DPH treated cheeses increased by 15%, suggesting losses of 55% DPH during pressing ($P < 0.05$). Water soluble extracts (WSE, < 3kDa) of treated cheeses showed more diverse peptide profiles than those of control cheeses after 1 d of ripening when tested by reversed-phase high performance liquid chromatography. In addition, WSE of DPH treated cheese increased ACE inhibition up to 28% more than control cheese WSE ($P < 0.05$). While DPH retention decreased during pressing, adequate concentrations were retained in supplemented cheeses to qualitatively alter the WSE peptide profile and increase ACE inhibition at 1 d ripening. Further research will investigate different DPH treatment levels and the effects of cheese ripening on ACE inhibitory properties.

Key Words: cheese, whey, peptide

2266 Improving the functionality of frozen and superchilled shredded cheese during extended storage. P. M. Eberly*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], R. A. Ibáñez[2], M. E. Johnson[2], and J. A. Lucey[1,2], *[1]University of Wisconsin–Madison, Madison, WI, [2]Wisconsin Center for Dairy Research, Madison, WI.*

For convenience in foodservice, low moisture part-skim (LMPS) Mozzarella cheese is shredded, mixed with anticaking agent (ACA) and may be frozen until needed. Past studies observed defects (e.g., clumping, fat leakage) with freezing cheese shreds, but the effects of ACAs during extended storage are unknown. This study aimed to determine the effects of different storage temperature (ST), times and ACA on shred performance (including on pizza). LMPS Mozzarella obtained from a commercial manufacturer was stored for 2 wk at 4°C, then shredded. Shreds were mixed with 4 ACAs at 2% wt/wt levels: control (no ACA), powdered cellulose, potato starch or corn starch ($n = 4$). Within each treatment, the shreds were subdivided and stored refrigerated (4°C), superchilled (−2°C) and frozen (−20°C). The composition, pH, stretchability of melted shreds (texture analyzer with an extensibility rig), and sensory properties of intact shreds and shreds baked on pizza (quantitative descriptive analysis) were tested during storage (0 d, after 1 and 3 mo). The −2°C and −20°C samples were tempered for 1 wk at 4°C before testing. Split-split-split plot design and multiple comparison of means ($\alpha = 0.05$, Duncan test) were used for statistical analyses. Moisture content and pH values were similar for all shreds during storage. Stretchability was quantified through work to extend (texture analyzer), and it did not change during storage at −20°C but decreased at the other ST ($P < 0.05$). ACA and ST affected the shred and pizza properties of the cheese. Using ACA and lower ST reduced matting. When baked on pizzas, adding ACA increased blister quantity (BQ) and blister color (BC) but decreased free oil (FO) compared with control when baked on pizzas. Cellulose reduced FO the most; however, this ACA had the highest BQ and BC values ($P < 0.001$). BQ and BC gradually increased during storage at 4°C and −2°C ST but did not change at −20°C for all ACAs. Overall, freezing with a 1-wk thaw time maintained the functionality of cheese shreds during storage. Cellulose was effective in reducing defects such as matting and FO with the downside of intensified BQ and BC.

Key Words: shredded cheese, anticaking agents

2267 Continuous microwave-assisted extrusion for high moisture texturized foods. B. Graf*, F. Schmidt, C. Kern, and J. Hinrichs, *Institute of Food Science and Biotechnology, Department of Soft Matter Science and Dairy Technology, Stuttgart, Baden-Wuerttemberg, Germany.*

High moisture extrusion (water content >40%) is well suited to texturize proteins. Texturization involves the application of thermal treatment in combination with mechanical stress. A thermo-mechanical treatment can be achieved by a waterless single screw extruder with double-jacketed hot water cycles as heat sources. However, upscaling of such an extruder faces challenges like complex material composition and uniform heat distribution. The latter is required for proper texturization but is hindered within upscaling using indirect heating because the volume increases in third power, whereas the area only increases quadratically. Microwave technology with its volumetric heating shows high potential for upscaling applications of an extruder. It offers high efficiencies, the possibility to use green energy sources, and is hypothesized to maintain a homogenous temperature distribution throughout the material. The aim of this study was to present a process design for microwave-assisted extrusion in technical scale. A technical scale set-up for microwave-assisted extrusion of high moisture, texturized food systems is demonstrated. A cheese model food system has been plasticized in duplicate by continuous microwave extrusion. The product outlet temperature and chemical composition have been investigated with varying screw speeds ($n = 10, 15, 20$ rpm) and power inputs ($P = 0.8$–1.4 kW). Finally, theoretical considerations evaluated the potential for scale-up. A preliminary stationary study resulted in specific microwave tuner positions, giving an area of desired process parameters. This area was successfully transferred to a continuous product application, where desired gel-sol transition ($\vartheta_{\text{Gel-Sol}} = 56$–58°C) of the cheese model food system was achieved. With increasing power input, the product temperature increased, while it decreased with increasing screw speed and constant power input. The fat, protein, and dry matter content of the cheese model system did not differ significantly throughout all trials. Considerations for scale-up showed that microwave-assisted extrusion is a promising approach for industrial scale applications. Mass flows of up to 2,000 kg/h with microwave powers up to 86 kW ($f = 915$ MHz) and screw diameters of 236 mm are realizable.

Key Words: casein-based gel, gel-sol transition, scale-up

2268 A survey of biogenic amines and amine oxidase activity in commercial cheese. J. Larsen*[1], R. Ibáñez[2], J. Lucey[1,2], and M. Johnson[2], *[1]University of Wisconsin–Madison, Madison, WI, [2]Wisconsin Center for Dairy Research, Madison, WI.*

Biogenic amines (BA) are low molecular weight organic bases that may negatively impact organoleptic qualities as well as trigger allergic-like reactions upon consumption. BA are generally formed in fermented food products from free amino acids by microbial amino acid decarboxylase enzymes. Amine oxidases are enzymes with the capacity to degrade BA. There are 2 classes of amine oxidase enzymes, monoamine oxidase (MAO) and diamine oxidase (DAO). Bacteria containing amine oxidase enzymes have been successfully used in other food systems to degrade BA. The impact of amine oxidase enzyme activity on the concentration of BA in cheese is unclear. This study aims to determine levels of BA and amine oxidase activity in 79 commercial cheeses of several varieties (from the US and other countries) to establish a relationship between them. The levels of BA in cheeses were quantified by reversed-phase high performance liquid chromatography (RP-HPLC) following purification by solid phase extraction (SPE) and derivatization with dansyl chloride. Amine oxidase activities of water-soluble cheese extracts were assayed fluorometrically using enzymatic kits for MAO and DAO. Total BA content in surveyed cheese samples ranged from 4 mg/kg to 2447 mg/kg, with a mean concentration 217 mg/kg total BA. (Listed in descending order of occurrence) histamine, tyramine, spermine, spermidine, cadaverine, putrescine, tryptamine, and 2-phenylethylamine were detected. 83% of surveyed cheeses exhibited DAO activity, 39% exhibited MAO activity, and 29% demonstrated both MAO and DAO activity [maximum 43 mU/g cheese]. No significant ($P > 0.05$) Pearson correlation could be established between amine oxidase activity and BA content in surveyed cheeses. Principal component analysis (PCA) showed that activity of MAO and DAO was related to reduced levels of total BA in cheese. Further study is required to determine the relationship between amine oxidase activity and BA content in cheese, and future work will investigate the impact of amine oxidase-positive bacteria on flavor as well as their ability to degrade BA in a cheese system.

Key Words: biogenic amines, enzyme activity, cheese quality

2269 Effect of stretching temperature on alkaline phosphatase in the raw-milk pasta-filata cheese manufacture.

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Alkaline phosphatase (ALP) used to check possible milk heat treatment in raw-milk cheeses could be inactivated due to the use of hot water or scotta-whey (>80°) during stretching. To this purpose, an experimental trial was carried out, in triplicate, using 3 different thermal treatments of "scotta"-whey (74.5 (T70), 83.6 (T80), and 90.2°C (T90)) for the stretching phase of traditional "Caciocavallo" pasta-filata cheese. For each thermal treatment, the stretching was carried out in a thermal tank and 6 block cheeses were produced and sampled at different stages (after stretching, 4h, after brine, 30d, 60d, and 90d) for ALP determination using a fluorimetric method, both outside and core portions. Data on ALP activity were analyzed with JMP 16 software (SAS 2022) using a GLM mixed model to test the effect of the temperature. Starting from the acidified cheese curd (ALP value 1475.6 mU/g), both T80 and T90 curds had ALP activities significantly lower ($P < 0.0001$) after stretching, compared with T70 treatments (3.84, 5.94 and 1,305.1 mU/g respectively). In all remaining stages and for all the 3 thermal treatments, there was a drastic reduction on ALP activity (>99%). Moreover, in T70 treatment there was a significant location effect ($P < 0.001$), with a greater reduction in the core portion compared with the outside in 4h cheeses (11.23 vs. 88.80 mU/g). Thus, even with the use of raw milk, ALP values found in the pasta-filata cheese in all treatments seem to be comparable to ALP values reported in literature for pasteurized-milk cheeses (<10 mU/g).

Key Words: alkaline phosphatase, raw-milk, pasta-filata cheeses

2270 Relating consumer preferences to descriptive visual stringiness attributes of string cheese.

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Consumers expect String cheese (SC) to peel apart, therefore, stringiness is a main performance indicator for SC. We developed 2 descriptive sensory scales for analyzing the visual stringiness (VS) of SC during storage: feathering (F) and fiber quantity (FQ). Our objective was to understand how VS results relate to consumer preferences and use that information to determine a consumer acceptance range (CAR) for both attributes. We conducted a consumer panel using 4 major retail SC samples, Brands A-D. Adult participants (n = 97) evaluated quantity of fibers and firmness (5-point, Just About Right scale), assessed overall liking and likelihood to purchase (9-point hedonic scale), ranked the samples, and indicated whether the SC met their expectations. Our trained descriptive panel also evaluated VS of the 4 SC samples, and cheese composition was analyzed. Statistical analysis was performed using paired, 2-sample *t*-tests ($P < 0.05$). Brand B scored very highly and met expectations of 93% of participants; a few said it was too stringy. Brands C and D performed slightly lower than Brand B, but similarly to one another, however Brand C had slightly lower quantity of fibers than Brand D. Brand A scored the lowest overall and only met 46% of the consumers' expectations, likely due to an off flavor and pasty texture. There were compositional differences between the SC brands, however it was unclear how the composition impacted the consumer's preferences. Brands A and C had the lowest VS scores (F~1, FQ~2 of 15 pts) from the descriptive panel, Brand D had slightly higher scores (F~2, FQ~3.5), while Brand B had the highest (F~6, FQ~6.5). Considering the similar acceptance of Brands C and D and that they met many of the consumers' expectations (75 and 80%, respectively), Brands B-D were used for developing the CARs for the stringiness attributes, F and FQ, based on the descriptive results. The CARs for F and FQ were set at 2–6 pts and 3–7 pts out of 15 (scale), respectively. Application of these CARs to our previous work helped to interpret our descriptive VS results in terms of likely consumer acceptance.

Key Words: string cheese, consumer panel

2271 Evaluating impact of adding capsular and exo polysaccharide producing cultures in a model Mozzarella cheese system.

S. Pant*, T. Oberg, and P. Sharma, *Utah State University, Logan, UT.*

Microbial polysaccharide (PS) is currently used in modern food manufacturing and studies have shown that PS deliver interesting technological properties such as water retention and viscosity leading to improved yield and functionality of cheese. In this study, PS producing bacterial cultures were evaluated in a model cheese system to understand the functionality imparted by microbial PS to pasta filata cheese. Two commercial *Streptococcus thermophilus* cultures which produce capsular- and exo-PS were selected for the study. A non-PS producing *S. thermophilus* strain (DM10) was used as a culture negative control. The strains were grown in M17+2% lactose media to a concentration of 10^8 cfu/mL. The PS was then concentrated by heating the cells to 90°C for 15 min followed by cold centrifugation. A model cheese system was used where curd was produced and collected before the pasta filata process then flash frozen. The frozen curd was thawed for 5 d, and the extracted PS was hand-mixed at controlled temperatures (80°C) to mimic the pasta filata process at benchtop scale. Extracted PS was added at 0.3% and 0.5%. A starch sample (same levels) was prepared as a reference for functionality along with a negative control of curd only. Samples were tested for free oil (Babcock), Schreiber Melt, expressible serum, stretchability (Texture Analyzer) and viscoelastic properties (Oscillatory Rheometer). The testing was run in triplicate and analyzed using *t*-tests. The free oil and Schreiber Melt results showed that samples containing starch and capsular PS released less free oil and exhibited higher melt resistance. This can be attributed to increased oil binding by starch or capsular PS. The amount of work (force) needed to stretch the cheese to a specified height was less than control when exo-PS was added to the cheese at both levels and the lowest when capsular PS was added at 0.5%. Exo-PS showed the highest Loss Factor of all samples for the viscoelastic parameters when added at 0.5%, which is indicative of the melting properties of cheese. These results suggest that PS producing culture can be used for modifying functionality of Mozzarella cheese.

Key Words: polysaccharide, exo, capsular

Joint NMC (National Mastitis Council) and ADSA Lactation Biology Symposium: Unlocking the Potential of the Bovine Mammary Gland—Recognition of the Contribution of ADSA Fellow Mike Akers

2272 Physiological regulation of lactogenesis and early lactation: Implications for milk and colostrum production. T. B. McFadden*, *University of Missouri, Columbia, MO.*

The mammary gland is a truly remarkable organ. As the final stage of successful reproduction in mammals, lactation has proven to be a highly successful adaptation and reproductive strategy. Indeed, the Cenozoic Era, spanning the past 65 million years to the present, has been dubbed the Age of Mammals. As befits the wide range of mammalian species, mammary glands and milk vary tremendously in function and composition. Thus, lactation physiology presents a rich tapestry through which to explore evolutionary biology and function. In terms of biological limits, modern dairy animals continue to raise the ceiling of perceived limits to productivity. At the start of my career, training under Mike Akers at Virginia Tech in the late 1980s, we spoke of cows producing 30,000 lbs of milk in a lactation as a reach goal. Yet, over the past several years the world's top cows have produced over 75,000 lbs in a single lactation. Across all of this variation, many of the key regulatory features of lactogenesis and lactation are common among mammals and these have been described by researchers such as Mike Akers, among many others. The aim of this presentation is to review some of the range of lactation physiology, the core regulatory features, and opportunities to improve milk composition and productive efficiency, with emphasis on contributions by Dr. Akers and his collaborators.

Key Words: lactation, lactogenesis, milk

2273 Foundational studies on the role of nutrition on prepubertal mammary growth and development. K. Sejrsen*, S. Purup, and M. Vestergaard, *Aarhus University–Viborg, Department of Animal and Veterinary Sciences, Tjele, Denmark.*

Observations of a positive relationship between body weight at calving and milk yield suggested that age at calving can be reduced without negative effect on milk yield if weight at calving is maintained. This was investigated in several experiments, including studies using identical twins in US and Denmark. Surprisingly the high feeding level required to lower age at puberty and obtain a normal weight at calving resulted in a dramatic reduction in subsequent milk yield. This was supported in subsequent Danish experiments. Scrutiny of the data indicated that the effect mainly was due to the high feeding level in the early part of the rearing period coinciding with the period of allometric prepubertal mammary development. A later study confirmed that high feeding level reduced growth of mammary parenchyma before puberty but not after. The results also showed a positive relationship between circulating level of growth hormone and prepubertal mammary growth. A significant relationship with prolactin disappeared after correction for growth hormone. The importance of growth hormone for prepubertal mammary growth was supported by a positive effect of exogenous growth hormone. However, a role of circulating levels of growth hormone in mediating the effect of level of nutrition is questionable because GH receptors are absent in mammary tissue and IGF-1 – the main mediator of GH action – is increased not decreased by increasing level of nutrition. Furthermore, the response of serum on primary cell cultures depends on IGF-1 and not GH content. So, why is prepubertal mammary growth decreased not increased by high feeding level? This is largely still an open question but using primary cell cultures from parenchymal tissue has added some insight. Adding mammary parenchymal extract from heifers fed high feeding to the primary cells stimulate cell proliferation less than extract from heifers fed moderate feeding level despite a higher content IGF-1. Evidence suggest that inhibitory effect of locally produced IGFBP-3 is involved, but a role for other locally produced factors cannot be excluded.

Key Words: mammary growth, nutrition, epithelial cells, heifers, IGF-1

2274 Nuances of pre-pubertal mammary gland development and the role of nutrition. H. L. M. Tucker*, *Novus International Inc., Saint Charles, MO.*

The prepubertal period establishes the foundation of the mammary gland structure for a heifer. At the macro level the mammary gland is separated into parenchyma and the mammary fat pad that will eventually merge. At the micro level there are many cell types and structural proteins that eventually develop to support production of milk. The integration of parenchyma into the mammary fat pad results in numerous coordinated cellular interactions that must occur during growth and development of the gland. These cells and proteins are heavily influenced by hormones deriving from the ovaries despite occurring before estrus onset. When heifers are ovariectomized, mammary gland mass decreases resulting in increased estrogen receptor 1 positive cells, change in number and morphology of myoepithelial cells, and decrease in proliferating cells. Ovariectomized heifers also have an abundance of change in the extracellular matrix where fibronectin is higher while laminin is lower. With an ovariectomy, the disruption of the complicated web of interactions between different hormones makes it difficult to pinpoint specific hormonal actions. More specifically, when estrogen is blocked during the prepubertal period a reduction in size of the overall gland results, coinciding with a reduction in protein expression of estrogen receptor 1 and number and size of myoepithelial cells. The stromal tissue is influenced by increased collagen deposition and an increase in number of putative epithelial stem cells observed. Moreover, there is still the added complication of systemic versus local influences of hormones that are difficult to distinguish. Beyond the influence of hormones on the mammary gland, nutrition can also elicit an effect. Studies have shown through elevating different nutrients the macro level of the mammary gland can be influenced, which results in an impact on the micro level of the gland. With a wide range of feeding practices during the prepubertal period across the industry the influence of nutrition is of importance for the future productive life of the cow.

Key Words: mammary development, myoepithelial, mammary epithelium

2275 Contributions of the mammary physiologist to the mastitis researcher. B. D. Enger*, *Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.*

Mastitis is considered to be the most common and expensive disease in the US and global dairy industries. Significant advancements over the past 50 to 70 years have markedly reduced the occurrence and the negative physiological and financial consequences of mastitis. Many of the key advancements that have resulted in better prevention, treatment, and an understanding of mastitis have arisen from key contributions of mammary gland physiologists. Central among these contributions is the mammary physiologist's unique knowledge of mammary tissue architecture and cell type organization, particularly how the heterogeneous tissue and cell types work in concert to accomplish the functional role of milk synthesis and secretion. Joint efforts by mammary physiologists and mastitis researchers have elucidated how milk secretion and composition are changed during mastitis, which is primarily a result of alterations in epithelium integrity and cellular competency. The exploitation of the compositional changes has been fruitful, allowing for the development of improved diagnostic approaches to identify affected cows and assess treatment outcomes from both a disease and physiology-based perspective. Histological evaluations by mammary physiologists have improved the immunologist's and mastitis researcher's understanding of the dynamics of immune cell recruitment and clarified the spatial localization of the recruited immune cells in tissue compartments. Joint efforts have also been successful identifying how the teat canal and teat sphincter prevent mastitis, which has improved equipment and management decisions aimed at maintaining the key defense mechanisms of the mammary gland. Continued efforts and collaborations will be important as key knowledge gaps remain regarding the pathophysiology of mammary gland inflammation and advancements are needed to determine how to better influence the immune response to desired outcomes. A better understanding of the interplay between physiology and mammary gland host defense can improve animal health and welfare and reduce the deleterious impacts of mastitis on mammary gland productivity.

Key Words: intramammary infection, inflammation, pathophysiology

2276 Understanding mammary physiology and histology: A story told in pictures.

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How do you decide a research direction or more narrowly a research question? What sparks your interest or your scientific curiosity? Maybe you need to ask, am I actually curious at all? In the end, what motivates you to be in this discipline, even in this symposium, at this moment in time? Distilled and concentrated, all our sequential professional actions are the latest in a line of questions posed and corresponding answers each of us had to conjure. I will describe some of twists and turns that I have taken in my career. What were the some of the rocks that I overturned and what led me to those particular stones? It has to be clear that like all other academics or scientists, any success that I have had rests on the efforts of my former teachers and mentors, in particular Dr. William Heald and Dr. Alan Tucker, and the many colleagues and students I have known. Without each of you, there would be no career in lactation biology to review. As a discipline, we all stand on the shoulders of those who came before us. It is often, rightly noted that many of the central questions in our branch of lactation biology, have not actually changed. Rather the tools and approaches to attempt to answer the questions have evolved. It is often repeated that a picture is worth a thousand words. Images have been an important part of my career and I will share some of those that have punctuated research over the years.

Key Words: mammary gland, research, lactation

Physiology and Endocrinology 1

2277 RNA-Seq Analysis reveals the transcriptional profile of bovine follicular wall cells treated with nerve growth factor-beta. S. Salman*[1], K. Conner[1], A. Morton[2], A. Denicol[2], P. Dini[1], D. Melo[1], T. Marques[1], and F. Lima[1], [1]*Department of Population Health and Reproduction, University of California, Davis, CA,* [2]*Department of Animal Science, University of California, Davis, CA.*

The study aimed to characterize differentially expressed genes in follicular wall cells treated with nerve growth factor-β using RNA-Seq analysis. Twelve biological replicates (wells containing follicular walls with respective granulosa and theca cells from 3 different follicles) were performed for each treatment, representing 36 preovulatory follicles. Follicular walls were cultured at 38.5°C in a humidified incubator gassed with 5% CO2:95% air until reaching 80% confluence. The follicular wall granulosa and theca cells were treated with either phosphate buffer solution (Control, n = 12); or 100 ng/mL of purified NGF (NGF, n = 12). Media was withdrawn (75%) and replaced with fresh media at 6, 24, 48, and 72 h of culture, and at the end of the 3 d, follicle cells and media were collected separately, flash frozen, and stored at −80°C for RNA extraction and RNA-Seq. RNA-Seq data with the main effects of treatment were analyzed with DeSeq2 in R, and genes were considered differentially expressed when adjusted $P < 0.05$. A total of 19,234 genes were identified, with 18 differentially expressed between NGF and control. The mRNA expression of key steroidogenic Hydroxyl-delta-5-steroid dehydrogenase 3-β (HSD3B), Hydroxysteroid 17-β dehydrogenase (HSD17B), 17a-hydroxylase/17e20 lyase (CYP17A1), Aromatase (CYP19A1) was similar. The luteinizing hormone receptor (LHCGR) was upregulated ($P < 0.05$) for NGF, but the follicle-stimulating hormone receptor (FSHR) was similar. The mRNA expression of angiogenic markers was upregulated ($P < 0.05$) in VEGFR1 and FGF2 for NGF. The top differentially expressed genes also included NGF upregulation of SNCAIP (synuclein α interacting protein) and ESAM (endothelial cell adhesion molecule), and NGF downregulation of RAD51 recombinase, a gene involved in halting cell death. RNA-Seq analysis revealed that NGF increased mRNA abundance of LH receptor and angiogenic markers ESAM, VEFGR, and FGF2. The results suggest that NGF may support luteinization in cattle.

Key Words: angiogenesis, theca cells, granulosa cells

2278 Kidney function and nitrogen excretion in Brown Swiss and Holstein dairy cows. E. C. Kessler, R. M. Bruckmaier, and J. J. Gross*, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland.*

Brown Swiss (BS) cows have higher urea concentrations in milk and blood than Holstein (HO) cows. The hypothesis was tested that BS and HO cows differ in kidney function and nitrogen excretion. Blood, saliva, urine, and feces were sampled in 31 pluriparous BS and 46 HO cows kept under identical feeding and management conditions. Samples were collected at different lactational stages after the monthly DHIA control test-day. Concentrations of urea and creatinine were measured in serum, urine, and saliva. Symmetric dimethylarginine (SDMA) was determined in serum, which is an established indicator for glomerular filtration rate (GFR). Feces were analyzed for dry matter content and nitrogen concentrations. Data on milk urea and protein concentrations, and daily milk yield were obtained from the monthly DHIA test-day records. The effects of breed, time, and parity number on blood, saliva, urine, feces, and milk parameters were evaluated with the GLM procedure with breed, time, and parity number as fixed effects. Differences between BS and HO were assessed by the Tukey-corrected *t*-test at $P < 0.05$. Concentrations of urea, creatinine, and SDMA in serum, were higher in BS than in HO cows ($P < 0.01$) with 5.46 ± 0.19 vs. 4.72 ± 0.13 mmol/L for urea, 105.96 ± 2.23 vs. 93.07 ± 1.50 mmol/L creatinine, and 16.78 ± 0.69 vs. 13.39 ± 0.44 µg/dL SDMA, respectively. BS cows showed greater urea concentration 25.8 ± 0.7 vs. 21.8 ± 0.7 mg/dL, and protein content in milk 3.70 ± 0.08 vs. 3.45 ± 0.07% than HO ($P < 0.01$). Urea and creatinine concentrations in urine and saliva did not differ among breeds. No differences between BS and HO were observed for milk yield, fecal DM and nitrogen content. Dry matter intake and body weight were similar in BS and HO cows ($P > 0.05$). Despite greater urea and creatinine concentrations in blood, and milk urea content in BS compared with HO, excretion of these parameters did not differ between breeds in urine. In conclusion, our results suggest a differential renal GFR between BS and HO cows.

Key Words: kidney, nitrogen, dairy cow

2279 Palmitic acid alters pyruvate carboxylase expression and carbon flux in Madin Darby bovine kidney cells. L. M. Beckett*[1], N. E. Sunny[2], T. M. Casey[1], and S. S. Donkin[1,3], [1]*Purdue University, West Lafayette, IN,* [2]*University of Maryland, College Park, MD,* [3]*Oregon State University, Corvallis, OR.*

Fatty acids regulate expression of pyruvate carboxylase (PC), which encodes a key gluconeogenic enzyme. PC increases the pool of oxaloacetate (OAA), whereas phosphoenolpyruvate carboxykinase pulls OAA from the tricarboxylic acid (TCA) cycle toward glucose production. Our objective was to determine the impact of varying ratios of palmitic (C16:0) and α-linolenic (C18:3n-3) acid on PC expression and carbon flux through the TCA cycle in Madin Darby Bovine Kidney (MDBK) cells. We hypothesized that C16:0 alone would decrease *PC* expression and decrease flux into TCA cycle, which would adversely affect the OAA pool, and addition of C18:3n-3 in the presence of C16:0 would block *PC* repression and maintain flux. MDBK cells were grown to 80% confluency in triplicate and pretreated for 21 h with: 1 m*M* C16:0 + 0 m*M* C18:3n-3, 0.75 m*M* C16:0 + 0.25 m*M* C18:3n-3, 0.5 m*M* C16:0 + 0.5 m*M* C18:3n-3, 0.25 m*M* C16:0 + 0.75 m*M* C18:3n-3, 0 m*M* C16:0 + 1 m*M* C18:3n-3, and control (no fatty acid addition). Media was replaced with [U-^{13}C] pyruvate, [1-^{14}C] palmitic acid, or [U-^{14}C] lactate media and incubated for 3 h. [$^{14}CO_2$] and acid soluble products were measured using liquid scintillation counting. Mass isotopologues of TCA cycle intermediates were measured by gas chromatography mass spectrometry and mass isotopologue distribution was calculated and standardized by DNA content of cells. Expression of *PC* mRNA was decreased due to 1 m*M* C16:0 and 0.75 m*M* C16:0 compared with control ($P < 0.05$), but other treatments were not different from each other. Enrichment of Citrate M+5 was 50% higher due to 1 m*M* C16:0 compared with every treatment ($P < 0.05$), but none of the other flux measurements were different ($P > 0.10$). Treatment with 1 m*M* C16:0 decreased lactate oxidation by 67% compared with control and 0.25 m*M* C16:0, but was not different from 0.75 m*M* C16:0 or 0.50 m*M* C16:0. [1-^{14}C] palmitic acid full and partial oxidation were unaffected by treatment ($P > 0.10$). Our hypothesis was supported that *PC* expression controls carbon flux, and that fatty acids regulate *PC* and carbon flux into the TCA cycle to support energy production.

Key Words: palmitic acid, α-linolenic acid, tricarboxylic acid cycle flux

2280 Pyruvate carboxylase overexpression increases carbon flux of the tricarboxylic acid cycle. L. M. Beckett*[1], J. Laguna[1], N. E. Sunny[2], T. M. Casey[1], and S. S. Donkin[1,3], *[1]Purdue University, West Lafayette, IN, [2]University of Maryland, College Park, MD, [3]Oregon State University, Corvallis, OR.*

Dairy cattle synthesize 90% of their glucose requirements. Oxaloacetate (OAA) synthesis is a critical step in gluconeogenesis and energy metabolism in tricarboxylic acid (TCA) cycle. Pyruvate carboxylase (PC) catalyzes the synthesis of OAA, an intermediate in the TCA cycle to affect both gluconeogenesis and TCA cycle activity. The objective of this study was to determine the impact of PC knockdown (PCKD) or PC overexpression (PCOE) on carbon flux into the TCA cycle. We hypothesized PCKD would impair and PCOE would increase carbon flux into the TCA cycle. Madin Darby Bovine kidney (MDBK) cells were transduced with shRNA that targeted *PC* to create PCKD. To generate PCOE, MDBK cells were transduced with PC lentiviral plasmids and the rtTA plasmid for doxycycline induction of expression. PC shRNA scramble (PCshRNA) served as a negative control. Respective knockdown and overexpression of PC were confirmed with RT-qPCR and Western blot analysis. Cells lines were grown to 80% confluency in 35 mm dishes in triplicate and incubated in [U-^{13}C] pyruvate, [1-^{14}C] palmitic acid, or [U-^{14}C] lactate for 3 h. Conversion to ^{14}C substrate (nmol•μg cell DNA^{-1}•3 h^{-1}) to ^{14}CO$_2$ and acid soluble products was quantified using liquid scintillation counting and found not different between the lines ($P > 0.10$). Flux of ^{13}C pyruvate was measured using gas chromatography mass spectrometry followed by mass isotopologue distribution (MID) analysis. MID was standardized to cell DNA and values of PCshRNA. Level of *PC* expression in PCOE was greater (7.5 AU) than PCKD (0.5 AU; $P < 0.05$). [U-^{13}C] pyruvate flux to Aspartate M+3 was 6 times higher in PCOE than PCKD ($P < 0.05$), and flux to Malate M+3 and Fumarate M+3 was 4 times higher in PCOE compared with PCKD ($P < 0.05$) indicating greater supply of TCA cycle intermediates that can support the OAA pool when *PC* expression is increased. These findings supported our hypothesis that increased *PC* expression increases carbon flux to OAA, and call for design of additional studies aimed at identifying potential modulators that target PC expression to alter gluconeogenesis and energy metabolism.

Key Words: pyruvate carboxylase, tricarboxylic acid cycle, metabolic flux

2281 Effects of hyperketonemia on metabolism and inflammation following an lipopolysaccharide administration in a ruminant model. S. Rodriguez-Jimenez*[1], M. A. Abeyta[1], B. M. Goetz[1], J. Opgenorth[1], A. D. Freestone[1], H. K. J. P. Wickramasinghe[1], J. A. R. D. N. Appuhamy[1], J. L. McGill[2], and L. H. Baumgard[1], *[1]Department of Animal Science, Iowa State University, Ames, IA, [2]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA.*

Objectives were to investigate the metabolic and inflammatory consequences of hyperketonemia in immune activated Holstein heifers. Using a completely randomized design, heifers (n = 16; 310 ± 23 kg BW) were enrolled in a 56 h hyperketonemic clamp and assigned to 1 of 2 i.v. infusion treatments: 1) saline and pair-fed control (CON; n = 8) and 2) β-hydroxybutyrate (BHB) solution and ad libitum-fed (KET; 2.2 *M*; NaBHB; Lesen Bio-Technology Co., Ltd.; n = 8). Blood samples were obtained every 10 min and immediately analyzed for BHB concentrations utilizing a hand-held meter (PortaCheck). Rate of BHB infusion (ROBI) was adjusted to achieve a "clamped" BHB concentration of 1.3 ± 0.3 mmol/L. After 8 h of KET, LPS (0.085 μg/kg BW) was administered i.v. to all animals. The statistical model included treatment, time, and their interaction as fixed effects, and heifer as a random effect. By design, BHB levels were increased in KET relative to CON heifers (4-fold; $P < 0.01$). Regardless of treatment, LPS bolus gradually increased circulating NEFA ($P < 0.01$). LPS acutely caused hyperglycemia but hypoglycemia ($P < 0.01$) from 3 to 12 h in both treatments. Circulating insulin increased (22-fold; $P < 0.01$) 3 h after LPS in both treatments before returning to baseline from 6 h onwards. Tumor necrosis factor-α, interferon (IFN)-γ, macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein-1 and IFN-γ-induced protein-10 markedly increased ($P < 0.01$) following LPS but were not affected by BHB infusion. LPS bolus markedly increased circulating IL-6 for both treatments in the first 3 h (32-fold) but it remained elevated in KET cows through 6 h while it progressively decreased in CON ($P = 0.06$). IL-10 (an anti-inflammatory cytokine) increased at h 1 post-LPS in all heifers, however it was more elevated in KET relative to CON (57%; $P < 0.01$). Following LPS, LPS-binding protein and serum amyloid A gradually increased in both treatments ($P < 0.01$). Circulating haptoglobin was increased 24 to 48 h after LPS-bolus but KET decreased (41%; $P < 0.01$) this response. In summary, hyperketonemia appears to ameliorate some aspects of inflammation following immune activation in ruminants.

Key Words: immune activation, ketones

2282 Effects of an intramammary endotoxin challenge on production, metabolism, and inflammation in early versus mid-lactation dairy cows. J. Opgenorth*[1], M. A. Abeyta[1], B. G. Goetz[1], S. Rodriguez-Jimenez[1], A. D. Freestone[1], R. P. Rhoads[2], R. P. McMillan[2], and L. H. Baumgard[1], *[1]Iowa State University, Ames, IA, [2]Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Objectives were to evaluate how early lactation (EL) relative to mid-lactation (ML) cows spare nutrients for immune activation and milk synthesis following an intramammary lipopolysaccharide (LPS) challenge. Multiparous EL (20 ± 4 DIM; n = 11) and ML (155 ± 40 DIM; n = 10) cows received 10 mL sterile saline with 100 μg *Escherichia coli* O111:B4 into the left rear mammary gland. The trial consisted of 2 periods (P); P1 (5 d) served as baseline during which a muscle and liver biopsy were obtained on d 1. At the beginning of P2 (3 d), LPS was administered, and cows were biopsied 12 h later. Data were analyzed with PROC MIXED in SAS 9.4. All cows became febrile following LPS, but EL had increased peak rectal temperature relative to ML (41.6 vs. 40.8°C at 5 h; $P < 0.01$). Cows had decreased DMI (14%), milk yield (MY; 26%), and energy corrected MY (24%) on d 1 after LPS but did not differ by lactation stage (LS; $P > 0.20$). LPS induced marked neutrophilia which was further increased in EL relative to ML (54%; $P < 0.01$), but the increase in haptoglobin did not differ by LS ($P > 0.77$). Insulin concentrations increased after LPS which were heightened in ML relative to EL (2-fold; $P < 0.01$), and glucose concentrations were maintained in EL but reduced in ML (0 and −4% relative to baseline, respectively; $P = 0.04$). Further, LPS decreased free fatty acids (20%) but did not differ by LS ($P > 0.92$). Metabolic flexibility, a proxy of fuel oxidation preference from pyruvate to palmitic acid, increased in EL relative to ML during P1 (25 and 44% in liver and skeletal muscle, respectively; $P \leq 0.04$). LPS reduced metabolic flexibility to 18% in both tissues regardless of LS ($P > 0.83$), and the decrease from P1 in EL liver and muscle was 49 and 40%, respectively, whereas in ML was 38 and 17%, respectively. In summary, despite exacerbated fever and neutrophilia, EL maintained similar milk production relative to ML following an intramammary LPS challenge. Further, although LPS decreased metabolic flexibility in both EL and ML cows, data suggest

EL cows conserve some glucose-sparing capacity for the mammary gland during immune activation.

Key Words: lactation stage, immune activation

2283 Inter-animal variability in systemic inflammation status in early lactation and its relation to the cow's metabolic status and reproductive performance.

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Around parturition, inflammation occurs in almost all cows. Generally, this situation resolves within the first week after calving. A prolonged inflammation, however, characterized by elevated/decreased levels of positive/negative acute-phase proteins (APP) may be detrimental. Unfortunately, variations in basal blood APP concentrations among herds, hamper the identification of critical blood APP thresholds to distinguish dairy cows that do or do not encounter prolonged inflammation. Accordingly, this study aims to apply a multivariate approach to cluster cows with a distinctive grade of inflammation at 21 d after calving and to describe their metabolic status and reproductive performance. This experiment involved 99 individual transition dairy cows and 109 observations (10 cows monitored in 2 consecutive lactations), under the same management. Blood was taken at −7, 3, 6, 9, and 21 d relative to parturition, and glucose, BHBA, NEFA, insulin, IGF-1, and fructosamine were analyzed. Additionally, APP including haptoglobin (Hp), serum amyloid A (SAA), and albumin-to-globulin ratio (A:G) were determined in the blood at 21 d. The 3 APP parameters were used in a k-medoids clustering and clinically healthy cows were grouped into 2 categories, acute phase response (APR, n = 39) and non-APR (n = 50). Disease cases (n = 20) were treated as a separate group. The parity median was smaller in the non-APR group (2.0) compared with the APR group (3.0) ($P < 0.05$). In the non-APR group, the highest A:G and lowest concentrations of SAA were observed compared with the other groups ($P < 0.05$). In addition, the non-APR group showed a more balanced metabolic status, characterized by a higher level of IGF-1 and fructosamine than the APR group ($P < 0.05$). The median of days open did not differ between APR and non-APR. A higher culling rate was noticed in the APR than in non-APR (51% vs. 28%, $P < 0.05$), however, this also may be, in part, related to the higher parity. These findings suggest that dairy cows that have an enhanced inflammatory status at 21 d after calving show more metabolic stress during the transition period.

Key Words: inflammation, metabolic status

2284 Heat stress and dietary organic acid and pure botanical supplementation alter the metabolome of lactating dairy cows.

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We previously demonstrated that heat stress (HS) evolves with increased gastrointestinal permeability (GP) and dietary organic acid and pure botanical (OA/PB) supplementation improved milk production of HS cows. Because the metabolome can be altered with increased GP, our objective was to identify metabolites that are related to GP and milk production in HS cows supplemented with OA/PB. Forty-six Holstein cows were enrolled in a study with a completely randomized design. After 7 d of acclimation in thermoneutrality (temperature-humidity index [THI] 68), cows were assigned to 1 of 4 groups: thermoneutral conditions (TN-Con, n = 12), HS conditions (HS-Con, n = 12; diurnal THI 74 to 82), TN conditions pair-fed to match HS-Con (TN-PF, n = 12), or HS fed OA/PB top-dress (HS-OAPB, n = 10) for 14 d. GP was assessed with an oral Cr-EDTA challenge. Samples of liver and rumen fluid were collected on d 12 and plasma and cecal contents were collected on d 14. Metabolome profiles were assessed with untargeted metabolomics using quadrupole time-of-flight mass spectrometry. Means were analyzed using a mixed model with a false discovery rate (FDR) correction applied. Correlation between metabolites, production and GP was assessed with Pearson correlations. HS-Con had higher relative abundance (RA) of plasma and hepatic lysophosphatidylcholines (LPC; e.g., LPC-16:1 and −18:0), ruminal aspartate, and hepatic and cecal glutamate, relative to all treatments (FDR <0.01); and these metabolites were negatively correlated with milk yield and energy-corrected milk yield (ECM; r = −0.34 to −0.68; $P < 0.05$). While Met was negatively correlated with GP (r = −0.36, $P = 0.02$) and positively correlated with ECM (r = 0.42, $P < 0.01$), we observed higher Met RA in HS-OAPB, relative to HS-Con (FDR <0.01). HS-OAPB had reduced RA of hepatic palmitate, relative to HS-Con (FDR <0.01); and importantly, hepatic palmitate was strongly correlated with ECM (r = −0.61, $P < 0.01$) and moderately with GP (r = 0.31, $P = 0.04$). Changes in the metabolome of HS cows are associated with reduced milk production and increased GP.

Key Words: heat stress, metabolome, organic acid

2285 Effects of heat stress and dietary organic acid and pure botanical on hepatic one-carbon metabolism.

V. Sáinz de la Maza-Escolà*[1,2], A. B. P. Fontoura[1], A. Javaid[1], N. S. Salandy[1,3], S. L. Fubini[1], E. Grilli[2,4], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy,* [3]*Tuskegee University, Tuskegee, AL,* [4]*Vetagro S.p.A, Reggio Emilia, Italy.*

Heat stress (HS) increases plasma urea-nitrogen in cows. Urea reduces the activity of glycerophosphocholine (GPC) phosphodiesterase, the enzyme that regulates the degradation of phosphatidylcholine (PC) into GPC and choline. Our objective was to evaluate the effects of HS and dietary organic acid and pure botanical (OA/PB) supplementation on liver one-carbon and phospholipid metabolism. Following a 7 d acclimation in thermoneutrality (temperature-humidity index [THI] 68), 46 pregnant late-lactation Holstein cows were randomly assigned to 1 of 4 groups: thermoneutral conditions (TN-Con, n = 12), HS conditions (HS-Con, n = 12; diurnal THI 74 to 82), TN conditions pair-fed to match HS-Con (TN-PF, n = 12), or HS fed OA/PB (HS-OAPB, n = 10; 75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlus R; Vetagro S.p.A) for 14 d. Liver biopsies were performed on d 6 of acclimation (baseline) and d 12 of environmental conditioning. Hepatic choline metabolites were analyzed by LC/MS. Data were analyzed using a general linear mixed model including fixed effects of treatment and block, and the random effect of cow. Planned contrasts included HS-Con vs. TN-Con, HS-Con vs. TN-PF, and HS-Con vs. HS-OAPB. Liver choline concentrations were lower in HS-Con compared with TN-Con and TN-PF ($P < 0.05$). Liver betaine and total PC concentrations were greater in TN-PF compared with HS-Con ($P < 0.01$). HS-Con had greater hepatic GPC concentrations compared with TN-Con, TN-PF, and HS-OAPB ($P < 0.05$). Hepatic GPC:choline ratios were greater in HS-Con, relative to TN-Con and TN-PF ($P < 0.01$). Liver *S*-adenosyl methionine (SAM) tended to be lower in HS-Con compared with TN-Con ($P < 0.10$). Although treatment did not modify *S*-adenosyl homocysteine (SAH) concentrations, HS-Con had lower liver SAM:SAH ratios, relative to TN-Con ($P = 0.05$) and HS-OAPB ($P = 0.06$). We conclude that HS develops with methyl donor deficiency in parallel with impaired N metabolism and dietary supplementation of OA/PB appears to maintain remethylation capacity in bovine liver during heat stress.

Key Words: heat stress, liver, one-carbon metabolism

2286 Effect of high vs. low body condition score at dry-off on calcium homeostasis, neutrophil function, and energy balance in the subsequent parity of Holstein lactating cows. T. O. Cunha*[1], P. L. J. Monteiro Jr.[1], W. S. Frizzarini[1], L. A. C. Ribeiro[1], L. Lewandowski[1], H. Hanling[1], R. Zhu[1], N. N. Teixeira[1], M. Z. Toledo[3], R. D. Shaver[1], J. P. N. Martins[1,2], M. C. Wiltbank[1], and L. L. Hernandez[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [2]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI, [3]Purina Animal Nutrition, Madison, WI.*

Delineation of the physiological mechanisms underlying the possible interactions between hypocalcemia and body condition score (BCS) loss postpartum and their effects on immune function and metabolic diseases is critical to improvement of cow health. Our objective was to evaluate the effect of BCS on periparturient calcium (iCa) homeostasis, neutrophil function, metritis, and subsequent energy balance. Beginning 75d before dry-off multiparous cows (BCS 3.16 ± 0.03, 148 ± 0.5 d post-AI) were blocked by lactation and BCS and randomly assigned to one of 2 diets, CON ($NE_L = 1.57$ Mcal/kg; n = 44) and HighBCS ($NE_L = 1.82$ Mcal/kg; n = 45). All cows were fed a common ration during the dry and fresh periods. BCS was recorded weekly, intake and milk production were recorded daily. Blood samples were collected daily from −7 to 7 DIM and 2×/wk until 35 ± 3 DIM. Flow cytometry was used to evaluate blood circulating neutrophil function at 0, 2, and 7 DIM. Presence of metritis was assessed by Metricheck at 7 and 14 DIM. Data were analyzed by MIXED procedure of SAS. At dry-off, HighBCS cows had a greater BCS than CON ($P < 0.01$; 3.72 ± 0.1 vs. 3.28 ± 0.1). HighBCS cows had decreased feed intake the last 10 d of gestation compared with CON ($P < 0.001$). Plasma iCa concentration at 1 DIM was decreased in HighBCS ($P = 0.04$; 0.94 ± 0.03 mmol/L, n = 34) compared with CON (1.02 ± 0.02 mmol/L, n = 31). Treatment did not affect serum magnesium ($P = 0.53$) and phosphorus concentrations ($P = 0.53$) from −1 to 4 DIM. Blood neutrophil counts ($P = 0.89$), oxidative burst ($P = 0.14$), and phagocytosis ($P = 0.25$) did not differ between treatments. Metritis was diagnosed in 8/83 cows (HighBCS: 5/41 vs. CON: 3/43). Mean BCS loss from 0 to 35 DIM was greater for HighBCS than CON ($P = 0.005$; 0.66 ± 0.6 vs. 0.43 ± 0.6). No differences were detected between treatments for serum Beta-hydroxybutyrate (−5 to 35 DIM), milk production, or dry matter intake (1 to 35 DIM). In summary, high BCS at dry-off affected energy balance, and postpartum iCa homeostasis, but it did not affect neutrophil function and metritis incidence.

Key Words: body condition score, calcium homeostasis, neutrophil function

2287 Effects of three different prepartum diets on mineral concentrations in saliva and feces in multiparous Holstein cows. W. Frizzarini*, J. Campolina, A. Vang, P. Monteiro, and L. Hernandez, *University of Wisconsin, Madison, WI.*

The objective of this study was to assess salivary calcium (Ca), phosphorus (P), and magnesium (Mg) concentrations, and soluble phosphate (PO_4) concentrations in feces, in cows fed 3 different prepartum diets. Multiparous Holstein cows (n = 122) were randomly assigned to one of 3 different diets, 21 d before the expected due date: control (CON; +190.24 mEq/kg; n = 40), negative DCAD (DCAD; −64.71 mEq/kg; n = 41), and a diet with sodium aluminum silicate (XZ; +277.40 mEq/kg with 500 g/day X-Zelit). Prepartum diet was given in automatic feeders. Salivary Ca (mmol/L), P (mmol/L), and Mg (mmol/L) were measured before treatments, and daily beginning 14 d before parturition until parturition, and subsequently on d 1, 2, 3, 6, 9, 12, 15, 18, 21, 35, and 49 postpartum in all animals. Before mineral analysis, salivary samples were scored 1 to 4 according to color. Fecal samples were collected across 3 d at 8-h intervals covering each 4-h clock period to make a composite sample per cow in a subset of 24 cows (n = 8 per treatment). Data were analyzed using PROC MIXED procedure of SAS. For salivary minerals, day was used as a repeated measurement, the models included treatment, day, lactation, score, treatment × day, and treatment × lactation; for fecal PO_4, the model included treatment, lactation, and treatment × lactation. Cows fed XZ had the greatest salivary Ca ($P < 0.01$; 1.70 ± 0.04, 1.41 ± 0.04, and 1.43 ± 0.04 for XZ, DCAD, and CON respectively) and Mg ($P < 0.01$; 3.17 ± 0.07, 2.66 ± 0.07, and 2.57 ± 0.07 for XZ, DCAD, and CON respectively) during prepartum. During prepartum, cows fed XZ had the lowest salivary P ($P < 0.01$; 6.22 ± 0.18), and cows fed CON had decreased concentration compared with DCAD (8.52 ± 0.21 and 10.02 ± 0.18, respectively). During postpartum, cows fed CON had the lowest salivary P ($P < 0.01$; 9.09 ± 0.21), and no differences were detected between DCAD and XZ (9.90 ± 0.19 and 9.90 ± 0.18, respectively). Cows fed XZ had the greatest fecal PO_4 concentrations compared with DCAD and CON ($P < 0.01$; 623.76 ± 31.51 mg/kg, 419.05 ± 34.51 mg/kg, and 425.45 ± 31.51 mg/kg respectively). Cows fed XZ diet had increased amounts of undigested PO_4 in the feces.

Key Words: transition period, dairy cow

1543T The association of delayed milk ejection with milking performance in Holstein dairy cows milked 3 times per day: A cohort study. M. O. Dahl, A. Singh*, M. E. Spellman, and M. Wieland, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

The objective was to study the association between delayed milk ejection (DME) and milking performance in Holstein cows. We hypothesized that DME would be associated with decreased milk yield (MY) and increased milking unit-on time (MUOT). In this retrospective cohort study, a total of 2,937 cows milked 3 times daily were followed over the course of 1 week resulting in a total of 61,677 cow milking observations. Electronic on-farm milk flow meters were used to record milk yield, MUOT, and the 30-s to 60-s milk flow rate. Delayed milk ejection was considered present if the 30-s to 60-s milk flow rate was ≤ 3.1 kg/min. Multivariable linear regression models were fitted to study the associations of DME with (1) MY (kg/d), and (2) MUOT (min/session). Cows were in their 1st (1,098, 37.3%), 2nd (769, 26.2%), and 3rd or greater lactation (1,070, 36.4%), and between 10 and 749 DIM (mean ± SD, 170 ± 103) on the day of inclusion into the study. For MY, we observed an interaction between DME and parity, such that the largest differences between cows with and without DME, respectively, were observed in cows in parity 3 or greater. Milk yield [LSM (95% CI)] for a hypothetical cow with 0 or 21 milking observations exhibiting

DME, respectively, were 38.8 (37.8–39.7) and 35.4 (34.7–36.1) kg for a cow in parity 1, 46.8 (45.8–47.8) and 38.4 (37.4–39.3) kg for a cow in parity 2, and 48.8 (48.1–49.5) and 35.3 (34.4–36.3) kg for a cow in parity 3 or greater. There was an interaction between DME and DIM for MUOT such that the differences in MUOT were smaller for cows with 201 DIM and greater. Milking unit-on time for a hypothetical cow with 0 or 21 milking observations exhibiting DME, respectively, was 3.5 (3.47–3.60) and 4.8 (4.77–4.91) min for a cow between 1 and 100 DIM, 3.5 (3.40–3.51) and 4.6 (4.49–4.61) min for a cow between 101 and 200 DIM, and 3.4 (3.34–3.46) and 4.4 (4.39–4.49) min for a cow with 201 DIM and greater. The novelty of our study was the serial assessment of DME over a 1-week period. Our results extend the current literature suggesting a dose-response relationship of DME with MY and MUOT.

Key Words: bovine, bimodal, milk ejection

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2289 Automatic milking system decision support tool for southern dairy businesses. A. McCalmon*, Y. Zhao, C. Martinez, and E. Eckelkamp, *University of Tennessee, Knoxville, TN.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).

Decision support tools have provided end-users with options for decision-making under different scenarios. The objective of this decision tool was for Southeastern producers considering an automatic milking system (AMS) to input their information and determine AMS investment impact. Data were sourced from 62 farm observations across Tennessee, Kentucky, and North Carolina through the Dairy Gauge Program (2021 and 2022). Mean data across years included farm incomes and expenses, such as milk production, feed, and labor costs, hauling, veterinary expenses, etc. Investment in AMS was modeled to determine net present value (NPV), internal rate of return (IRR), and payback period (PP). Three sensitivity analyses considering low, mean, and high options were compared against the default model assumptions including 1) construction style, 2) herd size, and 3) loan rate and AMS useful life. Three additional sensitivity analyses through Simetar in Excel analyzed changes in 4) milk production, 5) labor cost, and 6) purchased versus raised feed costs. Scenario 1 considered the construction costs of a retrofitted barn, an addition to a current barn, and a completely new barn holding other inputs at the default value. Scenario 2 considered changes in herd size (60, 240, 480, and 720 lactating cows, respectively). Scenario 3 considered different loan rates (0%, 25%, 50%, and 100%) and the useful life of the AMS in years (10, 15, and 20, respectively). Scenarios 4 through 6 ran stochastic simulations from all data points from Dairy Gauge with endpoints at the minimum and maximum values. These values minimum, mean, median, and maximum are as follows: milk production (12.5, 27.3, 28.5, and 37.0 kg per cow per day, respectively), labor cost ($0, $532, $588, and $1,123.29 per cow per year, respectively), purchased feed cost ($3.38, $8.76, $8.72, and $13.92 per CWT, respectively), and raised feed ($0, $2.87, $2.83, and $10.82 per CWT, respectively). Higher salvage values and longer useful life increased NPV, IRR, and shorter PP. Higher production led to greater feed costs, but still supported a positive NPV. New construction had a lesser NPV and IRR with the longest PP. In most Southeast dairy scenarios, AMS investment positively impacted financial indicators.

Key Words: decision tool, precision technology, economics

2290 The association of production outcomes with continuous blood BHB concentrations in the first or second week post-partum in dairy cows. A. D. Ravelo*[1], G. Cramer[1], S. Mann[2], and L. S. Caixeta[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN,* [2]*Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY.*

Postpartum blood β-hydroxybutyrate (BHB) concentration is often considered as a categorical variable with a typical cutoff of 1.2 mmol/L to evaluate associations with production outcomes. Our objective was to characterize the association of continuous BHB concentration with milk production and reproductive outcomes. A data set with blood BHB concentrations collected during the first (1 to 7 DIM; BHB1) or second wk (8–14 DIM; BHB2) for dairy cows (n = 3,375) from 7 farms between 2017 and 2020 was used to evaluate our outcomes of interest: milk production at the fourth wk of lactation (W4MK), peak milk production (PEAK), days open (DOPN), and risk of pregnancy at first service (P1AI). For the analysis of W4MK, PEAK, and DOPN, linear mixed models were used in R 4.2.2 with the fixed effects of continuous BHB, continuous BHB as a quadratic term, lactation (1, 2, 3+), and the random effect of farm. For risk of P1AI a log binomial regression was considered with the same fixed and random effects. There was no linear association of BHB1 or BHB2 with any of the outcomes of interest. There was a negative quadratic effect of BHB1 on W4MK ($P < 0.01$), as well as PEAK ($P < 0.01$), but there was no quadratic effect observed on the number of DOPN. A negative quadratic effect was observed when investigating the association of BHB2 and W4MK ($P < 0.01$), PEAK ($P < 0.01$), and DOPN ($P = 0.01$). Continuous BHB1 or BHB2 were not associated with risk of P1AI. For BHB1 and W4MK and PEAK, milk production was greatest with a BHB of 1.0 and 1.7 mmol/L, respectively. For BHB2 and W4MK and PEAK, milk production was greatest with a BHB of 1.6 and 1.9 mmol/L, respectively. Quadratic association of continuous BHB in the first 2 wk of lactation with production parameters such as W4MK and PEAK show that both low and high BHB concentrations were associated with lower milk production. These data modeling BHB as a continuous variable improve our understanding of the biological relevance of BHB concentrations in early lactation.

Key Words: hyperketonemia, quadratic, milk production

2291 A computer vision strategy to alleviate cow mastitis and improve dairy farm sustainability. C. P. J. Chen*, M. Das, and G. Ferreira, *Virginia Tech, Blacksburg, VA.*

Dairy farmers use water flushing systems to wash out manure left in the alleyway, but this can result in splashes of manure directly contacting the cow's udder, increasing the risk of mastitis. We hypothesized that a computer vision (CV) system can be used to synchronize the water flushing system: The flushing system would only be triggered when the least number of cows are standing in the alleyway, thus reducing the risk of mastitis. To make this technology more affordable to dairy farmers, we used a budget camera system, Amazon Ring, as the monitoring sensor. The camera was installed at one end of the alleyway, facing the other end. Thanks to the Ring camera's well-developed data transmission protocol, we were able to transmit real-time video snapshots to a farmer's computer via Wi-Fi. The number of cows in the snapshot was then counted by the CV model. If the number of cows reached a specific threshold (e.g., less than 5), the water flushing system would be triggered. The current CV system was validated after testing 50 images that contained from 4 to 18 standing cows in the alleyway at Virginia Tech Dairy Complex. The mean difference between the actual and the CV-counted number of cows was 3.8 cows. The errors were mostly due to the low resolution (1,280 × 720) of the camera, which blurred the contours of the cows standing further away from the camera. Potential solutions include installing more cameras around the alleyway, using a higher resolution camera, or fine-tuning the CV model, which would require more human effort to label the cow positions in a snapshot.

Key Words: computer vision, mastitis, flushing system

2292 Using kinematic to quantify gait attributes and predict gait score in dairy cows. C. Julliot[1,2], G. M. Dallago*[3], A. Nejati[2], A. B. Diallo[3], and E. Vasseur[2], [1]*Institut Agro Rennes-Angers, Rennes, France,* [2]*McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada,* [3]*Université du Québec à Montréal, Montreal, Quebec, Canada.*

Early identification of gait abnormalities could reduce lameness in dairy cows, but current assessment methods are laborious, making their use prohibitive for continuous monitoring. The objective of this pilot study was to predict the gait scores of dairy cows based on the gait attributes obtained using kinematic data. Data were collected from 12 Holstein cows between January 18 and February 12, 2021, using reflective markers attached to 20 anatomical locations. Cows were walked multiple times in a 7-m passageway corridor with 3 cameras on each side, totaling 69 passages of 3 steps long each. Five gait attributes of distance, duration, and velocity were calculated using 3D coordinates of the hoof markers. Range of motion was measured based on the angle between the stifle, hock, and fetlock markers of the rear legs. A trained observer scored cows' gait on a 5-point numerical rating system (NRS), with scores ranging from 1 (sound) to 5 (severe lameness). Passages with NRS scores of 1.5 (n = 1) and 4 (n = 2) were removed since there were insufficient samples to represent these scores. The data were split into training and validation sets following a 70:30 ratio stratified by the distribution of the NRS scores. The machine learning algorithms RPART, GBM, XGBM, RF, and SVM with a radial basis kernel were trained using leave-one-out cross-validation. In addition to the original data, weighted classification and synthetic minority over-sampling technique (SMOTE) were also tested due to uneven NRS distribution. The models were evaluated according to their accuracy, sensitivity, specificity, F1 score, and balanced accuracy on the validation split. The best model was the XGBM trained using the original data, which achieved an overall accuracy of 0.66 (95% CI = 0.53–0.78). Conversely, the weighted RPART classification model had the lowest overall accuracy of 0.44 (95% CI = 0.31–0.57). The insights from this pilot study contribute to developing an automatic monitoring system to identify and treat cows that have impaired locomotion but are not yet clinically lame, allowing for improved welfare and profitability of dairy cows.

Key Words: gait prediction, animal welfare, machine learning

2293 Automated extraction of domain knowledge for transition-cow management.

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In dairy cattle, the transition period (±3 weeks from calving) represents a challenging time for management. Vast changes in a cow's physiology, housing, and feeding often result in metabolic or reproductive diseases, leading to a drop in production. Because most metabolic processes are intricately linked, dairy producers and their advisors may have difficulty drawing concise conclusions concerning transition-cow management. To help in this, machine-learning techniques and knowledge-graph theory were explored with a view to creating a decision-support system that could provide producers and their advisors with knowledge from domain literature. Specifically, knowledge was modeled as entities and relationships in knowledge-graph theory, and natural language models were developed to extract information as knowledge graphs. A data set comprising 1,152 sentences from 20 papers was created and split into 922 sentences for training and 230 sentences for testing. Two deep-learning models were trained to extract entities and relationships, respectively. For testing, a bi-lstm model was applied to the entity extraction task and obtained an F1 score of 80%. With regard to relationship extraction, a transformer-based model was deployed but yielded a low F1 of 23%. Therefore, a pre-trained transformer model with 80.8% accuracy was deployed. After feeding the domain literature into the deep-learning models, a knowledge graph of 1,576 nodes and 3,456 edges was constructed and stored in a Neo4j graph database. Subsequently, a semantic parsing method was used to allow users to query the knowledge graph using natural language. To determine the quality of the responses, answers were sampled and evaluated based on human evaluation. On average, the answers scored 7.5 out of 10 and proved informative with respect to the original literature. Although the final interactive results demonstrated a high degree of visualization and scalability, this study primarily sought to demonstrate its feasibility. For tailored industrial applications, further improvements could be implemented in specific knowledge-graph expansion and reasoning.

Key Words: machine learning, transition-cow management, knowledge graphs

2294 Using hyperspectral imaging to predict particle size distribution in total mixed rations fed to dairy cows.

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Near-infrared spectroscopy (NIR) has been widely used to assess the nutritional composition of feed ingredients and total mixed rations (TMR). However, NIR usually requires ground samples, which destroy all physical properties related to ingredients and TMR, such as particle size (PS) and distribution. NIR Hyperspectral Imaging (HSI) combines NIR spectroscopy with digital imaging, enabling both spatial (localization) and spectral (identification) information to be obtained simultaneously. HSI thus have the potential of describing the distribution of constituents within a sample. The Penn State Particle Separator (PSPS) is the gold-standard method to quantify PS distribution. Our objective was to develop a computer vision system to predict PS distribution in TMR fed to dairy cows through NRI HSI. We scanned a total of 20 fresh TMR samples from 7 diets and separated using a PSPS. The percentages of TMR retrieved in each screen (U: >19 mm, M: 8–19 mm, L: 4–8 mm, and B: <4 mm particle size, respectively) were predicted using Lasso regression. Leave-one-out cross validation was used for model assessment. We grouped pixels based on their spectrum using k-means clustering and, for each cluster, the average area, circularity, perimeter, and eccentricity were calculated as features for the regression model. The R^2 for screens U, M, L and B were 0.84, 0.58, 0.03, 0.83, respectively, and the root mean squared errors were (%) 26.4, 6.7, 6.6, and 22. Our preliminary data suggests great potential of NRI HIS to predict PS distribution, which can contribute to on-farm tools for optimal management decisions.

Key Words: computer vision, hyperspectral imaging, particles

2295 Descriptive evaluation of camera-based dairy cattle lameness detection technology paired with artificial intelligence.

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The study objective was to describe the association between average weekly autonomous camera-based (AUTO) mobility scores and cows with a lesion for the first time to see if this technology can detect first lameness occurrence earlier. Data were collected from 1 farm from April to December 2022 and included cow ID, mobility score (0–100), and observation date and time. Historical farm hoof lesion data were used to determine cow lesion history and date of lesion diagnosis (LD). To remove the confounding impact of chronicity, the study focused on cows with no history of lameness. Cows were categorized as: having a first-time LD (LAME) or as seen by a hoof trimmer without a LD (TRIM). These categories were compared based on when the trimming occurred: within 7 d of dry off (DOT) or at a random time based on farm staff observation (RT). AUTO scores were summarized into weekly scores and included if cows had at least 1 observation/wk in the 4 wk before LD. All weekly and categorical AUTO scores were reported as median [IQR]. Comparisons were made for the LAME cows by lesion type. The lesion types for DOT (n = 60) were 93% TRIM, 3.3% toe ulcer (TOE), 1.7% white line disease (WLD) and 1.7% sole ulcer (SU). For RT (n = 239), 63% of lesion types were TRIM, 17% digital dermatitis (DD), 7.5% SU, 7.1% WLD, 4.2% foot rot (FR) and 4.2% TOE. Four wk before RT, LAME had a similar median score (38.6 [16.8]) to TRIM (38.0 [15.7]). One wk before RT, LAME had a higher median score (43.0 [18.3]) compared with TRIM (39.2 [16.2]). For DOT, 4 wk prior LAME had a higher median score (64.2 [13.2]) than TRIM (38 [14.3]) and this pattern persisted through 1 wk prior. FR had the highest score (47.3 [22.9]) at 4 wk prior, followed by SU (40.3 [11.2]) WLD (40.1 [15.2]) and DD (35.1 [12.4]). One wk prior, these scores were increased for FR (57.1 [22.9]), SU (45.5 [11.2]), WLD (47.8 [15.2]) and DD (39.4 [12.4]). The results indicate AUTO scores may have the potential for the earlier detection of some lesions. However, variation between cows and weeks presents a challenge that needs to be addressed.

Key Words: dairy technology, lameness, precision livestock farming

2296 Development of an automated diagnostic and anomaly detection system for milk profiles in dairy herds.

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Bulk tank milk components reflect herd and feeding practices. Abnormal trends in the milk profile can provide valuable insight into important changes occurring on farm. However, interpreting changes in the milk profile can be difficult given the multitude of milk components measurable on routine basis. Our objective was to develop an automated tool that can detect anomalies in the milk profile and propose corrective actions for timely interventions on farm. The proposed approach consists of identifying anomalies based on 3 indicators: the deviation from the breed-specific population average, short-term changes (i.e., the variation between 2 milk collections) and long-term changes (i.e., the gradient over 10 d) in the milk components. For each anomaly indicator a herd rank (0–100) is computed which allows to parameterize the severity of an observed anomaly and trigger user-specific alerts. A python package was developed to compute anomaly indicators daily for fat, protein, MUN and fatty acids in bulk tank milk, and identify potential anomalies in real time. Starting from the anomaly indicators, an additional diagnostic alert system with recommendations was developed using a rule-based expert system approach. Diagnostic rules (*if-then* rules) for several wide-spread events related to herd and rumen health management (e.g., potential rumen perturbations, risk of acidosis) were created with the help of domain experts. To facilitate timely interventions on farm, patterns in the milk profile were investigated during the 10 d leading to an event (i.e., detected diagnostic rule). For these analyses we analyzed bulk tank samples from 1,324 dairy herds in Quebec and New Brunswick, Canada, with potential anomalies detected between January and September 2022. Important changes in the milk profile were observed starting up to 6 d before firing a diagnostic rule. Overall, these preliminary analyses suggest that early detection of anomalies may be possible allowing for timely farm interventions. Further work is needed to determine whether the observed changes in the milk profile can accurately predict a future diagnostic event.

Key Words: bulk tank milk, prescriptive analysis

2297 From averages to individuals: A data cleaning dashboard for automatically collected feed intake data.

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Dairy research that reports feed intake typically compares means between treatment groups with the assumption that enough replication will allow statistical models to detect treatment effects. However, as we move toward developing individualized precision feeding protocols, we place a heavier importance on each recorded feeding event. The Insentec system (Insentec, Marknesse, NE) is an automated roughage intake control system used in dairy barns globally. We found 90 publications from 2019 to 2022 that used Insentec data from dairy cows, but only 22% of these mentioned a data cleaning protocol. Therefore, this work aimed to develop 1) a pipeline to clean automatically collected feed intake data that could be used in a closed-loop precision feeding model, and 2) an open-source online dashboard for researchers to clean and explore feed intake data. Historical Insentec data (Jan–Dec 2021) from the Ontario Dairy Research Centre (Elora, ON, Canada) were used to develop a 2-step cleaning pipeline that 1) flags and corrects 7 types of errors based on logical tests of chronological entries, and 2) regresses feed intake and duration for individual cows to identify and re-estimate outliers. This pipeline was evaluated on data from 98 cows over 4 weeks (100 bins) using a mixed model with method (cleaned or raw) and week (1–4) as fixed effects, and cow nested within week as random effect. Weekly means of daily feed intakes were 0.36 ± 0.66 kg/d (mean ± SD) lower ($P < 0.001$) after cleaning compared with raw data. In addition, 13% of cows had mean daily intakes that differed from −3.5 to +8.1 kg/d in cleaned vs. raw data ($P < 0.05$). Therefore, this pipeline is particularly relevant for individualized precision feeding applications. The proposed pipeline is released as an open-source R package with a user-friendly R Shiny webapp that allows researchers to upload raw feed intake files to collate, filter, clean, annotate and visualize them in a consistent and reproducible workflow. Precision feeding will require accurate data and, thus, we are releasing this application to encourage critique of routinely collected feed intake data.

Key Words: precision feeding, Insentec, feed intake

2298 Using late lactation milk composition to predict cow's transition success: An exploratory study.

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The transition cow index (TCI) gives a quantitative evaluation of success of the transition period (dry period and early lactation). Yet, assessing the success at the beginning of the lactation is already too late to allow any preventive measures for the cow. The aim of this study was to identify if milk composition from the last milk recording test date (TD) could explain the success of transition cow index score (i.e., TCI). Last TD records were obtained from the Lactanet (Canadian Network for Dairy Excellence) database from 2,809 herds from December 2020 to November 2022, for a total of 142,577 cows and 591,221 records. Data were filtered for Holstein cows with completed records of late lactation (LL). Four categories of TCI (CTCI) were created based on the mean and ± 1 SD of the TCI by parity (2, 3 and 4+), i.e., the thresholds used for CTCI differed by parity. Data were analyzed using one-way ANOVA and Tukey's test was used to determine significant differences. Milk yield was excluded from the analysis as TCI calculation is based on it. Results show that CTCI with the high TCI (3 and 4) had lower LL lactose (4.52 and 4.53%, respectively) than CTCI 1 and 2 (4.55 and 4.54%; $P < 0.001$). Likewise, LL fat percentage was low for CTCI 3 (4.25%) and 4 (4.23%) compared with CTCI 1 (4.28%) and 2 (4.27%; $P < 0.01$). Also, LL SCC were significantly lower for CTCI 3 and 4 (213.8 and 202.3, cells × 1,000/mL; $P < 0.01$) compared with CTCI 1 and 2 (251.2 and 225.9, cells × 1,000/mL; $P < 0.01$). High CTCI (3 and 4) had high LL de novo fatty acids (FA; 26.87, 26.86 g/100 g of total FA; $P < 0.001$) than CTCI 1 and 2 (26.62 and 26.72; $P < 0.001$). Same results were found for LL mixed FA for CTCI 3 and 4 (36.10, g/100 g of total FA; $P < 0.01$) and CTCI 1 and 2 (35.65 and 35.87, g/100 g of total FA). The contrary was found for LL preformed FA, CTCI 3 and 4 had lower (37.03, g/100 g of total FA) than CTCI 1 and 2 (37.43 and 37.41, g/100 g of total FA; $P < 0.01$). Results show that there are significant differences in most of the variables evaluated but the differences were relatively small. The next phase of this study is to finetune the analysis to better identify some key variables specific to LL status that could help predict the outcome of the next lactation.

Key Words: test day, late lactation, milk composition

2299 Benchmarking first-lactation organic Holstein, Jersey, and crossbred cows for production, somatic cell score, and days open in the United States.

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The objective of the study was to evaluate certified-organic Holstein, Jersey, and crossbred cows for milk production, somatic cell score (SCS), and days open. Data were from organic dairy herds in the US from DHIA data acquired from Dairy Records Management Systems (DRMS; Raleigh, NC) that include complete herd and cow lactational data from 35 states from years 2012 to 2018. States were categorized into regions: Midwest, South, Northeast, and West. The 305-d production and days open (DO) was analyzed for 25,764 first-lactation Holstein cows and 7,516 first-lactation Jersey cows and 6,642 first-lactation crossbred cows. Holstein, Jersey, and crossbred cows were analyzed separately. Independent variables for statistical analysis were the fixed effects of age at calving (20 to 36 mo), year of calving (2012 to 2018), and region. Holstein cows in the Western US had greater ($P < 0.05$) 305-d milk production (8,073 kg) compared with cows in the Midwest (7,604 kg), Northeast (7,201 kg), and the South (7,284 kg). However, Holstein cows in the Western US had lower ($P < 0.05$) 305-d combined fat and protein production compared with cows in the Midwest, Northeast, and the South (391 kg vs. 524 kg, 459 kg, and 452 kg, respectively). Days open was greater ($P < 0.05$) for cows in the South (190 d) and West (183 d) compared with the Midwest (162 d) and Northeast (162 d). Jersey cows had greater ($P < 0.05$) 305-d milk and 305-combined fat and protein in the Midwest and Northeast compared with the South and the West. The SCS was highest for Jersey cows raised in the South. Jersey cows in the Midwest and Northeast had fewer ($P < 0.05$) DO (154 d) compared with cows in the South (167 d) and the West (203 d). Crossbred cows in the South and West had greater ($P < 0.05$) 305-d milk and 305-d combined fat and protein production compared with cows in the Midwest and Northeast. However, SCS for crossbred cows was lowest ($P < 0.05$) in the Northeast (2.33) compared with the Midwest (2.63), South (2.89), and the West (2.58). The DO was similar across regions for crossbred cows. Breed and regional differences for production and fertility exist for organic dairy cows in the United States.

Key Words: organic, Holstein, somatic cell count

2300 Relationship between actual vs. targeted weight at first calving and lactation performance.

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The objective was to quantify the relationship between differences in actual vs. targeted weight at first calving and subsequent 305-d milk production. Data from birth through 3 lactations were obtained for all animals born during 2015 to early 2019 on a single Holstein dairy. Animals had to calve at least once and have body weights recorded at the time of calving along with genomic and production data. From an initial population of 3,333 heifers, 1,751 entered their 3rd lactation and had a body weight recorded after calving. Mature body weight (MBW) is defined as the weight of 4th-lactation cows at 100–200 DIM. Since no weights were available for the 4th lactation, adjustments were made to the 3rd-lactation weights to estimate expected MBW. Cows were assumed to add an additional net gain of 2% in body weight from the start of 3rd lactation until maturity. These weights were regressed against genomic body composite (BDC) resulting in the following equation: MBW (kg) = 802 + 36.4 × BDC. Using this derived equation, a predicted MBW (pMBW) was created for each animal that calved at least once. The product of 0.85 and pMBW was used as the targeted weight at first calving. Subtracting targeted weight from the actual weight resulted in the first-lactation weight difference (L1 Wt Diff). From this L1 Wt Diff, 3 groups were created for the original 3,333 heifers representing "Light" animals at calving (L1 Wt Diff = −181 to −35 kg, n = 2549), "Targeted" animals (−34 to 34 kg, n = 631) or "Heavy" (35 to 136 kg, n = 163). The Targeted category cutpoints were selected around 0 through trial and error to include a reasonable group size and to reflect the imprecision associated with the estimated target. The other cutpoint naturally followed. Linear mixed models were used to evaluate 305-d milk within group using genomic milk, DIM, DIM^2, age at first calving, and age at first $calving^2$ as fixed effects, and month and year of calving as random effects. In the Light group, each additional kilogram of weight at first calving was associated with 3.4 kg more 305-d milk in lactation 1 ($P < -0.01$) and 1 kg more in lactation 2 ($P < 0.01$). There were no significant effects of L1 Wt Diff in the Targeted or Heavy Groups ($P = 0.38$ and $P = 0.47$). This project demonstrates that failure to adequately grow heifers before calving limits their production potential in first and second lactation and represents a large lost economic opportunity.

Key Words: heifer growth, milk production, replacement

Ruminant Nutrition Symposium: Improving Rumen Fermentation Through Altering Rumen Microbiota

2301 The rumen microbiome and its function—Predators within and their implication in intraruminal recycling of microbial protein. Z. Yu* and M. Yan, *The Ohio State University, Columbus, OH.*

Microbial protein accounts for > 80% of the protein metabolizable by dairy cows. Intraruminal recycling of microbial protein decreases the metabolizable protein reaching the abomasum and small intestines and thus is an important barrier for nitrogen utilization efficiency. Although proteolytic bacteria participate in intraruminal recycling of microbial protein, rumen protozoa are considered the major culprit as they directly engulf microbial cells and degrade the cellular protein. Recent genomic studies have identified the genes encoding the "digestive enzymes" of rumen protozoa, while transcriptomic studies confirmed the expression of these genes. Besides confirming the "guilt" of rumen protozoa in lysing microbial cells and degrading microbial protein, the genomic and transcriptomic information also identified protozoal digestive enzymes as targets for specific inhibition of rumen protozoa to decrease intraruminal recycling of microbial protein. In addition to rumen protozoa, rumen viruses, especially those of bacteria (i.e., bacteriophages), can infect and then lyse their host from inside by their lytic cycle, increasing the bacterial protein available for degradation by proteolytic bacteria. Therefore, rumen bacteriophages likely also contribute to the intraruminal recycling of microbial protein. In a recent metagenomic study, we developed a global rumen virus database by collecting and analyzing nearly 1,000 rumen metagenomes. We found diverse rumen viruses including phages infecting rumen bacteria, methanogens, and protozoa. Most of the species that can be infected by rumen bacteriophages belong to the phylum *Bacteroidetes* and *Firmicutes*, the most abundant phyla of the rumen microbiome, and lytic phages represent a high proportion of the rumen phages. These phages probably contribute significantly to the intraruminal recycling of microbial protein. Preliminary analysis also showed an association of the rumen virome with feed efficiency and methane emissions. Future research on intraruminal recycling of microbial protein should include rumen viruses.

Key Words: rumen protozoa, rumen viruses, intraruminal recycling of microbial protein

2302 New biochemical pathways for forming short-chain fatty acids during fermentation in rumen bacteria. T. Hackmann*, *University of California, Davis, Davis, CA.*

Fermentation is a major type of metabolism and carried out by over 2,300 species of bacteria. Though fermentation is widespread, the biochemical pathways underpinning it are not always clear. Pathways are written in textbooks and other resources, but they are based on study of a few model bacteria. Recent work suggests textbook pathways do not apply to many bacteria, and new pathways await discovery. In one study, we searched genomes of bacteria from the cattle rumen, and we found 44% of them did not encode textbook fermentation pathways. For example, 8% of them encoded a pathway for forming acetate not previously known in bacteria. In subsequent studies, we have found biochemical evidence for new pathways. In propionibacteria, for example, we confirmed the existence of a pathway for forming acetate previously unknown in bacteria. The pathway involves 2 enzymes, succinyl coenzyme A:acetate CoA-transferase and succinyl-CoA synthetase. The enzymes are common in bacteria, but not previously recognized as having this role in fermentation. Similar studies have confirmed new steps in pathways for forming propionate and butyrate. Discovering these new steps and pathways provides new targets for manipulating fermentation, which will improve animal productivity.

Key Words: rumen, bacteria, fermentation

2303 Microbiome-guided strategies to improve cattle production. P. Fan*[1] and K. Jeong[2,3], [1]*Department of Animal and Dairy Sciences, Mississippi State University, Starkville, MS,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Emerging Pathogens Institute, University of Florida, Gainesville, FL.*

Trillions of microorganisms inhabit the gastrointestinal tract (GIT) of animals, forming a dense and diverse microbial ecosystem. The GIT commensal bacteria co-evolve with the host and exert numerous functions influencing animal digestion, metabolism, immunity, and behavior. The aim of this presentation is to highlight the roles of "sensor" and "motor" of GIT microbiota played in the mutualistic relationship and propose potential strategies of using microbial features as biomarkers for animal breeding and early prediction and as direct targets to improve cattle performance. Our previous finding showed a significant impact of host genetics on the abundance of butyrate-producing bacteria in the hindgut gut of cattle throughout life, which were correlated with cattle weight gain and plasma IgG level, and identified specific host SNPs in genes related to metabolism and immunity associated with these bacteria. Integrating the GIT microbiota feature in cattle breeding is also supported by evidence from other studies reporting the genetic effects on rumen microbiota and interactions with feed efficiency and methane production. Besides, we observed distinct bacteria-bacteria co-occurrence networks between healthy and diarrheic calves and isolated beneficial bacteria from healthy calves that inhibited the pathogens, suggesting microbiota from diarrhea-resistant could be a potential source for probiotic development. In addition, a dramatically higher abundance of antimicrobial resistance genes in GIT of newborn calves compared with adult calves as well as antimicrobial resistant bacteria in colostrum were detected in both our and other studies, indicating a necessity to optimize calf diet and management to develop healthy GIT microbiota. Future research should evaluate the efficiency of using microbiota features for genetic selection and phenotype prediction and exploit the causative effects of specific commensals on cattle physiology.

Key Words: gastrointestinal tract, microbiota, biomarker

Ruminant Nutrition 3: Carbohydrates and Lipids

2304 Dietary fiber source and direct fed microbial supplementation effects on performance of high-producing dairy cows. M. R. Pupo*, E. C. Diepersloot, C. Heinzen Jr., M. P. Rodrigues, and L. F. Ferraretto, *University of Wisconsin–Madison, Madison, WI.*

Sixty-four multiparous Holstein cows (76 ± 22 DIM) were randomly assigned to 1 of 4 treatments in a completely randomized design with a 2 × 2 factorial arrangement for an 11-wk trial (1 wk acclimation, 2 wk covariate, and 8 wk treatment periods). Treatments were forage inclusion in the diet, 45% (LOW) or 60% (HIGH) of DM, and without (CON) or with the supplementation of a direct-fed microbial containing herbal extracts, *L. plantarum*, *L. buchneri*, and *Saccharomyces cerevisiae* (75 mL/head/day; AD). Data were analyzed with a covariate and using a mixed model with week of treatment as repeated measures. The model included fiber source, direct fed supplementation, week, and all 2- and 3-way interactions as fixed effects, and cow (treatment) as a random effect. Three-way interactions were observed for dry matter intake ($P = 0.001$) and feed efficiency ($P = 0.001$). Dry matter intake was 2 to 3 kg/d greater for LOW-AD than HIGH-CON and HIGH-AD during wk 2, 3, 5, and 8 of the treatment period. Feed efficiency was 0.2-units greater for LOW-CON than HIGH-CON during wk 4 and 7, whereas did not differ from other treatments during wk 4. Milk production was greater ($P = 0.002$) for LOW than HIGH forage diet (56.9 vs. 54.8 kg/d, respectively), which affected ($P = 0.01$) lactose yield (2.7 vs. 2.6 kg/d, respectively). Milk fat ($P = 0.02$; 3.52% and 3.34% DM, respectively) and MUN ($P = 0.02$; 11.9 mg/dL and 11.4 mg/dL, respectively) concentrations were greater for HIGH than LOW. Conversely, milk protein concentration was lower ($P = 0.04$) for HIGH than LOW (2.95 vs. 3.01% DM, respectively). Morning respiration rate ($P = 0.02$) was 3.2 breathes/min lower with AD supplementation. Daily rectal temperature ($P = 0.02$) was approximately 0.1°C greater for LOW than HIGH forage diets. Moreover, TMR containing AD had ($P = 0.01$) 1 h greater aerobic stability than TMR without AD. Feeding a low forage diet increased actual milk yield but not component-corrected milk yields by dairy cows. In addition, AD supplementation reduced respiration rate during the summer.

Key Words: direct-fed microbial, forage, feed efficiency

2305 Effects of isoacids supplementation in lactating cows' diet varying in forage fiber level on performances, feed efficiency and milk fatty acids profile. S. Ahmed*[1], M. R. A. Redoy[1], M. L. Bulnes[1], J. B. Urbina[1], D. H. Kleinschmit[2], and M. E. Uddin[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Zinpro Corporation, Eden Prairie, MN.*

Our objective was to determine the effects of isoacids (ISO; IsoFerm, Zinpro Inc.) on the lactation performances, feed efficiency and milk fatty acids (FA) profile of Holstein cows under 2 forage neutral detergent fiber (NDF) levels (FL). For a 10-wk long (2-wk for covariate) randomized complete block design study, 64 mid-lactation cows [662 ± 71 kg body weight, 119 ± 51d in milk (DIM), 2 ± 0.9 parity] were blocked by parity, DIM, and prior milk yield (MY) or genetic merit. Cows were randomly assigned to 1 of the 4 diets (n = 16) which were arranged as 2 × 2 factorial, with 2 FL containing 18 (LF) and 23% forage NDF (HF) without (WIA) or with ISO (IA, 40 g/d per cow). Diets were formulated to meet NASEM (2021) requirement containing similar crude protein (16.5%), NDF (28%), and net energy for lactation (1.72 Mcal/kg DM). Dry matter intake (DMI) and MY were recorded daily, and milk composition were analyzed weekly. Data were analyzed in R using lme4 where final model included wk, FL, ISO, and FL × ISO as fixed effects and block as random effect. The ISO did not affect DMI which was greater in LF than HF (27.1 vs. 25.8 kg/d, $P = 0.048$). Compared with HF, LF cows had greater MY (36.6 vs. 32.9 kg/d, $P = 0.001$) and fat-and-protein corrected milk (FPCM, 37.6 vs. 35.1 kg/d, $P = 0.001$), whereas ISO increased MY (14%, $P < 0.001$) and FPCM (10%, $P = 0.003$) in HF but not in LF diet (i.e., FL × ISO interaction). We only found a FL × ISO interaction for feed efficiency (MY/DMI and FPCM/DMI) i.e., ISO increased feed efficiency in HF but decreased in LF diet regardless of expressions. Treatment did not affect milk protein, but milk fat was lower in LF than HF (4.1 vs. 4.4%, $P = 0.007$) and tended to be lower in IA than WIA group (4.1vs. 4.3%, $P = 0.092$). Compared with HF, LF also had lower total and de-novo FA whereas ISO only decreased total FA ($P = 0.007$). The FL did not affect milk urea nitrogen but was decreased by ISO (12.8 vs. 14.0 mg/dL, $P = 0.001$) under LF diet only (i.e., FL × ISO) suggesting a lower urinary urea nitrogen excretion. In conclusion, IA supplementation improved lactation performances and efficiency depending on FL.

Key Words: Holstein, milk fat, de-novo fatty acid

2306 Fiber evaluation methods of novel feed ingredients for dairy cows: The case of macroalgae. E. Chasse*[1], M. V. Curtasu[1], K. E. Bach Knudsen[1], A. Bruhn[2,3], and M. O. Nielsen[1,3], [1]*Department of Animal and Veterinary Sciences, Aarhus University, Foulum, Denmark,* [2]*Department of Ecoscience, Aarhus University, Aarhus, Denmark,* [3]*Center for Circular Bioeconomy, Aarhus University, Foulum, Denmark.*

Macroalgae have attracted significant attention as potential feeds or additives, since their cultivation is not associated with use of arable land suitable for human food production, and because of the ability of some species to reduce enteric methane formation from ruminants. There is only limited information available on rumen degradability of macroalgae CHO fractions, specifically the non-starch polysaccharides (NSP). Traditionally, CHO analyses of ruminant feeds provide information about contents of starch and sucrose, and insoluble fiber classified into neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin. The conventional fiber analysis does not provide any information about the actual composition of fibers or their physiochemical properties. The aim of this project was to compare fiber content of seaweed samples determined by both the NDF (Ankom 2000 Fiber Analyzer) and NSP (Bach Knudsen, 1997) methods. We analyzed 20 macroalgae species (12 brown, 2 green and 6 red) from Denmark and Australia. Samples were analyzed in triplicates. Data were analyzed using correlations between variables. Results showed that fiber content of seaweeds was overestimated by 28% on average when using the NDF method compared with the NSP method. The difference between NDF and total NSP content positively correlated with total galactose ($r = 0.5238$, $P = 0.004$), soluble galactose ($r = 0.5698$, $P = 0.002$), total arabinose ($r = 0.4940$; $P = 0.008$), soluble arabinose ($r = 0.5350$; $P = 0.003$) and insoluble mannose ($r = 0.5396$; $P = 0.003$). Galactose, arabinose and mannose are all components of side chain ramifications in macroalgae fibers. Moreover, galactose is the main component of agar which has structural resemblance to pectin. The NDF method is known to give poor estimates of fiber content of pectin-rich ingredients because pectins are washed away with the detergent. In conclusion, the NSP method is more appropriate to determine fiber content of macroalgae compared with the NDF method because it includes the soluble fraction and provides details about the components of the fibers.

Key Words: macroalgae, fiber, carbohydrate fermentation

2307 Fatty acid supplementation interacts with starch content to alter production responses during the immediate postpartum in dairy cows. J. E. Parales-Giron*[1], A. C. Benoit[1], J. M. dos Santos Neto[1], J. de Souza[2], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue Agribusiness, Salisbury, MD.*

We evaluated the interaction between increasing dietary starch content and fatty acid (FA) supplementation on production responses of early-lactation cows. Sixty multiparous cows were used in a randomized complete block design with a 2×2 factorial arrangement of treatments. Treatment diets were fed from 1 to 24 DIM and contained 22% or 28% diet DM starch (LS and HS) and 0% or 2% diet DM supplemental FA (NF and HF). The FA supplement was a Ca-salt containing 70% palmitic and 20% oleic acid. Treatment diets were formulated to contain 17% CP and 22% forage NDF. The statistical model included the random effect of block, cow within block and treatment, Julian date, and the fixed effects of starch content, FA supplementation, time, and their interactions. Results are presented in the following order: LSNF, HSNF, LSHF and HSHF. We observed interactions between dietary starch and FA supplementation for the yields of milk (43.1, 47.4, 43.4, 43.6 kg/d; $P < 0.05$), milk fat (1.95, 2.12, 2.13, 2.07 kg/d; $P = 0.06$), and 3.5% FCM (50.3, 55.1, 53.7, 52.7 kg/d; $P < 0.05$) because FA supplementation increased the yields of milk fat ($P < 0.05$), and tended to increase the yield of 3.5% FCM ($P = 0.09$) in the low starch diet but decreased milk yield in the high starch diet ($P < 0.05$). Overall, high starch increased the yield of milk ($P < 0.05$), tended to increase milk lactose yield (2.08, 2.27, 2.11, 2.17 kg/d; $P = 0.07$), and reduce milk protein content (3.46, 3.30, 3.38, 3.33%; $P = 0.06$), and had no effect on the yields of milk fat and protein. Overall, FA supplementation increased milk fat content (4.57, 4.48, 4.83, 4.71%; $P < 0.01$) and had no effect on the yields of milk fat and protein. In conclusion, feeding high starch diets increased milk yield during the immediate postpartum. The effect of FA supplementation on the yields of milk fat and 3.5% FCM of early-lactation cows depended on dietary starch level.

Key Words: starch, fatty acid, early-lactation

2308 Effects of rumen-protected sugar supplementation on milk yield, subclinical ketosis and reproduction of dairy cows in the transit period. C. Brock*[1], H. Wenjuan[2], D. Zhuangzhao[3], and A. Robinson[3], [1]*Berg + Schmidt GmbH & Co. KG, Hamburg, Germany,* [2]*Beijing Mingrida Trade, Beijing, China,* [3]*Berg + Schmidt Asia Pte Ltd., Singapore.*

In the transition period, most dairy cows suffer from subclinical ketosis (SCK). Therefore, the objective of the present study was to reduce SCK and improve milk and reproductive performance by supplementation of rumen-protected sugar (RPS). In a trial 449 Holstein cows were divided into 2 groups: control (CON), or supplementation of RPS (LipoAktiv Glu 60, Berg + Schmidt GmbH & Co. KG, Hamburg) with 150 g/cow/day from wk −3 before expected calving through calving and 200 g/cow/day from calving to wk 3 of lactation. Daily milk yield was measured until wk 9 of lactation, blood glucose levels were measured at calving and at 6 d in milk, β-hydroxy butyric acid (BHB) levels were measured at wk 1 of lactation. Days to first insemination and number of services until confirmed conception were measured. Post-calving data were analyzed using statistical software R version 4.1.1 (2021). Treatment, time, interaction of treatment and time were included as independent variables, cow nested within treatment as random effect in a linear mixed model. Supplementation of RPS increased milk yield numerically compared with CON (44.6 kg/day CON vs. 45.3 kg/day RPS). Blood glucose concentrations were lower in CON than in RPS at calving ($P < 0.001$), but equal at wk 1 of lactation. Blood BHB concentration was lower in RPS than in CON at wk 1 of lactation (0.72 vs. 0.66, $P < 0.05$), where 9.49% and 5.41% of cows in CON and RPS had SCK ($P = 0.11$); threshold used to define SCK was blood BHB levels ≥1.2 mM. In the RPS group 81.5% of animals had blood BHB levels ≤0.8 mM, in CON only 69.0%. Blood BHB levels >0.8 mM are associated with decreased migration of circulating polymorphonuclear neutrophils (PMN). Average days to the first insemination were not different between groups (62.8 CON vs. 62.6 RPS, $P = 0.9$), number of services until confirmed conception were not different (1.52 CON vs. 1.47 RPS, $P = 0.2$). In conclusion, feeding transition cows rumen-protected sugar tendend to decrease subclinical ketosis in fresh cows with no effect on milk yield or reproduction performance.

Key Words: rumen-protected sugar, milk performance, subclinical ketosis

2309 Effects of adding field peas to the diet of lactating dairy cows on rumen fermentation and *de novo* milk fatty acids. J. C. Plaizier*[1], R. Gervais[2], K. H. Ominski[1], and C. Yang[1], [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*Laval University, Quebec City, QC, Canada.*

Field peas have a potential as a dairy cow feed. However, the possible impact of their high starch content on rumen fermentation and de novo milk fat synthesis requires further research. The objectives of this study were, therefore, to determine the effects of including field peas in the diet of lactating dairy cows on rumen fermentation, milk fat content, and the proportion of de novo fatty acids (FA) in milk fat. Twelve lactating, non-pregnant Holstein cows were used in a repeated 3 × 3 Latin square with 21-d experimental periods, with the last 7 d used for sample and data collection. Cows were fed a basal TMR (CTL), or partial mixed diets (PMR) to which 3.9% (LP), or 7.8% DM (HP) of field peas were added. The basal TMR contained on a DM basis 17.4% crude protein (CP), 4.5% crude fat, 28.0% starch, and an estimated 1.67 Mcal/kg of net energy for lactation (NE_L). The field peas contained 22.5% CP, 1.67% crude fat, 44.7% starch, and an estimated 1.81 Mcal NE_L/kg. As a result, the estimated starch intake of cows on the HP treatment was 800 g/d higher than that of cows on the CTL cows. Peas were coarsely ground and provided as a top dress. Rumen fluid digesta was collected by rumenocentesis on of d 18 of each period at 6 h after feed delivery. Adding field peas to the diet did not affect the rumen pH, rumen VFA concentrations, the milk fat yield, nor milk fat proportions of de novo FA (Table 1). However, feeding peas tended to increase the milk fat content ($P = 0.10$). This suggests that the additional intake of starch resulting from the feeding of field peas did not impact rumen fermentation and the de novo milk fat synthesis.

Key Words: field peas, milk fat, rumen fermentation

2310 Temporal changes in plasma choline and choline metabolite concentrations in response to an esophageal bolus of rumen-protected fish oil in early lactation cows fed rumen-protected choline. V. Sáinz de la Maza-Escolà*[1,2], M. F. Marchesi[1], M. Dei Cas[3], S. Casati[4], F. Piccioli-Cappelli[5], E. Trevisi[5], E. Grilli[1,6], and J. W. McFadden[2], [1]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy,* [2]*Cornell University, Ithaca, NY,* [3]*Department*

Table 1 (Abstr. 2309).

	CTL	LP	HP	SEM	Significance, *P*-values
Rumen pH	6.08	6.09	6.02	0.08	0.62
Acetate, mmol/L	54.2	57.0	57.0	2.51	0.43
Propionate, mmol/L	16.8	17.9	18.4	1.05	0.25
Butyrate, mmol/L	11.7	13.0	12.4	1.05	0.26
Total VFA, mmol/L	85.1	90.9	91.4	4.20	0.28
Ac/Pr	3.25	3.30	3.14	0.10	0.57
Milk fat, %	4.16	4.21	4.38	0.09	0.10
Milk fat, kg/d	1.49	1.53	1.56	0.04	0.29
Total *de novo* FA, g/100 g FA	23.4	23.3	23.3	2.31	0.99

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Fish oil (FO) is a rich source of docosahexaenoic acid (C22:6; DHA), which may be a preferable fatty acid (FA) for hepatic phosphatidylcholine (PC) synthesis via the actions of phosphatidylethanolamine *N*-methyltransferase. Our objective was to determine whether rumen-protected choline (RPC) feeding enhanced concentrations of circulating PC enriched with DHA in cows fed FO. Eighteen Holstein cows (3.6 ± 2 [mean ± SD] lactations) were randomly assigned to 2 groups (n = 9/group) at −21 ± 3 d prepartum: unsupplemented (CON) or supplemented (CHOL) with RPC (0 or 60 g/d, respectively; 25% choline chloride; Ruprocol; Vetagro S.p.A., Reggio Emilia, Italy) until d 35 postpartum. At d 27 ± 4 postpartum, all cows were provided a gelatin capsule containing 100 g of lipid-encapsulated FO as an esophageal bolus (36% FO; 10.2 g of omega-3 FA; Prototype 6; Vetagro S.p.A.). Cows were fed a total mixed ration and milked twice daily. Blood was sampled at 0, 10, and 24 h, relative to bolus delivery. Plasma was analyzed for PC, lysoPC (LPC), choline, and choline metabolites using LC/MS. Data were analyzed under a mixed model with the random effect of cow, and the fixed effects of treatment, hour, and their interaction. At h 0, plasma betaine concentrations were greater in CHOL, relative to CON ($P = 0.05$); however, choline concentrations were not modified by treatment. FO increased circulating trimethylamine *N*-oxide and dimethylglycine (DMG) by h 10 (time, $P < 0.03$). DMG and methionine were greater in CHOL cows, relative to CON ($P < 0.07$). Although no changes were detected for plasma LPC-22:5 or −22:6, total LPC, or PC-16:0/22:5 or −18:0/22:5, RPC feeding increased plasma PC-16:0/22:6, −18:0/22:6, and LPC-20:5, compared with CON, following FO bolus ($P < 0.07$). Plasma PC-18:0/20:5 and total PC concentrations were greater in CHOL cows by h 24, relative to CON (treatment × time, $P < 0.05$). We conclude that RPC feeding augmented the ability of dietary FO to enhance PC synthesis in early lactation cows.

Key Words: choline, fish oil, phosphatidylcholine

2311 Meta-analysis of the effects of the dietary inclusion of brewer's grain on feed intake, milk production, and feed efficiency of lactating dairy cows.

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Distillery byproducts like brewer's grains have been widely used in dairy cow diets for decades to reduce diet costs and improve nutrient efficiency. Thus, the objective of this study was to evaluate the magnitude of the effects of dietary supplementation with brewer's grain (BG) on feed intake (DMI), digestibility, milk yield (MY), and feed efficiency of lactating dairy cows. A total of 12 peer-reviewed manuscripts (n = 472 dairy cows) from 1983 to 2021 were collected systematically with the PRISMA method. The weighted raw mean differences (RMD) between dietary BG and control treatments were estimated with a robust variance estimation. Likewise, diet characteristics, like CP content, NDF content, type of brewer's grains (Wet = 1 and Dry = 2), days in milk (DIM), and BG inclusion rate (0 to 75%) were used as covariates in a meta-regression, subset, and dose-response analysis. Compared with the control, dietary BG decreased ($P < 0.05$) DMI (19.7 vs. 20.2 kg/d) and tended to increase ($P = 0.1$) MY (28.9 kg/d vs. 28.4 kg/d), but no effects were observed on fat-corrected milk (FCM) yield (29.1 vs. 28.8 kg/d), milk fat (3.8 vs. 3.7%), and milk protein (3.2 vs. 3.2%) concentrations. However, dietary BG increased ($P < 0.05$) feed efficiency (1.45 vs. 1.34 FCM/DMI) without influencing total-tract DM (61.9 vs. 55.7%) and NDF (49.7 vs. 54.5%) digestibility. Increasing the inclusion rate of BG linearly increased ($P < 0.01$; $R^2 = 55.4$) dietary NDF. Thus, the dose-response analysis revealed that feeding up to 29.3% BG (dietary NDF = 40%) to lactating dairy cows increased milk yield and feed efficiency. Overall, this meta-analysis supports the hypothesis that dietary BG improved feed efficiency in lactating dairy cows.

Key Words: brewer's grain, performance, dairy cows

2312 Production performance and nutrient digestibility in grazing dairy cows fed an extruded flaxseed-based supplement.

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Eighteen multiparous and 2 primiparous Jersey cows (128 ± 52 DIM) were used in a randomized complete block design to investigate the effects of an extruded flaxseed-based supplement (LinPRO-R) on production performance and nutrient digestibility during the grazing season. Cows grazed a mixed grass-legume pasture (herbage allowance = 15 kg of DM/cow daily) overnight and received a partial total-mixed ration (pTMR) during the day. The pTMR were formulated to contain (DM basis) 37.5% mixed, mostly legume baleage and 62.5% of a soybean meal/ground corn-based concentrate mash. Cows were randomly assigned to 1 of 2 diets: (1) pasture plus pTMR (control = CTRL) or (2) pasture, pTMR, and 6% LinPRO-R (LIN). Ground corn and soybean meal were replaced with LinPro-R in the LIN diet. Diets were formulated to be isonitrogenous and to yield a 60:40 forage to concentrate ratio.

Table 1 (Abstr. 2313).

	Treatment[1]						
	CTL	PA	HT	CS	SO	SEM	*P*-value
Milk yield, kg/d	42.1^{b}	44.1^{ab}	44.0^{ab}	44.2^{ab}	45.4^{a}	1.3	<0.01
ECM, kg/d	42.9^{b}	46.3^{a}	45.5^{ab}	44.3^{ab}	43.1^{b}	1.5	0.01
Fat, %	4.08^{ab}	4.31^{a}	4.22^{ab}	4.01^{b}	3.58^{c}	0.16	<0.01
Fat, kg/d	1.70^{bc}	1.89^{a}	1.84^{ab}	1.76^{abc}	1.61^{c}	0.08	<0.01
True protein, %	3.32^{a}	3.33^{a}	3.27^{ab}	3.19^{b}	3.19^{b}	0.08	<0.01
True protein, kg/d	1.39	1.46	1.43	1.41	1.44	0.05	0.16
MUN, mg/dL	13.1^{ab}	13.6^{a}	12.5^{ab}	11.9^{b}	9.7^{c}	0.6	<0.01
$D_{4,3}$, µm	4.04^{xy}	4.12^{x}	4.07^{xy}	4.05^{xy}	3.79^{y}	0.11	0.09
Casein micelle size, nm	161	166	161	163	163	4	0.48
Lipolysis, mEq/100g fat	0.48	0.63	0.48	0.62	0.50	0.08	0.23

[1] Means within a row with different superscripts differ at $P \leq 0.05$ (a,b,c) or $0.05 < P \leq 0.10$ (x,y).

Pasture averaged 17.5% CP and 53% NDF, and pTMR 9.7% CP and 15% NDF. The experiment lasted 12 wk with 2 wk for a covariate period followed by 3 sampling periods during wk 4, 7, and 10. Individual herbage intake was estimated using Cr_2O_3 and in vitro DM digestibility of feeds. Fecal grab samples were taken 8 times over 5 d in each sampling period. Data were analyzed using the MIXED procedure of SAS with repeated measures over time. Herbage intake was lower (7.52 vs. 6.92 kg/d; $P < 0.01$) in LIN compared with CTRL, and pTMR intake tended to increase with feeding LIN (14.9 vs. 14.5 kg/d; $P = 0.07$). However, total intake of DM, OM, CP, and NDF was not affected by treatments. Similarly, milk yield (mean = 27 kg/d), concentrations and yields of milk fat and milk protein were not changed when cows received CTRL or LIN. In contrast, MUN concentration was lower ($P < 0.001$) in LIN (8.38 mg/dL) than CTRL (11.0 mg/dL). Treatments had no effect on the apparent total-tract digestibilities of DM (mean = 61%), OM (mean = 63%), CP (mean = 56%), and NDF (mean = 53%). Furthermore, no treatment effects were observed for BW change and BCS. In summary, LinPRO-R fed at 6% of diet DM did not affect milk yield and nutrient digestibility in grazing dairy cows.

Key Words: digestibility, flaxseed, grazing.

2313 Effects of dietary lipid supplements on milk production and raw quality in dairy cows.

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This study was conducted to evaluate the effects of 4 lipid supplements on milk production and technological properties. Ten multiparous Holstein cows (64 ± 21 DIM) were used in a replicated 5 × 5 Latin square design and fed a basal TMR without supplementation (CTL) or with 2% (DM basis) fatty acids (FA) provided as soybean oil (SO), calcium salts of palm FA (CS), hydrogenated tallow FA (HT) or palmitic acid-enriched supplement (PA). Periods lasted 21 d, with the last 5 d used for milk sampling and production measurements. Data were analyzed using the MIXED procedure of SAS, with cow as random effect. Intake of DM was similar between treatments (29.5 ± 0.6 kg/d; $P = 0.14$). Milk yield was increased with SO compared with CTL, whereas CS and PA tended to increase milk yield compared with CTL (Table 1). Milk fat content was decreased with SO compared with any other treatment and increased with PA compared with CS. Milk true protein content was greater for PA and CTL compared with CS or SO, but true protein yield was not affected. Energy-corrected milk (ECM) and fat yield were increased by PA compared with CTL or SO. Milk urea nitrogen (MUN) was lower with SO compared with any other treatment and was also lower for CS compared with PA. Fat globule diameter ($D_{4,3}$) tended to be greater for PA than SO. Casein micelle size and milk lipolysis (increase in milk free FA content after 24 h of storage at 4°C) was not affected by treatment. Overall, HT had limited impact on milk composition, while CS and SO decreased milk protein content and PA increased ECM compared with the CTL diet.

Key Words: dairy cow, lipid supplement, raw milk quality

2314 Hepatic metabolome of grazing dairy cows with or without environmental control during lactation.

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Twenty spring-calved multiparous Holstein cows (588 ± 26 kg body weight, 2.8 ± 0.1 body condition score) were used in a randomized block design. Cows grazed a mixed pasture (one session from 17:00 to 5:00 h, 35 kg of dry matter (DM)/d of herbage allowance), and were supplemented with a total mixed ration (9.5 kg DM/d, 30:70 forage:concentrate) in a compost barn (with fan and soakers; CB-GRZ; n = 10) or in an open pen (OP-GRZ; n = 10). The aim of this work was to characterize differentially enriched hepatic metabolic pathways, by means of metabolomic analysis, in mid-lactation cows exposed to different environmental conditions during summer. Milk production and composition, BW, and BCS were recorded and liver biopsies were collected at 135 ± 11 d in milk. Four days before biopsy collection the temperature humidity index (THI) ranged from 58 to 73 and was at least 9 h per day ≥70. The metabolome was analyzed using a targeted metabolomic approach through a gas chromatography/time-of-flight mass spectrometry method and results analyzed with, an orthogonal partial least squares discriminant analysis, the variable importance to projection (VIP) score was calculated, and metabolites with VIP score >1 were selected to perform a set enrichment analysis (MSEA) using Metaboanalyst 5.0. Volcano plots were used to determine differentially abundant metabolites among treatments. Energy corrected milk (ECM), BW and BCS were analyzed using a model which included treatment as a fixed effect. ECM yield (31.9 vs. 30.64 ± 1.1 kg/d for CB-GRZ vs. OP-GRZ), BW and BCS (665 vs. 669 ± 36 kg, 2.8 vs. 2.8 ± 0.1 body condition score, $P > 0.05$) did not differ between treatments. MSEA showed an enrichment of the gluconeogenic pathway (FDR <0.05). Phosphoenolpyruvate tended to be more abundant ($P = 0.06$) while, 3-phosphoglycerate, and glucose were more abundant ($P < 0.05$) for OP-GRZ than CB-GRZ cows (FC > 1.4). Despite similar ECM yield BW and BCS, our results showed an enrichment of the gluconeogenic pathway during mid lactation in summer, especially for cows exposed to environmental conditions during supplementation.

Key Words: liver, metabolites, gluconeogenesis

2315 Impact of heat stress and dietary lipids on plasma oxylipids in dairy cows.

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Heat stress can trigger inflammatory responses in dairy cows, which in turn may be modulated by unique oxylipids synthesized from dietary lipid substrates. Our objective was to evaluate the effects of heat stress and dietary lipid type on plasma lipoxygenase- (LOX), cyclooxygenase- (COX), and Cytochrome P450-derived oxylipid concentrations. Twelve lactating Holstein cows (38.5 ± 9.8 kg milk/d; 85 ± 33 DIM) were randomized into treatment in a replicated incomplete Latin square design with two 10-d periods. Treatments were: 1) Heat stress + fish oil (HS/n3; 8.3% EPA, 19% DHA; Max THI = 84), 2): Heat stress + corn oil (HS/n6; 55% linoleic acid;) and 3): Thermoneutral pair feeding + corn oil (TN/n6; Max THI = 64). Oils were abomasally-infused daily in 2 boluses (160 g/d). Blood samples were collected 4 h after abomasal infusion on d 0, 5, and 10 for lipid analysis by LC-MS/MS. Data were analyzed in a mixed model with repeated measures including cow and period as random effects, and treatment, time, and their interactions as fixed effects. Relative to HS/n6, HS/n3 resulted in reduced rectal temperatures and respiratory rates ($P < 0.05$), while increasing plasma concentrations of EPA, DHA and cytochrome P450-derived DiHDPA and EpDPE (>3-fold; $P < 0.05$). Relative to HS/n3, both HS/n6 and TN/n6 exhibited increased LOX-derived oxylipids such as 9-HODE, 9-oxo-ODE, 13-HODE (>30%; $P < 0.05$), whereas 15-HETE concentrations were reduced (−37%; $P < 0.05$). There was no effect of treatment on plasma isoprostanes or COX-derived lipids ($P > 0.30$). Partial alleviation of hyperthermia by fish oil was associated with changes in plasma oxylipid profiles in dairy cows under heat stress.

Key Words: fish oil, omega-3, lipidomics

Animal Health 3

2400 Supplementation with postbiotic products from *Saccharomyces cerevisiae* fermentation alters the lung transcriptome of preweaning calves given an experimental viral-bacteria coinfection. T. W. Maina[1], P. O. McDonald[2], B. E. R. Samuel[3], M. I. Sardi[1], I. Yoon[4], A. Rogers[1], and J. L. McGill*[3], [1]*Biotechnology R&D, Cargill, Minneapolis, MN,* [2]*Department of Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI,* [3]*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA,* [4]*Diamond V Mills Inc., Cedar Rapids, IA.*

Bovine respiratory disease causes morbidity and mortality in cattle of all ages. Supplementation with postbiotic fermentation products from *Saccharomyces cerevisiae* (SCFP; SmartCare and NutriTek; Diamond V) has been shown to improve the outcome of respiratory infections in preweaning calves. The objective of this study was to determine effects of SCFP supplementation on the transcriptional response to co-infection with bovine respiratory syncytial virus (BRSV) and *Pasteurella multocida* (*PM*) in the lung using RNaseq. Holstein-Angus cross calves (1–2 d old) were assigned to 2 treatment groups: 1) control: base milk replacer and calf starter; or 2) SCFP treated: milk replacer with 1 g/d SmartCare and calf starter top-dressed with 5 g/d NutriTek. Calves were infected with ~10^4 (Median Tissue Culture Infectious Dose, $TCID_{50}$) BRSV on d 21, followed 6 d later by intratracheal inoculation with ~10^{10} cfu of *PM*, strain P1062. Bronchoalveolar lavage (BAL) was collected antemortem (d 14, before BRSV infection) and postmortem (d 10 post viral infection) from control (n = 12) and SCFP supplemented (n = 11) calves. Lung tissue samples were collected from the same calves for RNA extraction and sequencing. Sequencing reads were aligned to the bovine reference genome (UMD3.1) and differential gene expression (DEGs) analysis was conducted using edgeR version 3.32.1 using a general linearized model with control and treatment as factors. The Benjamini and Hochberg correction was used to estimate the false discovery rate. The top enriched pathways in SCFP-treated lungs were associated with decreased expression of inflammatory genes and increased expression of plasminogen, supporting effective lung repair. A linear model analysis of BAL samples identified 254 DEG (FDR <0.05) that differed in the response to coinfection due to SCFP supplementation. The BAL samples revealed that calves treated with SCFP had milder transcriptional responses and different transcriptional patterns when responding to the coinfection compared with controls. The data suggest SCFP supplementation modulates immune function in the lungs and provides insight into the potential for SCFP products to serve as alternatives to antimicrobials.

Key Words: *Saccharomyces cerevisiae* fermentation products, bovine respiratory disease, preweaning calves

2401 Developmental adaptations of γδ T cells in blood and intestinal mucosa from birth until weaning in Holstein bull calves. L. R. Cangiano*[1,2], K. Lamers[2], M. F. Olmeda[2], C. Villot[3], D. C. Hodgins[4], B. A. Mallard[4], and M. A. Steele[2], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, ON, Canada,* [3]*Lallemand Animal Nutrition, Blagnac, France,* [4]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to characterize how systemic and colon tissue resident gamma delta (γδ) T cells develop from birth until weaning, and how weaning influences the intestinal microbiota and immune responses in the colon. A total of 36 of Holstein bull calves were enrolled in a longitudinal study, and blood and colon biopsy samples were collected on d 2, 28, and 42 of life. Lymphocytes were extracted from blood and colon biopsies and stained with fluorescent antibodies to determine the proportions of various γδ T cells subsets by flow cytometry. Additionally, colon biopsies were used to isolate microbial DNA. On d 2 of life γδ T cells accounted for 58.5% of the total lymphocytes in blood, and gradually decreased until weaning on d 42 to 38.2% ($P < 0.01$). Moreover, we further classified γδ T cells into different functional subsets according to the level of expression intensity of the coreceptors WC1.1 (effector function) and WC1.2 (regulatory function). The proportion of γδTCR$^+$ lymphocytes expressing WC1.1 decreased from d 2 to d 28 ($P < 0.01$), whereas no change was observed in the expression of WC1.2 on γδTCR$^+$ lymphocytes ($P = 0.11$). The proportion of γδTCR$^+$ intra-epithelial lymphocytes in colon increased by 50% during weaning on d 42 compared with d 28 of life ($P < 0.01$), and the expression of WC1.2 on γδTCR$^+$ lymphocytes doubled from d 28 to 42 ($P \leq 0.01$). Microbial diversity of the epimural microbial community increased from d 2 to d 28, as denoted by the chao1 ($P = 0.03$) and Shannon ($P < 0.01$) indices. A reduction in microbial diversity was observed during weaning denoted by the Chao1, Shannon, and Phylogenetic Diversity indices ($P < 0.01$). The reduction in microbial diversity during weaning was correlated with the increase in all γδ T cell subsets in colon ($P \leq 0.02$, Spearman' $\rho \geq 0.40$). These data suggest that ontological adaptations during early life coordinate expansion of γδ T cells to provide early systemic protection, as well as to steer immune tolerance at birth. Additionally, the increase of colonic γδ T cells on d 42 suggests a protective role of these cells during weaning.

Key Words: neonatal immunity, gut health

2402 Lysophosphatidylcholine administration increases circulating lysophosphatidylcholine and haptoglobin concentrations in calves. B. N. Tate[1], M. M. Deys[1], P. Deme[2], N. J. Haughey[2], and J. W. McFadden*[1], [1]*Cornell University, Ithaca, NY,* [2]*Johns Hopkins University School of Medicine, Baltimore, MD.*

Endotoxin exposure decreases circulating lysophosphatidylcholine (LPC) concentrations in dairy cattle. Moreover, subcutaneous administration of LPC impairs growth and induces an acute febrile response in calves. We aimed to define how LPC administration impacts circulating LPC and haptoglobin concentrations. In 43 healthy Holstein heifer calves (age 7 ± 3 d), 1 of 4 treatments were randomly assigned (n = 10–11/treatment): unsupplemented control (CON), mixed LPC (mLPC; 69% LPC-16:0, 25% LPC-18:0, 6% other), LPC-18:0 (pLPC), or milk replacer containing 3% lysolecithin (LYSO) for 5 wk. Calves received 5 subcutaneous (s.c.) injections of vehicle (10 mL of phosphate-buffered saline containing 20 mg/mL bovine serum albumin; CON and LYSO) or vehicle containing mLPC or pLPC to provide 10 mg of LPC/kg of body weight (BW)/injection every 12 h during wk 3 of life. Calves were fed a milk replacer containing 27% crude protein, 24% fat at 1.7% of BW per d (dry matter basis) until wk 6 of life (start of weaning). Starter (22% CP) and water were provided ad libitum. Blood samples were collected 0, 5 and 10 h, relative to the final injection. Circulating LPC and haptoglobin concentrations were measured using LC-MS and immunoassay, respectively. Data were analyzed using a mixed model with repeated measures including fixed effects of treatment, time, and their interaction. A Dunnett's test was used to compare treatments to CON. Plasma total LPC concentrations were greater for calves administered mLPC and LYSO at h 5 and 10, relative to CON ($P < 0.05$). Similar observations were observed for plasma LPC-16:0, −18:0, and −18:1. pLPC calves did not experience elevations in plasma LPC post final injection, relative to CON. Treatment did not modify plasma LPC-18:3. −20:5, or −22:5 concentrations. Serum haptoglobin concentrations were elevated with mLPC at h 5 and 10 post-final injection, relative to CON ($P < 0.05$). mLPC and LYSO did not modify serum haptoglobin concentrations, relative to CON. We conclude that mLPC and LYSO administration increased plasma LPC concentrations in pre-weaned calves, and the ability of mLPC to induce serum haptoglobin concentrations aligns with our prior evidence for immune activation.

Key Words: calf, lysophosphatidylcholine, haptoglobin

2403 Serum natural antibody IgM titers in colostrum-deprived and conventionally raised neonatal dairy calves.

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Colostral maternal immunoglobulin (Ig) is responsible for providing initial protection to neonatal calves as there is a lack of placental transfer of maternal Ig. Colostral Ig isotypes include IgG, IgM, and IgA. Natural antibodies (NAb), typically isotype IgM, are produced by the dam without exogenous antigenic stimulation. The ability of maternal NAb to bind conserved antigenic epitopes, paired with the high binding efficiency of IgM, may play an important role in protecting against neonatal infections, such as septicemia, in the first days of life. Keyhole limpet hemocyanin (KLH) is commonly used as a surrogate antigen to evaluate NAb since exposure to KLH is uncommon in cattle. The objective of this study was to assess and compare the concentration of serum NAb IgM in conventionally raised and colostrum-deprived (CD) calves. Blood was collected from 40 conventionally raised dairy calves aged 2 to 5 d. Banked sera were also available from 16 CD dairy calves aged 0 to 2 d. Serum NAb IgM binding KLH was measured using an enzyme-linked immunosorbent assay. Titers are reported as the $\log_2$ of the reciprocal of the calculated titer (limit of detection = 2.80). Titers from conventional and CD calves were compared using the Wilcoxon-Mann-Whitney nonparametric test. In conventional calves, serum NAb IgM $\log_2$ titers ranged from <2.80 to 8.93, with a median of 6.82. In conventional calves, 1 of the 40 calves had a titer below the limit of detection. In CD calves, NAb IgM titers were low and all below the limit of detection (<2.80). Titers from CD calves were significantly lower than those of conventionally raised calves ($P < 0.01$), showing the importance of colostral transfer. Further investigations of the endogenous production of NAb IgM in calves and the protective benefits are needed.

Key Words: natural antibody, colostrum-deprived, colostrum

2404 RNA-Seq reveals induced calves' mucosal immune response to *Cryptosporidium parvum* infection.

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Cryptosporidium parvum (*C. parvum*) is a protozoan parasite best known for causing diarrhea in pre-weaning calves. Parasite clearance requires a coordinated host immune response involving complex immune-associated signal transduction and communication between epithelial and hematopoietic immune cells, which are not yet fully understood. Herein, we used next-generation sequencing to identify the key molecular players associated with establishing host immune reactions to *C. parvum* infection. Neonatal calves (Holstein-Friesian) were either infected (n = 5) or not (CTRL, n = 5) by oral application of 2×10^7 *C. parvum* oocysts (LE-01-Cp-15 strain) at d 1 of life. The infection was confirmed by clinical observation of calves' fecal consistency and quantification of fecal shedding using an immunofluorescence assay kit MERIFLUOR Cryptosporidium (Meridian Bioscience). Total RNA was extracted from the jejunal mucosa of slaughtered calves on d 8, and sequenced using an Illumina HiSeq 2500 (Illumina). The reads were mapped against the bovine reference genome (ARS-UCD 1.2). Differential expression analysis between the 2 groups was performed with DESeq2 and the list of upregulated genes [$\log_2$ (fold change) >1.5, false discovery rate <0.05] was used for pathway enrichment analysis using the ClueGO (v2.5.9) in Cytoscape. In total, 12,908 genes were expressed in calves' mucosa, of which 193 genes were downregulated and 87 were upregulated in the infected group. A UniProt immune system process database highlighted that upregulated genes, including chemokine (C-C motif) ligand (CCL)3, CCL4, CCL24, and CCL26, SAM and HD domain-containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1), interferon-gamma (IFNG), and lactotransferrin (LTF) were associated with eosinophil, lymphocyte, monocyte, and neutrophil chemotaxis, B cell activation, and T cell differentiation. The up-to-date model suggests that in response to *C. parvum* infection, the host immune system initiates an inflammatory response by activating NF-KB, which then induces the transcription of inflammatory mediators, and triggers antimicrobial production. Our study provides an update on the molecular markers of immune responses and the interaction between innate and adaptive immune pathways in epithelial and hematopoietic immune cells in calves infected with *C. parvum*.

Key Words: transcriptome, immunity, chemotaxis

2405 Effects of transport age on hematological parameters and growth performance in dairy calves.

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Transport-related stress contributes to pre-weaning calf mortality and morbidity in the United States. Although recognized as a determinant of outcomes, evidence-based recommendations on the minimum age at transportation are still lacking. Therefore, our objective was to evaluate the impact of transport age on hematological parameters and growth performance over the first 7 d post-transport in Charolais × Holstein crossbred calves. Bull calves (n = 20) ages 2, 4, 8 and 14 d old were transported from a commercial farm to an on-campus calf rearing facility (410 miles). Blood samples collected from calves before transportation and on arrival were analyzed for hematological parameters. Calf body weight (BW) was recorded before departure, on arrival and 7 d post-transport. Milk replacer (MR) intake was recorded during the first 7 d post-transport. Data were analyzed using PROC MIXED of SAS. Except for red cell distribution width (RDW) count and proportion, which were lower ($P \leq 0.02$) for 2- and 4-d-old calves compared with 8- and 14-d-old calves, there were no differences in hematological parameters before transportation. However, on arrival, both lymphocyte count and proportion were lower ($P = 0.02$) for 2-d-old than 4-, 8-, and 14-d-old calves. Neutrophil proportion at arrival was greatest ($P < 0.01$) for 2-d-old calves, intermediate for 4-d-old calves, and lowest for 8- and 14-d-old calves at arrival. Monocyte count ($P = 0.10$) and proportion ($P = 0.07$) tended to be greater for 8-d calves at arrival compared with the rest. The RDW count was also lower ($P < 0.01$), whereas RDW proportion tended to be lower ($P = 0.08$) for 2- and 4-d-old calves compared with 8- and 14-d-old calves. Platelet count tended to be lower ($P = 0.10$); whereas mean platelet volume was lower ($P = 0.04$) for 2-d-old than 4-, 8-, and 14-d-old calves. However, transport age had no effect ($P \geq 0.34$) on MR intake, BW change, and average daily gain post-transport. Overall, transportation led to differences in the hematological profile across ages, suggestive of an impact on immune function; however, there were no differences in growth performance during wk 1 post-transport.

Key Words: calf growth performance, hematology, transport age

2406 Exploring preweaning dairy calf mortality risk factors in Ontario.

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The objective of this cross-sectional herd level study was to identify the association of calf management practices in dairy farms with preweaning mortality. From April to August 2022, a convenience sample of 100 dairy farms from Ontario, Canada were visited once. A questionnaire covering farm biosecurity, calving, colostrum management, preweaning nutrition and housing was also administered on-farm. Data regarding preweaning calf mortality was retrieved from each farm herd management software as the total count of calves that survived, culled, and died during the preweaning period for the 365 d prior to the farm visit. The preweaning period considered was from 3 to 60 d of life. Preweaning mortality was defined as the rate of calf deaths per 60 calf-days for each farm. The herd-level preweaning mortality ranged from ranged 0 to 3.0 deaths/60 calf-days with an average of 0.5 ± 0.71 (SE) deaths/60 calf-days. A multivariable Poisson regression model was used to assess factors associated with preweaning mortality. Raising calves in groups of 6 or more per pen increased the rate of preweaning mortality ($P < 0.01$). Compared with housing calves using a mix of indoor and outdoor approach, consistent indoor housing ($P < 0.01$) or consistent outdoor housing ($P < 0.01$) decreased the rate of preweaning mortality. Offering calves more than 6 L of milk/day after 3 weeks of life decreased the rate of preweaning mortality ($P < 0.01$). In operations where farmers had achieved a level of education higher than high school ($P = 0.02$) and the herd veterinarian inquired always ($P = 0.01$) or sometimes ($P < 0.01$) during every herd visit about calf health a decreased rate of preweaning mortality was observed. The current results highlight novel factors associated with preweaning mortality, such as the farmer level of education and the intervention of the herd veterinarian in calf health.

Key Words: neonatal, health, herd

2407 The effect of benchmarking reports on the health of surplus calves.

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Appropriate early-life care is necessary for optimal health of surplus dairy calves. Health data collected at calf dealers and delivered back to source dairy farms may motivate early-life care. We hypothesized that benchmarking reports would result in improved metrics for transfer of passive immunity, hydration, and navel health in surplus calves delivered to calf dealers. Farms delivering the largest number of calves to 2 calf dealers were allocated to receive benchmarking reports (intervention group, n = 6) or not (control group; n = 7). Farms were allocated to achieve balance based on herd size and frequency of failed transfer of passive immunity (FTPI) in previously delivered calves. Two calf dealers were visited 2 to 3 times/wk from May 2021 to March 2022. About 6 mo post enrollment, reports were delivered detailing metrics for passive transfer, hydration, and navel health relative to the other farms in the study. Health assessments and serum samples for total protein measurement were collected within 48 h after calf arrival at the dealer for a total of 282 and 371 calves from intervention and control farms, respectively. Generalized linear mixed models were fitted to observations collected at the calf level. Random effects specified farms as both the experimental unit for intervention and the blocking structure for a before-after assessment. Model estimates for overall probability of navel infection and FTPI were 20.5% (95%CI = [15.5, 26.5]) and 21.4% [13.9, 31.6], respectively, and were not significantly different between intervention and control farms at either time points. The estimated probability of calves with dehydration in intervention farms was 69.5% [51.4, 83.1] and 46.5% [29.2, 64.7] before and after the intervention, respectively. Following intervention, dehydration was marginally less likely in the intervened farms relative to controls (OR = 0.29 [0.07,1.13], $P = 0.07$). These preliminary results suggest that delivering surplus calf health data back to dairy farms may help prevent calf dehydration. Additional work is necessary to ensure generalizability and to understand underlying mechanisms.

Key Words: dairy calves, health, benchmarking

2408 Does providing a rest period mitigate the impact of long-distance transportation on markers of energy status in surplus dairy calves?

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Long-distance transportation of surplus dairy calves has been shown to impact blood parameters when measured on arrival to calf-raising facilities. The objective of this randomized controlled trial was to determine if providing a rest stop mitigates the impact of transportation on markers of energy status in calves transported by road for 16 h. This study was conducted between September to November 2022 and included surplus dairy calves ≥7 d old from a commercial dairy farm in Ontario, Canada (n = 64). Calves were enrolled 1 d before transportation and randomly assigned to one of 2 treatment groups: 1) continuous transportation by road for 16 h or 2) 8 h of transport, 8 h of rest, then a further 8 h of transport to a single calf-raising facility. Calves that received a rest stop were fed 2 L of milk replacer at the time of unloading for the rest period and again before reloading for the second leg of transportation. Blood samples were collected immediately before loading and after unloading at the destination, as well as d 1, 2, and 3 following unloading. Serum was analyzed at a commercial laboratory for β-hydroxybutyrate (BHBA) and nonesterified fatty acids (NEFA), while glucose concentration was assessed using an iStat Alinity and CG8+ cartridge within 1 h of collection. Mixed models with repeated measures were built to evaluate the impact of providing a rest stop on these markers of energy status. Immediately following transportation, BHBA (−60.75 mmol/L, $P = 0.001$, 95% CI −95.02 to −26.49) and NEFA (−0.19 mmol/L, $P < 0.001$, −0.26 to −0.11) were lower in calves provided a rest stop compared with continuously transported calves. Furthermore, glucose was higher on arrival in rested calves (0.48 mmol/L, $P < 0.001$, 0.23 to 0.73). These results demonstrate that providing a rest stop can reduce markers of energy mobilization and may improve the condition of calves on arrival to veal and dairy-beef industries.

Key Words: male dairy calf

2409 Effect of group housing of preweaning dairy calves on health and fecal shedding of antimicrobial resistant *Escherichia coli* and *Enterococcus* spp. M. J. Breen*[1], D. R. Williams[1], E. M. Abdelfattah[1,3], B. M. Karle[4], T. W. Lehenbauer[1,2], and S. S. Aly[1,2], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare, CA,* [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA,* [3]*Department of Animal Hygiene, and Veterinary Management, Faculty of Veterinary Medicine, Benha University, Moshtohor, Qalyubia, Egypt,* [4]*Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Davis, Orland, CA.*

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The study objectives were to investigate the effects of group housing on bovine respiratory disease (BRD), diarrhea and acquisition of antimicrobial resistance (AMR) in fecal commensal *Escherichia coli* (EC) and *Enterococcaceae* (ES) in preweaning dairy calves on a California dairy. The experimental arm of our study randomized calves to either individual (IND; n = 21) or group (GRP; n = 21) housing, using a California wooden hutch with its partitions removed, creating a group of 3 calves. The study period lasted from birth to 56 d. Calves were health scored daily and incidence estimated for disease. A prospective cohort study assessed AMR outcomes; fecal samples were collected from each calf 3 times/week and minimum inhibitory concentrations (MIC) for EC and ES were evaluated using the broth microdilution method. Treatment records were used to select calves exposed to an antimicrobial drug (AMD) treated calf. In GRP, exposure occurred when a calf was a hutchmate with an AMD-treated calf. In IND, exposure occurred when a calf neighbored an AMD-treated calf. Proportions of AMR among exposed and unexposed calves were used to compare AMR between GRP and IND. Group calves had 1.94 times the BRD treatment hazard of IND calves. There was no difference in incidence of BRD or diarrhea between GRP and IND [BRD (%, SE); GRP: 66.66%, 10.29, IND: 47.62%, 10.90]. All but one calf was diagnosed with diarrhea. For AMR in EC isolates, unexposed GRP calves had lower AMR to ceftiofur compared with exposed calves {Unexposed [% (95% CI)]: 17.24 (3.49–30.99), Exposed: 60 (40.80–79.20)}. There were no differences in proportions of ES AMR between GRP and IND across the study AMD. This study showed an increase in BRD hazard for GRP and demonstrated that GRP calves are capable of transiently acquiring AMR from treated hutchmates, when compared with GRP calves unexposed to AMD treatment.

Key Words: group housing, bovine respiratory disease, antimicrobial resistance.

2410 Seasonality of colostrum Brix values and total serum protein of newborn dairy female calves in a temperate climate. B. Gonçalves da Costa*, K. Sharpe Moser, M. Endres, and B. Heins, *University of Minnesota, Minneapolis, MN.*

Newborn calves obtain their primary immune protection from immunoglobulins (IgG) in the colostrum. Successful absorption of enough IgG depends on colostrum IgG concentration, quantity of colostrum intake, and timing of intake. The aim of this study was to investigate if total serum protein (TSP) of newborn dairy female calves varied between fall and spring calving seasons. The experiment was conducted at the University of Minnesota West Central Research Outreach Center (WCROC), Morris, Minnesota. The WCROC has 2 calving seasons per year: fall (September to December) and spring (March to June). In this study were enrolled 263 female calves from 3 different breeds: Grazecross (n = 62), ProCross (n = 130) and Holstein (n = 71) born during fall 2020 (n = 69), spring 2021 (n = 44), fall 2021 (n = 57), spring 2022 (n = 30), and fall 2022 (n = 63). Calves were separated from dams during the first day of life, weighed (BW), and fed 4 L of colostrum from their dams in the first 12 h after birth. Colostrum samples were collected during the first milking after calving and a Brix value was obtained using a MISCO digital refractometer. Blood samples were collected 24 to 48 h after birth, centrifuged, and TSP were obtained using a MISCO digital refractometer. For statistical analysis, the fixed effects included season (fall or spring) and Brix and breed was a random effect. Year was also included as a fixed effect for Brix analysis. Calves born during fall (5.8 g/dL ± 1.2; BW: 38.4 kg ± 6.1) had higher ($P < 0.01$) TSP than calves born during spring (5.4 g/dL ± 1.0; BW: 40.7 kg ± 5.3). Breed and Brix values did not affect TSP ($P > 0.05$). Colostrum Brix values did not vary across fall and spring seasons ($P = 0.78$). The Brix value from fall 2021 was lower than all other seasons ($P < 0.05$), except for spring 2021 ($P = 0.14$). The results suggest that seasonality may influence IgG absorption.

Key Words: colostrum, seasonality, IgG

2411 Social housing: Impacts on health scores and gut microbiome of dairy calves. B. Gonçalves da Costa*, K. Sharpe Moser, M. Endres, A. Gomez, and B. Heins, *University of Minnesota, Minneapolis, MN.*

The aim of this study was to investigate the effects of social housing on the health scores of pre-weaning dairy calves and changes in the gut microbiome during weaning. A total of 343 Holstein (n = 79), ProCross, (n = 169), and GrazeCross (n = 97) calves were evaluated from fall 2020 to fall 2022. At 3 d of age, calves were divided into 4 housing groups: individually housed (n = 87), pair-housed (n = 86), group-housed (6 calves per pen; n = 84), and dam-reared (6 dam-calf pairs per paddock; n = 69) until weaning at 63 d of age. Health scores were weekly collected using the Wisconsin Calf Health Scoring Chart until weaning. Fecal samples were collected from dam-reared (n = 11), group-housed (n = 19), and individually housed (n = 18) calves before and 3 d after weaning in fall 2020 and sent for 16S rRNA amplicon sequencing. Statistical analyses for health scores were performed using PROC GLIMMIX of SAS with housing type, age, calf, breed, and season as fixed effects. Gut microbiome sequence data were analyzed using Qiime2 and the R statistical interface for ecological analyses. There were no significant breed effects on the treatments ($P > 0.05$). Respiratory scores were affected by season ($P = 0.03$), but not by housing type ($P < 0.05$). Across treatments, calves had higher fecal scores during the spring and fall of 2022 ($P < 0.05$), and fecal scores increased after wk 2 of life. Dam-reared (6.7 ± 1.16%) and group-housed (7.2% ± 1.19%) calves had higher fecal scores than pair-housed (3.1 ± 0.62%) and individually housed (2.9 ± 0.61%) calves. Before weaning there were no differences in microbiome diversity between treatments ($P > 0.05$). After weaning, individually housed calves had the tendency to have lower richness (Kruskal-Wallis $P = 0.04955$; W = 0.08). The microbiome of dam-reared and group-housed calves showed enrichment of gut bacteria such as lactobacilli and bifidobacteria, before and after weaning. Individual housing did not appear to have specific stimulating roles in the calf gut microbiomes. Our results suggest that social housing might affect fecal scores, while simultaneously stimulating the abundance of potentially beneficial bacteria in the gut.

Key Words: social housing, calves, gut microbiome

Breeding and Genetics Symposium: Breeding for Resilience in Dairy Animals

2412 Getting to grips with resilience: Toward large-scale phenotyping of this complex trait. N. C. Friggens*[1], G. Lenoir[2], and M. Ithurbide[3], [1]*INRAE, Campus Agro Paris-Saclay, Palaiseau, France,* [2]*AXIOM, Azay-sur-Indre, France,* [3]*INRAE, GenPhySE, Université de Toulouse, Castanet Tolosan, France.*

This paper will discuss the issues involved, and review recent work, in phenotyping resilience, i.e., an animal's ability to respond to and recover from environmental disturbances. As there is no single direct measure of resilience several proxy measures have been proposed for use in population level phenotyping. These are frequently estimates of perturbations in performance trajectories, which can be quantified in different ways. For such proxies to be of value they need to have been shown to be related to the accumulated consequences of a good resilience. These accumulated consequences may be measures such as productive longevity, total number of recorded disease events, etc. The rationale for this, and limitations, are discussed. Further, given that there is no single direct measure of resilience there are recent initiatives advancing the state-of-the-art in deriving resilience measures from multivariate analyses of time-series measures of multiple indicators. Examples are presented using functional PCA coupled with hierarchical clustering. Finally, prospects for inclusion in selection programs are discussed.

Key Words: resilience, modelling, proxies

2413 When, why, and how to breed for disease resilience in livestock. A. Doeschl-Wilson*[1], P. W. Knap[2], M. Ghaderi-Zefreh[1], and R. Pong-Wong[1], [1]*The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Estate, Scotland, UK,* [2]*Genus-PIC, Schleswig, Germany.*

Infectious diseases are a major threat to sustainable production of high-producing animals such as dairy cattle. Breeding for increased disease resilience has been a research focus for many years, but effective implementation into practical breeding programs is still lacking as this complex genetic trait is extremely difficult to estimate. Here we investigate how future breeding programs may benefit from recent research on disease resilience. We consider 2 alternative existing definitions of individual disease resilience as either a reaction-norm of host performance on environmental pathogen burden, or as the animal's capacity to resist or recover from the perturbation caused by an infection. We investigate the corresponding data requirements for accurately estimating individuals' genetic merit for disease resilience and the potential implications on individual and herd health and performance. In particular, we demonstrate that in the case of infectious diseases, herd disease resilience is more than the sum of the resilience of its herd members, but also depends on host traits affecting pathogen transmission. We conclude that the most promising break-throughs in genetic improvement of disease resilience rely on technological innovations such as automated data generation, and on methodological developments for analysis.

Key Words: disease, resilience, breeding

2414 Genetics of heat tolerance in dairy cattle. F. Peñagaricano*, *University of Wisconsin–Madison, Madison, WI.*

Heat stress negatively impacts the performance of dairy cows, causing huge economic losses to the dairy industry. The first objective of this study was to reveal the genetic basis of thermotolerance in Holstein cows. Multi-trait repeatability test day models with random regressions on a function of temperature-humidity index values were used for the analyses. Heritability estimates for milk production and conception under heat stress were around 20% and 3%, respectively, suggesting that the ability of a dairy cow to produce and conceive under heat stress is influenced by genetic factors, and hence it could be improved by genetic means. Notably, genetic correlations between general and thermotolerance additive genetic effects were negative, indicating an unfavorable relationship between cows' ability to either produce milk or conceive under thermo-neutral versus thermo-stress conditions. Therefore, the continued selection for greater productivity and fertility ignoring heat tolerance will result in even greater susceptibility to heat stress. Whole-genome scans identified genomics regions that harbor genes directly implicated in the cellular response to stress, heat shock protein binding, regulation of DNA repair, and oxidative stress. Our second objective was to examine the effects of intrauterine heat stress on the epigenome of the offspring. Intrauterine insults, such as thermal stress, can induce permanent changes in the structure, physiology, and metabolism of the offspring. This phenomenon is known as fetal programming and could have significant implications on food animal production. Our results showed that in utero heat stress alters the DNA methylation profile of fetal liver and mammary gland, and programs their morphology in postnatal life. These epigenetic changes may contribute to the poorer performance of in utero heat stressed calves. Overall, our findings contribute to a better understanding of the genetic and epigenetic basis underlying dairy cattle performance under heat stress.

Key Words: fetal programming, heat stress, temperature-humidity index

2415 Gene editing for improved health and resiliency. T. Sonstegard*, *Acceligen, Eagan, MN.*

Genome editing in the genetic stocks of food animals has tremendous potential as a breeding method best-suited to introduce traits not readily available for intensive selection in commercial breeding populations, especially those affecting disease resistance and adaptation to climate. To date, gene editing has been used to alter prolactin receptor and produce heat-tolerant, registered Angus for commercial production of semen and embryos that rapidly allows beef production improvement in the tropics. More recently, genome alterations based on rationale design have been deployed and tested to demonstrate genetic improvement of resistance to porcine reproductive and respiratory syndrome virus and bovine viral diarrhea virus in swine and cattle, respectively. This presentation will highlight the results of these new traits to breed healthier commercial animals that will eventually change global market dynamics of livestock production.

Key Words: gene editing, disease resistance, heat tolerance

Dairy Foods Symposium: Continued Challenges in Controlling Dairy Spoilage

2416 Understanding next-generation dairy spoilage bacteria. T. S. Oberg*, *Utah State University, Logan, UT.*

Defects in aged cheese continue to be a problem in the industry even with advances in cheese production technology, starter and adjunct culture selection, and modern microbial isolation and identification technologies. One reason is the difficulty in growing and isolating the causative microorganisms of these defects so that we can understand the mechanisms they utilize for growth, which can generate defects in cheese. Recently, *Paucilactobacillus wasatchensis*, a novel heterofermentative non-starter lactic acid bacteria (NSLAB) which causes late gas defects in commercially aged cheese has been identified. This bacterium grows very slowly under normal laboratory testing protocols and, hence, would go undetected utilizing most NSLAB isolation methodologies. Whole-genome sequencing of *Pa. wasatchensis* identified several potential strategies that the bacteria can use to grow and thrive in an aged cheese including the ability to grow on ribose as the sole carbon source, the ability to ferment gluconate, and the presence of several amino acid decarboxylases. In vitro testing and challenge studies have validated these pathways and shown that *Pa. wasatchensis* can cause late gas defects in cheese through the production of CO_2 even when the typical hexose sugars generally implicated in gas production are not present. These studies have shown that *Pa. wasatchensis* can be useful as a type strain for the study of hard-to-grow microorganisms present in cheese and can increase our understanding of their source, how these organisms thrive in aged cheese, and how modern dairying and manufacturing processes can unintentionally increase the risk of problem products.

Key Words: *Paucilactobacillus wasatchensis*, gas production, non-starter lactic acid bacteria

2417 Sources, transmission, and tracking of sporeforming bacterial contaminants in dairy systems. N. Martin*, *Cornell University, Ithaca, NY.*

Sporeforming bacteria are pre- and post-pasteurization contaminants in dairy systems. At the farm, spores transfer from soil, water, manure and other sources into raw milk primarily at the point of milking. In processing facilities, spores may persist for long periods of time in equipment or in the environment, where they can subsequently contaminate dairy products. Implications of spore contamination include premature spoilage in fluid milk and cheese, reduced specification compliance in dairy powders and ingredients, and represent a biological barrier to shelf-life extension for reaching new markets. Molecular subtyping for sporeforming bacteria in dairy systems represents a key tool for tracking and eliminating sources of spore contamination. In particular, sequencing of the *rpoB* gene has been used successfully for tracking spore contaminants throughout the dairy continuum. Here we explore case studies for the successful implementation of *rpoB* subtyping to reduce spore contamination in dairy products, best practices for developing baseline spore subtyping data, and digital tools that amplify the impact of this subtyping data.

Key Words: spores, subtyping, tracking

2418 Moo-deling the Dairy-verse: Using computer modeling to get more out of our testing results. A. Trmcic*, *Milk Quality Improvement Program, Cornell University, Ithaca, NY.*

The age of Industry 4.0 is upon us, and the dairy industry is moving forward with the rest of the world towards a more digitalized and automated future. With this move the volume of data that are being generated and the speed at which they are being generated is increasing exponentially. While the majority of these data are still being used for day-to-day activities by the Dairy Industry, there is an added value in them that is potentially being lost because using the data from the past can help us predict the future. This symposium will cover how these generated data can be used to build computer models that can be used by the Dairy Industry as support tools in decision-making. This symposium will cover spoilage prediction computer models that were developed in recent years for application in fluid milk, yogurt, and cheese manufacturing. The speaker will present on the process of development, validation and use of these prediction models, as well as how these models might impact the interaction the consumers have with our dairy products. The key goal of this symposium is to introduce the benefits of computer modeling and open the door to future innovations in the industry captured under the common term Digital Dairy.

Key Words: spoilage, computer modeling, digital dairy

2419 The application of protective cultures for yeast and mold control in fermented dairy products. S. Neuens*, *Chr. Hansen Inc., Milwaukee, WI.*

Bioprotection is the art of using natural microbial food cultures to inhibit unwanted contaminants—preventing food spoilage or improving food safety. The process of fermentation has been used from ancient times to the present day as a natural way of preserving and protecting the quality of foods. Chr. Hansen takes this a step further by identifying and selecting the best of the good bacteria to supply as commercial food cultures with bioprotective effect under the brand FRESHQ to the dairy industry. These cultures are used to enhance the inhibition of yeast and mold growth in fermented dairy products throughout shelf life. This presentation will focus on the application of bioprotective cultures for yeast and mold control in fermented dairy products. Explore how these cultures work in fermented dairy applications. Reveal the Chr Hansen's team of scientists' discovery of the main mechanism responsible for delaying the growth of yeast and mold. And further, provide a framework on how Chr Hansen works with dairy processors to not only demonstrate the effect of bioprotective cultures but to show the value of using such a product. The application of food cultures with bioprotective effect is becoming more widespread, and for good reason. Fermentation-enabled bioprotection, such as Chr Hansen's FRESHQ, helps fight spoilage to extend shelf life, reduce the need for artificial preservatives, cut down waste and enable consumers to get the most out of the foods they buy.

Key Words: bioprotection, yeast, mold

Dairy Foods 2: Dairy Products and Processing

2420 Withdrawn.

2421 Verifying origin claims on dairy products using stable isotope ratio analysis and random forest classification. R. O'Sullivan*, R. Cama-Moncunill, O. Schmidt, M. Salter-Townsend, and F. J. Monahan, *University College Dublin, Belfield, Dublin, Ireland.*

The ability to underpin geographic origin claims scientifically is necessary to improve consumer trust in food labels since origin labels are often used by consumers as an external indication of food quality. Stable isotope ratio analysis (SIRA) is an analytical technique that can be used to support the verification of the origin of food products based on naturally occurring differences in isotopic compositions acquired during the production process. In this international survey of commercially traded samples, SIRA of 5 relevant elements (C, H, N, O, S) was conducted on the casein fraction of butter (n = 60), cheese (n = 96) and whole milk powder (WMP; n = 42) samples. The samples were divided into 4 geographic regions based on their commercial origin: Ireland (n = 79), Other Europe (n = 67), Oceania (n = 30) and USA (n = 22). A random forest machine learning model was built using the $\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$ values of all dairy products (n = 198) which accurately (88% model accuracy rate) predicted the region of origin of the samples with class accuracy of 95% for Irish, 71% for Oceania, 86% for Other European and 89% for USA dairy products. It is concluded that SIRA and random forest classification can be used as an accurate method to underpin origin label claims on dairy products.

Key Words: traceability, origin, authentication

2422 Improved detection of antibiotic residues in near-infrared milk spectra using recursive feature elimination, principal component analysis, and machine learning clustering algorithms. K. Rodriguez*[1], S. LeBlanc[2], C. Baes[1,3], R. V. Ventura[4], J. Balieiro[4], and D. Tulpan[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada,* [3]*Vetsuisse Faculty, Institute of Genetics, University of Bern, Bern, Switzerland,* [4]*Department of Nutrition and Animal Production (VNP), School of Veterinary Medicine and Animal Science (FMVZ), University of São Paulo, São Paulo, State of São Paulo, Brazil.*

The use of antibiotics for preventing and treating illnesses in dairy cattle is a common veterinary practice. As such, even though antibiotic residues are rare in the milk leaving dairy farms, screening for these residues in shipments received at milk processing plants is essential. However, the sensitivity of traditional methods can lead to false violations of maximum residue limits. Our aim is to evaluate a novel alternative screening method: pairing near-infrared (NIR) spectroscopy with machine learning (ML) classification models for detection of residues of common, approved veterinary drugs in milk. If validated, this approach could offer automation potential and improved accuracy for the detection of non-saleable milk. In the preliminary stages of this study, NIR spectra from 26 milk samples artificially contaminated (spiked-in) with known concentrations (10 ppb, 50 ppb, 250 ppb, 1,250 ppb, 6,250 ppb, 6,650,000 ppb, 13,330,000 ppb) of amoxicillin were processed and analyzed. Three data sets (the full spectra, the spectra of manually selected wavelengths, and the spectra of wavelengths selected using recursive feature elimination [RFE) were subjected to qualitative analysis, principal component analysis (PCA), and 3 ML clustering algorithms: K-means, agglomerative, and spectral clustering. To evaluate the quality of clusters produced by the ML algorithms, the following metrics were considered: silhouette score, Davies-Bouldin score, completeness and homogeneity. Results revealed amplitude variation in the spectra that corresponded to amoxicillin concentration, as well as distinct separation between unadulterated samples, samples with low-level contamination, and samples with high-level contamination via PCA and ML clustering. Additionally, feature selection using RFE yielded equal or better performance across all evaluation metrics and clustering algorithms when compared with analyses applied to the full spectra, confirming an improved capability of distinguishing between samples with different contamination levels.

Key Words: near-infrared spectroscopy, antibiotic residues in milk, machine learning

2423 Evaluation of the biocompatibility of exopolysaccharide-producing lactic acid bacteria strains in a stirred yogurt model. A. Miteul*[1,2], A. Schera[1,2], M.-H. Lessard[1,2], M. Lafantaisie[1], S. Fraud[3], D. Miller[4], S. L. Turgeon[1], and S. Labrie[1,2], [1]*Department of Food Science, Institute of Nutrition and Functional Foods (INAF), STELA Dairy Research Centre, Université Laval, Quebec City, QC, Canada,* [2]*Laboratoire de Mycologie Alimentaire (LMA), Quebec City, QC, Canada,* [3]*Yoplait General Mills–Vienne Technical Center, Vienne, France,* [4]*General Mills, Minneapolis, MN.*

In response to the demand for clean label products this project aims to replace texturizing additives in yogurts without reducing their rheological qualities. A promising strategy is the incorporation of lactic acid bacteria (LAB) as adjunct strains. Some LAB are natural exopolysaccharides (EPS) producers: carbohydrate polymers with the intrinsic ability to modulate yogurt texture. From a panel of 537 LAB, 8 were pre-selected based on their EPS-related genomic features and ropiness. They were added independently, along with the commercial low-texturizing starter YO-MIX 511, to evaluate their impact on rheological properties of stirred yogurt. Flow curves were generated by recording shear stress as a function of shear rates from 0.1 to 250 s^{-1}, and fitted to the power-law model ($\eta = K\gamma^{n-1}$) to calculate apparent viscosity (η) and consistency index (K). Thixotropy was calculated as the area between the upward and downward flow curves. Statistical analyses were carried out by using generalized linear mixed models procedure. Compared with the low texturizing control, the addition of *Lacticaseibacillus paracasei* LMA-1793 significantly ($P < 0.05$) increased the viscosity, thixotropy and consistency index and *Streptococcus thermophilus* LMA-1781 showed a similar trend. *Lactiplantibacillus plantarum* LMA-1907 significantly decreased the consistency index and tend to decrease apparent viscosity and thixotropy. Biocompatibility of these adjuncts with commercial starters was evaluated during fermentation using massive amplicon sequencing (metabarcoding) targeting the 16S rDNA region V3–V4. At the beginning of fermentation, adjunct strain accounts for 15% of the total population (5×10^6 cfu/g) and reaches 3×10^7 cfu/g after 5 h ± 30 min (pH 4.6). Growth of starter strains was not affected by the addition of adjuncts, except for *L. plantarum* LMA-1907 that stimulated the growth of *S. thermophilus*. Ongoing transcriptomics data analysis will highlight changes in the metabolic activity of the yogurt ecosystem during fermentation and help understand expression profiles of genes related to the production of EPS.

Key Words: yogurt, exopolysaccharides, biocompatibility

2424 Modeling the meltdown behavior of ice cream. M. Azeem Ur Rehman Alvi and S. Martinez-Monteagudo*, *New Mexico State University, Las Cruces, NM.*

A desirable characteristic of any type of ice cream is its gradual meltdown as the environmental temperature increases. The meltdown behavior is commonly determined by a gravimetric test, and it is used to obtain the onset, rate, and maximum meltdown. However, these parameters are calculated ambiguously due to the lack of consistency in the reported methodology. This work aims at modeling the meltdown curves (weight vs time) of different commercial samples. About 40 g of ice cream were placed on a suspended wire mesh (6 holes per cm). The temperature of the ice cream and dripped weight were continuously recorded throughout the duration of the test. The meltdown test was conducted at room temperature. Each meltdown test generated more than 2,500 data points and was modeled using 4 equations: the logistic model, the Gompertz model, the Richard model, and the Hill model. All the meltdown curves were sigmoidal in shape, regardless of the type of ice cream. The experimental meltdown curves were adequately represented by the Logistic model, judging by several criteria ($R^2 = 0.999$, adjusted $R^2 = 0.998$, Akaike probability = 0.294, and F-value = 2.7×10^6). Thus, the logistic model is shown to be an effective tool for predicting the meltdown curves of ice cream, and it can be used to define unambiguously the onset, rate, and maximum meltdown.

Key Words: meltdown, modeling, ice cream

2425 A decision tool to determine economic feasibility of on-farm bottled milk operations. C. Zaring*[1], K. Jensen[2], A. Rihn[2], M. Morgan[3], and E. Eckelkamp[1], [1]*Department of Animal Science, University of Tennessee, Knoxville, TN,* [2]*Department of Agricultural and Resource Economics, University of Tennessee, Knoxville, TN,* [3]*Department of Food Science, University of Tennessee, Knoxville, TN.*

Few resources exist regarding US value-added dairy enterprises (VAD) and their start-up costs, equipment and maintenance costs, years-to-breakeven, and additional profits. A decision-making tool was created using Microsoft Excel to help current and prospective VAD determine the economic feasibility of a bottled milk VAD including: years-to-breakeven, milk processing equipment and time, and net present value (NPV). Seven scenarios were run with low, mean, and high numbers of lactating cows in the herd, mean production per cow per day, and percentage of the herd used in the VAD processing. Base data used were the 25th, mean, and 75th percentile values from summary statistics of a prior survey administered to the 9 operational VAD in Tennessee. Each scenario was tested against 4 equipment options, and 112 distinct outcomes were reviewed. Equipment options consisted of the most time-efficient (MEf), most economical (MEc), basic pasteurizer and bottler (BPB), and build your own (BYO). The MEf option included the highest capacity in-line pasteurizer, separator, homogenizer, and bottler, while the MEc option included the cheapest vat pasteurizer, separator, homogenizer, and bottler options. The BPB option included a small capacity vat pasteurizer and a small capacity bottler. The BYO option included a mid-range capacity vat pasteurizer, separator, homogenizer, and bottler. Only 32% of the options were determined profitable (NPV ≥0). Across the profitable scenarios, the mean years-to-breakeven was 5.1 ± 2.1 yr. The majority of profitable and feasible scenarios were the MEf options (5 of the 9) with a mean NPV of $2,410,669 and 4.9 years-to-breakeven. Of the 7 scenarios tested, only 2 were not profitable under any circumstance (low cow number and low percentage of the herd used for value-added dairy processing). The BPB options had the shortest years-to-break even (4.4 yr) and higher net-present values ($1,567,242) compared with the BYO and MEf options. Validation is ongoing using existing VAD data. This decision tool provided VAD with needed information on the economic viability of a bottled milk VAD operation.

Key Words: value-added, processing, economic decision tools

2426 Modulation of aerosol whipping cream microstructure by E 472b addition. M. Blankart* and J. Hinrichs, *Department of Soft Matter Science and Dairy Technology, University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany.*

Emulsifiers have to be applied to aerosol whipping cream (AWC) to stabilize the fat globule surface generated by homogenizing and to enable the foam generation and stabilization upon discharge from the aerosol can. E 472b, an emulsifier synthesized by the esterification of distilled saturated monoacylglycerides with lactic acid, depletes protein from the fat globule membrane and arranges in the form of an α-gel at the fat globule membrane, thus changing the fat globule stability.

Previous works showed that a certain amount of fat globule destabilization is required for the generation of the AWC foam structure. It was hypothesized that excess E 472b impedes the foam stabilization of AWC due to an increased fat globule stability and the formation of E 472b micelles. AWC with a fat content of 30 $g \cdot 100\ g^{-1}$ was processed out of cream and skim milk and homogenized at 80°C with 6/1 MPa. The E 472b concentration was varied from 0 to 2 $g \cdot 100\ g^{-1}$ and the emulsion (static light scattering and rheology) and foam properties (foam capacity and stability) were analyzed. Additionally, the content of extractable fat (CEF) before and after foaming was measured. For all analyses, a one-way ANOVA with subsequent Tukey test was performed (P-value = 0.05). The fat globule size and viscosity were found to decrease significantly up to E 472b concentrations of 0.6–0.8 $g \cdot 100\ g^{-1}$, accompanied by a significantly improved foaming capacity and foam stability and an increase of the CEF after foaming. E 472b concentrations >0.8 $g \cdot 100\ g^{-1}$ had no further effect on fat globule size and viscosity, but decreased the foam stability and the CEF after foaming. From this it was concluded that E 472b concentrations of 0.6–0.8 $g \cdot 100\ g^{-1}$ led to a mixed membrane, composed of E 472b and proteins, that enables the destabilization of fat globules and the foam stabilization of AWC. At 472b concentrations >0.8 $g \cdot 100\ g^{-1}$ a more and more complete α-gel layer around the fat globules is formed, which reduces the fat globule destabilization and the foam stability. Furthermore, E 472b micelles that are formed in the serum at excess concentrations decrease the foam stability. Therefore, the concept of critical micelle concentration, which states that excess emulsifier arranges in micelles and does not change the properties of emulsions, has to be expanded for the application of E 472b in AWC.

Key Words: aerosol whipping cream, foam structure, emulsifier

2427 The effects of whey protein isolate on the properties and biological activity of the bioaccessible fraction in camel milk yogurt, including its rheological properties and biological activity.

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The development of acid curd in camel milk (CM) yogurt is a critical challenge as the texture of yogurt is a major factor influencing consumer acceptance of the fermented product. Large size of casein micelles, low κ-casein, and absence of β-lactoglobulin in CM are the main reasons for the difficulty. This study aimed to investigate the impact of whey protein isolate (WPI) on the health-promoting benefits, texture profile, and rheological properties of CM yogurt after 1 and 15 d of storage. The study used 2 levels of WPI [3% (CMW3) and 5% (CMW5)] to supplement CM and compared it to bovine milk (BM) yogurt. Rheological and texture properties were assessed. The biological activities including antioxidant, antidiabetic, and ACE-inhibition activities of the bioaccessible fraction of the yogurt camel milk were evaluated. Experiments were repeated in triplicates and all biological assays were duplicated. One-way ANOVA was used to assess the significant impact of WPI. WPI increased the water-holding capacity (WHC) of yogurt samples since sample CMW3 had a higher ($P < 0.05$) WHC value. Antioxidant activity parameters of the yogurt before (water-soluble extract, WSE) and after (bioaccessible) in vitro digestion on d 1 and d 15 varied among the yogurts. They increased ($P < 0.05$) after the in vitro digestion, except for the superoxide anion scavenging and lipid oxidation inhibition. WSE of BM yogurt had the lowest ($P < 0.05$) degree of hydrolysis (DH%; 27.3% and 27.2% after 1 and 15 d, respectively) compared with CM yogurts. α-Amylase inhibitions decreased ($P < 0.05$) along with WPI addition. Bioaccessible fractions of digested samples exhibited significantly ($P < 0.05$) higher α-glucosidase inhibition values and BM yogurts had the lowest inhibition value. Addition of WPI increased the hardness, stringiness, and adhesiveness and decreased the resilience of the yogurt. The differences in the strength of CM and BM yogurt curd may be attributed to the lack of β-lactoglobulin and the low content of κ-casein in CM. Control yogurt displayed lower apparent viscosity, G′, and G″ values compared with BM yogurt and other WPI samples. The addition of WPI improved the rheological properties of CM yogurt.

Key Words: camel milk, in vitro digestion, rheological properties

2428 Impact of acoustic intensity on melting and meltdown characteristic of ice cream.

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Ice cream consists mainly of 3 phases (ice crystals, air cells, and fat globules) heterogeneously distributed within a concentrated frozen matrix made of proteins and carbohydrates. This complexity determines the melting and meltdown characteristics of the ice cream. In this work, we report the impact of ultrasound intensity on the melting and meltdown characteristics of regular ice cream. Ice cream mixes were sonicated at different acoustic intensities (11.18 ± 0.29, 14.37 ± 0.45, and 18.39 ± 0.82 W cm^{-2}) for 10 min. The impact of ultrasound on the flow characteristics of the ice cream was evaluated with a Discovery Hybrid rheometer (HR 30, TA Instruments). The melting characteristics of the resulting ice cream were measured through oscillatory thermorheometry, while the meltdown behavior was determined by the gravimetric method. Sonicated samples resulted in about 2.0-fold viscosity increment within the high spectrum of shear rate compared with the control samples. Overall, G′ and G″ changed with the temperature in a sigmoidal fashion, which resembled a typical melting curve of hard ice-cream. On the other hand, an ultrasound intensity of 18.39 ± 0.82 W cm^{-2} significantly delayed the onset of meltdown compared with the control ($P < 0.005$). Insights into the meltdown mechanism were obtained by the analysis of protein profile, particle size, and viscosity. The outcomes of this study help to gain insights into the melting and meltdown characteristics of ice cream and develop processing strategies for structuring frozen desserts.

Key Words: ice cream, ultrasound, rheology

2429 In vitro digestion of milk proteins present in liquid milk and cheddar cheese.

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To study the impact of food structure (solid and liquid) and protein type (renneted vs. soluble) 2 dairy foods, i.e., cheddar cheese and milk, were subjected to in vitro digestion using INFOGEST protocol to understand their disintegration and the protein release at different time intervals in each of the digestive stage, gastric phase, and intestinal phase. The structural breakdown of food materials in the oral phase was simulated by mixing artificial saliva with the sample using pestle mortar keeping protein content at 3.2%. The mixed bolus was then transferred to the stomach and intestinal phase and subjected to mechanical and enzymatic reactions at 37°C for further digestion and protein release. Pepsin enzyme (2000 IU/mL) was used to perform in the gastric phase responsible for protein disintegration at pH 2–3. Whereas trypsin (100 IU/mL) was used for the disintegration of protein into polypeptide fragments in the intestinal phase at pH 7. Samples were collected at different time points, for the gastric phase (0, 15, 30, and 60 min), and the intestinal phase (5, 30, 60, 120, and 180 min). Afterward, samples were centrifuged at 10,000 rcf for 20 min at 4°C. Both supernatant and pellet run on the SDS-PAGE and UREA-PAGE for studying the molecular breakdown of proteins. The digestion and protein release pattern for different types of dairy products showed a clear distinction between liquid and solid samples during invitro digestion at different time points. The disintegration and release of soluble protein compounds for liquid dairy products such as milk was significantly faster (60% soluble protein release in 1 h) in the gastric phase as compared with solid dairy products such as cheddar cheese (15% soluble protein release in 1 h). Both UREA and SDS gel images indicated that the rennetted caseins present in cheese were digested faster in the gastric phase as compared with the caseins present in the liquid milk. This can be attributed to the fact that renneted caseins in cheese have already been broken down due to proteolysis by residual rennet or microbial enzymes. While micellar casein in milk is susceptible to forming an acid gel initially which could retard substrate access to the digestive enzymes. The rate of protein breakdown in the intestinal phase in cheese and milk products were similar. Differences in the rate of protein breakdown during in vitro digestion could be attributed to the protein type and food structure. These results may assist the food industry to improve the nutritional quality and design functional dairy products by optimizing processing methods.

Key Words: dairy food, milk proteins, in vitro digestion

2430 Evaluating the effect of temperature and concentration on the steady shear rheological behavior of whey protein concentrate for use as a potential feed for filament extension atomizer. A. Parhi[1], D. Johnson[2], and P. Sharma*[1], [1]*Utah State University, Logan, UT,* [2]*Palo Alto Research Center, Palo Alto, CA.*

Filament extension atomizer (FEA) is a modern atomization technique capable of handling highly concentrated spray dryer feed. The objective of this study was to investigate the rheological behavior of reconstituted whey protein concentrate (WPC80) for potential application as feed in the FEA. Reconstituted samples were prepared by mixing WPC80 powder in DI water at 22°C using 2 sets of mixers: a combined overhead mixer and Hobart mixer ($OH^{+}PM$), and a high-shear mixer (SM). An Anton Paar 302 rheometer equipped with a 50-mm parallel plate was used for measuring the flow behavior of the samples at 28, 45, 50 and 55% WPC80 loadings. Samples were subjected to a steady shear rate sweep ($0.01–1{,}000 \cdot s^{-1}$) at 7, 22, 40 and 50°C. A commercial WPC80 spray drier feed containing 28% solids was also tested. The rheology data were fitted on Herschel Bulkley, Carreau-Yasuda, and Cross models to obtain yield stress (τ_0), consistency coefficient (K), flow behavior index (n), relaxation time (λ) and zero shear (η_0) and infinite shear viscosities (η_∞) which were further analyzed with 2-way ANOVA in OriginPro-2022 for obtaining the significant differences. With increasing solid loadings from 28 to 55%, τ_0 increased from 0.01(Pa) to 161.2(Pa) and K increased from 0.32 to 3314.1 $Pa.s^{n}$ at 7°C ($P < 0.05$). However, n decreased from 0.74 to 0.47 with 28 to 55% solid loading, indicating shear thinning tendencies at higher solid content. This could be due to a smaller intermolecular distance at higher solid concentrations, causing higher resistance to flow at slow shear rates following a sharp decline in resistance at higher shear rates because of structural failure. Similarly, with increasing temperature from 7 to 22°C at 55% solid loading, λ increased from 9.02s to 11.9s ($P < 0.05$) and n decreased from (0.47 to 0.27). This could be due to the gelation of whey proteins at higher temperatures and resulting formation of aggregates. High shear mixing produced a more homogeneous product than $OH^{+}PM$ mixing with slight differences in rheological behavior. Overall, this study would further the use of FEA for potential applications for dairy powder manufacturing, thus reducing the energy used in the process.

Key Words: dairy powders, atomization, spray drying

2431 Perception of consumers of the relevance of milk as a source of iodine. C. L. Manuelian*[1,2], G. Niero[1], and M. De Marchi[1], [1]*Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Legnaro (PD), Italy,* [2]*Group of Ruminant Research (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain.*

Milk, dairy products, and bread (due to iodized salt used) are the most important dietary iodine (IOD) sources based on daily average consumption. An online consumer survey was conducted among 16 countries across Europe, America, and Asia to evaluate consumers' awareness of milk consumption in meeting their IOD requirements. After deleting duplicates and inconsistent responses (6%), 4,513 questionnaires were retained (1% to 13% per country). Participants were mainly women (64%), <35 yr old (59%), had university studies (47%), and were employed (60%). Using a 7-point Likert scale, consumers associated milk more (mean ± SD) with minerals (5.35 ± 1.75) than carbohydrates (3.60 ± 1.75), and more with Ca (5.90 ± 1.62) than IOD (3.13 ± 1.75). They linked more IOD consumption with seafood (4.91 ± 1.90) or fish (4.88 ± 1.85) than milk and dairy products (3.32 ± 1.78). They considered that dietary IOD moderately influence their health status. Food groups relation with dietary IOD were also analyzed with a PROC MIXED of SAS that included country, gender, age, education level, and employment status as fixed effects. Milk and dairy products, meat, cereals, and vegetables and fruits perception as a source of dietary IOD were only influenced by the country ($P < 0.05$). On the other hand, all factors impacted ($P < 0.05$) the perception of fish (mean range, 4.27–6.05) and seafood (mean range, 4.35–6.42) as a source of dietary IOD, increasing awareness with age, education level, and being female, while decreasing when retired. In addition, all factors except age were relevant in modifying consumers' perception of dietary IOD influencing health status (mean range, 4.52–5.90), increasing awareness with higher education level and being female, and decreasing when retired. In conclusions, perception of food groups contribution to dietary IOD was mainly influenced by the country, and female seemed more informed than men. Whereas fish and seafood were identified as the main food group for dietary IOD, milk and dairy products were not considered a relevant dietary IOD source. Thus, efforts need to be made to inform consumers about the role of consuming milk to meet their IOD requirements.

Key Words: health, iodine, survey

Extension Education Symposium: Leading Extension Programs on Dairy Farms—Tribulations, Changes, and Successes

2432 Championing the science of behavioral change in dairy extension. N. Silva-del-Rio*, *Veterinary Medicine Teaching and Research Center, Tulare, CA.*

Are extension educators being effective change leaders? Stakeholder adoption of novel technologies or practices can be disappointing, even after solid research and well-orchestrated extension efforts support the efficacy and economic sustainability of these practices. On the contrary, well-designed advertising campaigns seem effective at changing consumers' behaviors. So, what makes change difficult? In this interactive presentation, we will study the social and psychological principles behind behavioral change. Participants will learn how to apply those principles in dairy production systems. Case studies will demonstrate how to lead change by overcoming physical barriers (path), designing effective action plans (rider), and engaging the emotional self (elephant).

Key Words: extension education, behavioral change, new practice adoption

2433 Dairy food safety training: Checking the box versus improving operational efficiencies. C. Stevenson*, *North Carolina State University, Raleigh, NC.*

The cost-versus-benefit analysis of dairy foods safety training programs is an ongoing consideration for dairy foods businesses. Food safety training is a costly business activity required by regulatory and third-party auditing entities. Such training requirements vary from introductory training for operators to more advanced training for managers. The design of food safety training also takes various forms such as slide decks, computer-based training, on-the-job training, etc. Whereas some organizations implement all their training in-house, others often leverage third-party training companies and university specialists' services. No matter the cost, design, or provider, it is worthwhile to question the impacts of the food safety training program on the company's day-to-day food safety program performance as well as its organizational culture of food safety. This presentation will highlight several studies of the impacts of different food safety training programs implemented at dairy foods companies. These studies include the following: (1) a comparison of the effects of training program delivery methods on food safety program performance across 70 dairy processing plants: (2) an analysis of behavioral psychology as it pertains to operators' experiences of training programs and their knowledge, performance, and attitudes about food safety, as well as (3) characteristics of dairy food safety training programs that lead to greater knowledge gains and conversion of knowledge into practice. The outcome of this presentation will be increased awareness about how training program design, cost, and delivery method impact overall food safety program performance.

Key Words: food safety, training, design

2434 Assessing the impact of dairy extension programs with stakeholders. L. Holden*, *The Pennsylvania State University, University Park, PA.*

Education and training is a little like a jigsaw puzzle with assessment being that final piece that completes the picture. Extension educators provide a wide range of programs and materials for dairy stakeholders including education for industry professionals, dairy farm owners, managers and employees. Understanding the impact of that education is critical not only for adding value to our end-users but also for continuing to develop even greater learning opportunities. The Penn State Extension Dairy Team has developed and delivered a variety of programs: training for Dairy Advisory Teams, customized producer educational programs, a Women in Dairy Conference, hands-on training at the farm for employees, and a variety of workshops, webinars and online courses. This session will highlight key aspects of some of these programs as well as the corresponding assessment data gathered following program delivery. Examples of "successes and struggles" for teams and data about impactful learning take-aways will be shared. So if you are a brand new extension educator or a seasoned veteran, come and join in the discussion. A better understanding of our stakeholders needs, through program evaluation, focus group data, pre-post or reflective appraisal approaches helps to complete the "jigsaw puzzle" of learning and makes our path forward for learning in the future more successful.

Key Words: dairy extension, assessment, education

2435 On-farm translational research and outreach through academic–extension–industry partnerships. D. Douphrate*, *Texas A&M University, College Station, TX.*

Dairy farming is among the most dangerous occupations and accounts for a disproportionately large percentage of all injuries in livestock-related agriculture. The US dairy industry continues to shift to a large-herd production model due to economies of scale. This shift has led to a higher risk of fatalities, injuries and work-related musculoskeletal disorders (MSD) due to task specialization, increased work demands and hazards. Owners and managers of growing farms are confronted with numerous human resource challenges which can compromise the health and safety of farm workers. Additionally, dairy owners are increasingly dependent on front-line supervisors to effectively manage a larger non-English-speaking workforce with novice workers who have minimal livestock experience before working on large-herd farms. Owners are increasingly seeking supervisors who demonstrate effective management and leadership skills, especially in relation to worker health and safety. Much research effort has been directed at developing safety management systems for large enterprises; however, there is a lack of development, implementation and evaluation of safety management systems for smaller enterprises, especially dairy farming operations. Despite increasing herd sizes with increasing numbers of hired workers, over 95% of dairy farms in the US remain private, family-owned operations. These small enterprises often have limited resources and lack formalization of safety management. This symposium presentation will outline translational research and outreach efforts to address dairy worker health and safety issues through academic, extension, and industry partnerships. Past research will be presented, as well as examples of partnerships with transdisciplinary researchers, dairy producers, extension specialists, and industry associations. Modern dairy farm human resource challenges will also be discussed, as well as methods to overcome these challenges to conduct research that can be translated into practice.

Key Words: partnerships, research, safety

Lactation Biology 1

2436 The effect of preparation lag time on teat tissue condition and milk yield in Holstein dairy cows. A. Singh*, M. O. Dahl, M. E. Spellman, and M. Wieland, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

The objectives were to study the effect of preparation lag time on (1) teat tissue condition and (2) milk yield. We hypothesized that cows subjected to a preparation lag time (i.e., period between the first tactile stimulus and the attachment of the milking unit) of 90 s would have 20 percentage points less (70 vs. 50%) adverse short-term changes to the teat tissue after machine milking (STC) and produce 1 kg/milking session more milk. In a randomized controlled crossover study, 148 Holstein dairy cows were assigned into treatment and control groups. Premilking udder preparation for the treatment group (LAG) consisted of (1) predipping of teats, (2) forestripping and wiping of teats, and (3) attachment of the milking unit. The preparation lag time was 90 s. The only difference in the control (CON) group's premilking udder preparation was that no further latency period was applied, and the milking unit was attached immediately after the wiping step. We assessed STC through visual assessment and palpation by means of discoloration of the teat skin (normal, red, or blue), swelling at the teat base (present or absent), and firmness at the teat end (present or absent). Milk yield (kg; MY) was measured with electronic on-farm milk meters. The effect of the treatment on the outcome variables was investigated using generalized linear mixed models. A random effect for cow crossed with day and study period was included to account for the clustered structure of the data. Treatment and order of treatment application were forced into the model as a fixed effect. Cows in group LAG had lower odds of STC compared with CON cows [odds ratio (95% CI) = 0.13 (0.08–0.20)]. Milk yield [least squares means (95% CI)] for cows in group LAG and CON, respectively, were 15.4 (14.8–15.9) and 15.3 (14.8–15.8) kg. We conclude that immediate attachment of milking unit following the tactile stimulation during premilking udder preparation may not provide sufficient time to elicit the cows' maximum milk ejection capacity. This may aggravate the negative effects of machine milking on the teat tissue, impact animal well-being, and negatively influence udder health.

Key Words: milking routine, oxytocin, animal welfare

2437 Effect of prepartum dietary energy density on serum glucose, insulin and immunoglobulin G and their associations with colostrum composition in Holstein dairy cattle. A. J. Fischer-Tlustos*[1], V. S. Fernandez[2], D. J. Seymour[2], J. P. Cant[1], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Trouw Nutrition Research and Development, Amersfoort, the Netherlands.*

Altering prepartum dietary energy density in Holstein cattle can impact colostrum composition; however, the specific alterations in dam physiology and metabolism responsible for these changes requires further investigation. The aim of this study was to determine how close-up diet (CUD) energy density affects serum concentrations of glucose, insulin, and IgG, and how these alterations may correlate to colostrum composition. From d −57 ± 5.8 before expected calving, multiparous (MP; n = 28) and primiparous (PP; n = 20) Holstein cows were fed a low-energy diet (LED; 1.10 Mcal NE_L/kg DM), and either remained on this diet until calving or were switched to a high-energy diet (HED; 1.52 Mcal NE_L/kg DM) from d −19 ± 4.0 until calving. Blood samples were collected at wk −8, −4, −3, −2, −1 and at calving (within 12 h; d 0). We quantified IgG by radial immunodiffusion, and insulin and glucose were quantified by enzymatic immunoassay. Data were analyzed using PROC GLIMMIX considering the fixed effects of parity, milking, CUD, and their interactions, and the random effect of cow and block. The HED cows tended ($P = 0.09$) to have 15% greater serum IgG from wk −4 to calving on average than LED cows although colostrum IgG concentration ($P = 0.67$) and yield ($P = 0.92$) did not differ. The HED-MP and LED-MP cows had a 70 and 67% increase ($P \leq 0.005$), respectively, in prepartum serum IgG than LED-PP cows, but did not differ ($P > 0.31$) from HED-PP cows. The CUD did not affect serum glucose ($P = 0.78$), but HED cows tended ($P = 0.08$) to have 1.3× greater serum insulin on average from wk −4 to calving than LED cows. Serum glucose at d 0 was moderately positively correlated with IgG (r = 0.67; $P < 0.01$), protein (r = 0.65; $P < 0.01$), fat (r = 0.53; $P < 0.05$), lactose (r = 0.59; $P < 0.05$), and TS (r = 0.64; $P < 0.01$) yields in PP cows. These results suggest that PP cows may require a high-energy diet to achieve serum IgG levels comparable to MP cows and that concentrations of circulating glucose around calving may be an important factor dictating colostrum composition of PP cows.

Key Words: close-up diet, immunoglobulin G, insulin

2438 Regulatory roles of acetate and butyrate on regulation of lipid metabolism genes in bovine mammary epithelial cells. A. Haile* and K. Harvatine, *Pennsylvania State University, State College, PA.*

Dairy cows predominantly utilize acetate for de novo milk fat synthesis. Acetate supplementation enhances milk fat synthesis in cows, but the mechanism is not clearly understood. The objective of the study was to determine the specific regulatory roles of acetate and butyrate on lipogenesis in mammary cells. The hypothesis was that acetate would increase the expression of lipogenic enzymes and factors. A bovine mammary cell line (MAC-T) was grown to 80% confluence on plastic, serum starved for 18 h, and treatments applied for 24 h. Treatments were either control (0), 4, 8, or 12 m*M* of glacial acetic acid, sodium acetate, butyric acid, sodium butyrate, or β-hydroxybutyrate. Osmolarity and pH were not adjusted between treatments. Key lipogenic enzymes and regulators were analyzed by quantitative RT real-time PCR. Data were analyzed using ANOVA and means separated using a protected LSD with significance declared at $P < 0.05$. Acetic acid linearly increased expression of *SREBP1c* (+94, 341, and 772% at 4, 8, and 12 m*M*), *ACSS1*, and *SCD1* and increased *INSIG1* at 12 m*M* and FASN at 8 m*M* but had no effect on *ACACA*. However, sodium acetate quadratically increased *SREBP1c* and linearly increased *SCD1* but had no effect on the other lipogenic genes. Sodium butyrate linearly increased expression of *SREBP1c* (+558, 1,861, and 4,371% at 4, 8, and 12 m*M*), *INSIG1*, *SCD*, *ACACA*, *FASN*, and *ACSS1*. However, butyric acid maximally increased the expression of *SREBP1c* (+3,500%), INSIG1, *ACACA*, *FASN*, *SCD*, and *ACSS1* at 4 m*M*. In contrast, β-hydroxybutyrate resulted in only small increases in some lipogenic genes, including a 100% increase in *SREBP1c* at 4 m*M*, up to 175% increase in ACACA at 8 m*M*, and a linear increase of up to 1,455% in FASN. In conclusion, acetic acid, sodium butyrate, and butyric acid were observed to have large effects on lipogenic genes and regulators, while sodium acetate and β-hydroxybutyrate had more limited responses. Generally, the acid form of treatment showed a stronger response at a lower dosage than the salt form and showed a maximal response at 4 m*M*. There was no observed change in morphology and cytotoxicity effects of treatments on cultured cells. Further work is needed to understand the effect of differences between sources and their impact on lipogenesis.

Key Words: milk fat, lipogenesis, short-chain fatty acids

2439 Killed *Staphylococcus aureus* intramammary challenge induces subclinical mastitis and clear changes in milk composition but not milk yield.

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Subclinical mastitis accounts for the greatest financial loss to producers, primarily due to reduced milk production. Despite the significant cost, the mechanisms of how subclinical mastitis decreases milk production remain largely unknown. The objective of this study was to assess the initial response during subclinical mastitis on mammary gland productivity by examining changes in milk yield and composition. Mid-lactation primiparous Holstein cows (n = 4) acclimated to tie stalls were milked 3×/d using a quarter milker 3 d preceding the beginning of the experiment on Day 0. On Day 0, one udder half of each cow was randomly selected, and fore and rear quarters were each infused with 2 billion cfu of formalin-fixed *Staphylococcus* (*Staph.*) *aureus*; the contralateral quarters were infused with saline. Udder half milk weights were recorded and udder half milk samples were measured by DHIA laboratory. Milk somatic cell score (SCS), composition, and yield were analyzed using PROC GLIMMIX in SAS; fixed effects were treatment, time, and their interaction, and cow was a random effect and repeated measure across time. No quarters exhibited signs of clinical mastitis. *Staphylococcus aureus* udder half SCS were equivalent to saline udder halves at challenge ($P = 0.7$) but increased and remained greater than saline halves at all 5 subsequent milkings ($P \leq 0.02$). Milk yield, fat percentage, and urea nitrogen did not differ between udder half treatments ($P \geq 0.3$), nor did energy-corrected milk ($P = 0.4$). Lactose and protein concentrations were similar between udder half treatments at challenge ($P \geq 0.7$), but *Staph. aureus* challenge significantly reduced lactose ($P \leq 0.03$) and increased protein percentages ($P \leq 0.01$) by 16 h post which were maintained throughout the study. In this udder half model, changes in milk composition were observed during the initial stages of subclinical mastitis but milk yield was not notably affected. Future studies should aim to understand why certain milk components are altered by subclinical mastitis to explain the consequent shift in mammary gland productivity.

Key Words: inflammation, infection, milk synthesis

2440 Effect of BlueLite and heat stress on productivity of lactating Holstein cows.

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Heat stress generates a negative effect on milk production and feed intake of dairy cattle. Strategies to ameliorate the effects of heat stress include osmolyte, electrolyte and energetic supplements in the diet. BlueLite (TechMix LLC) is a product with such characteristics that has been shown to improve responses to overcome heat stress. Thus, we hypothesized that BlueLite improves productive responses of early lactation dairy cows (~52 DIM) under heat stress. Cows (n = 52) were enrolled into one of 4 treatments for a 4-week study in a 2 × 2 factorial design; heat stress (HT, n = 13), heat stress–BlueLite (HT-BL, n = 13), cooling (CL, n = 13) and cooling–BlueLite (CL-BL, n = 13). All cows were housed in a sand-bedded freestall barn equipped with Calan gates to collect individual dry matter intake (DMI), and milked twice daily. Supplement treatment consisted of 113 g of BlueLite per day mixed with TMR and divided in 2 feedings (56.5 g a.m. and 56.5 g p.m.). A cooling system including shade, fans and soakers was provided to CL and CL-BL cows whereas HT and HT-BL cows only had shade. The average environmental temperature-humidity index (THI) in the barns during the study was 77.2 ± 1.3. Daily milk production, DMI, rectal temperature (RT) and respiration rate (RR) were collected during the entire study. In addition, milk fat, protein and lactose were measured using an AfiLab milk analyzer after each milking. Heat stressed cows had increased RR and RT ($P < 0.01$) relative to cooled cows (76.1 ± 2.3 vs 85.0 ± 2.2 breaths/min and 38.6 ± 0.1 vs 39.7 ± 0.1°C). We found that HT reduced DMI (19.9 ± 0.7 kg/day), relative to CL (24.8 ± 0.7 kg/day) and HT-BL reversed some of the effect (21.4 ± 0.7 kg/day; $P < 0.05$). Moreover, energy-corrected milk (ECM) yield averaged CL = 42.7, CL-BL = 41.3, HT = 36.9, and HT-BL = 39.5 ± 1.6 kg/d, and a significant time by treatment interaction was observed ($P < 0.05$). Finally, milk components were not significantly different among treatments ($P > 0.1$). These results suggest that Blue-Lite ameliorates the negative impact of heat stress on DMI and milk yield in lactating dairy cows.

Key Words: dairy cattle, heat stress, milk yield

2441 Proteome analysis exposes new insights into mammary gland adaptation to heat stress in dairy cows.

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Rapid global climate change is accompanied by rising ambient temperatures endangering health, feed intake, and milk production of dairy cows. However, the molecular mechanisms underlying the adaptation of the mammary gland (MG) to increased heat are not known yet. This study aimed to identify differences in the expression of MG proteins of dairy cows experiencing heat stress, or pair-feeding or ad libitum feeding at thermoneutrality. Methods: Eighteen primiparous, non-pregnant German Holstein cows (164 ± 40 d in milk) were evenly assigned to heat stress (HS, n = 6), control (CON, n = 6), or pair-feeding (PF, n = 6). First, all animals were kept in a climate chamber at thermoneutrality [16°C; 63% relative humidity (RH), temperature-humidity index (THI) 60] for 6 d and received a total mixed ration twice daily. Then, HS cows were exposed to 28°C with ad libitum feeding for 7 d (52% RH, THI 76). The CON and PF groups were exposed to 16°C (63% RH, THI 60), but only CON cows received ad libitum feeding. The PF cows were offered the amount of feed that HS cows ingested. After 7 d, MG parenchyma samples were taken and stored at −80°C. Protein extracts were run on a 1D gel. One lane per animal was cut into 10 slices and subjected to HPLC and mass spectrometry analysis. Group differences were analyzed in R with DESeq2 package and pathway enrichment was performed with DAVID. Results: On d 7, milk loss was higher in HS than CON and PF cows ($P < 0.05$), while dry matter intake was lower in HS and PF than CON cows ($P < 0.001$). Overall, 31 out of 880 proteins were differentially abundant between HS and PF cows, while 133 proteins were differentially expressed in HS and CON cows. The top functions of proteins differing between HS and PF cows were related to leucocyte migration, platelet activation, proteasome, and regulation of cytoskeleton. Differences in the carbon and protein metabolism were found between HS and CON cows. Conclusion: Heat stress in MG is accompanied by activation of the immune system, cellular conformational changes, and activation of the protein degradation machinery.

Key Words: heat stress, mammary gland, proteomics

2442 Bovine mammary epithelial cell number and cell losses after a short period of heat stress during lactation.

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This study aimed to evaluate the impact of heat stress on mammary epithelial cell (MEC) losses into milk and secretory mammary tissue structure. Sixteen multiparous Holstein cows (100 ± 14 DIM; 632 ± 12 kg BW) housed in individual climate-controlled rooms were paired by body weight and randomly allocated to one of 2 treatments, heat stress (HS) or thermoneutral (TN). Each cohort was subjected to 2 periods of 4 d each. In period 1 both treatments had ad libitum access to a common total mixed ration and were exposed to a controlled daily temperature-humidity index (THI) of 64. In period 2, HS cows were exposed to controlled cyclical heat stress (THI from 74 to 80), while for TN cows, THI remained at 64 and dry matter intake was matched to HS. Cows were milked twice daily and milk yield was recorded at each milking and compiled daily. Individual milk samples on the last day of each period were used for quantification of MEC losses by flow cytometry using butyrophilin as a cell surface marker. Mammary tissue was obtained from each cow on the final day of period 2 and fixed, paraffin-embedded, stained with hematoxylin and eosin, and analyzed for: alveolar area, alveolar number, and nucleated MEC number. Statistical analysis was performed using the GLIMMIX procedure of SAS. Milk yield was reduced by 4.3 kg in HS ($n = 7$) compared with TN ($n = 8$; $P = 0.001$). Independent of treatment, MEC in milk averaged 174 cells/mL (2.9% of total cells). There was no difference between HS vs. TN cows for MEC shed or concentration in milk. Alveolar area was reduced 25% by HS ($P = 0.002$) and HS had 4.1 more alveoli per unit area than TN ($P = 0.03$). Total nucleated MEC per area was greater in HS (389 ± 1.05) compared with TN (321 ± 1.05; $P = 0.01$). However, cell number per alveolus was similar between groups (25 ± 1.5 vs. 26 ± 1.4). These results suggest that even if milk yield differences and histological changes occur in the bovine mammary gland after 4 d of heat exposure, MEC loss into milk and nucleated MEC number per alveolus are unaffected. This study was supported by: USDA-NIFA Hatch Project VA-135995, Fulbright Visiting Scholar Program, and AFRI competitive grant no. 2019-06005.

Key Words: mammary gland structure, MEC loss and number, heat stress

2443 High lactate may reduce milk synthesis via interpreting the redox system and energy metabolism in the mammary gland of high-yielding dairy cows.

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In the early stage of various disease development in dairy cows, glycolysis is over-activated and excessive lactate is produced when cows are subjected to external stress or excessive tissue metabolic flux. However, the role of lactate in influencing lactation performance of dairy cows remains unclear. In this study, 62 high-yield dairy cows with similar DIM (79 ± 3 d), parity (2–3) and milk yields (49 ± 3 kg/d) were selected to learn the role of lactate in mammary gland health. Among all the experimental animals, cows with high lactate group (HL, $n = 15$, >0.88) and low lactate group (LL, $n = 15$, <0.51) are grouped. Milk samples and mammary vein samples at the peak lactation stage were collected for subsequent analysis. Milk samples were tested for milk composition and oxidative stress indexes. Mammary vein samples were tested for oxidative stress indexes, physiological and biochemical indexes, blood gas parameters and blood routine parameters. The results showed that milk yields of HL cows are lower, compared with that of LL cows ($P = 0.07$). In terms of oxidative stress indexes, milk eNOS concentration are higher in HL animals than those in LL animals. However, ROS ($P < 0.01$) and eNOS ($P < 0.05$) in mammary vein were lower in HL cows than those of LL animals. In terms of physiological and biochemical indexes, TBIL concentrations in mammary vein ($P < 0.05$) are lower in HL animals than those in LL animals, whereas contents of CREA ($P = 0.09$) and NEFA ($P = 0.08$) are lower than those in LL group. We also took mammary tissue of 3 cows in each group for transcriptome sequencing, and showed the differentially expressed gene GO functional enrichment and KEGG enrichment. The differences between the 2 groups were mainly concentrated in material transport (organic acid transport, anion transport), metabolism-related (fructose and mannose-metabolism, glycolysis/gluconeogenesis, carbon metabolism) and material synthesis (steroid synthesis, amino acid synthesis and lipid synthesis). Our data suggest that high lactate concentration in the milk may contribute to the decreased lactation performance in high-yielding cows, through inducing an imbalance in the redox system and altered energy metabolism.

Key Words: lactate, dairy cows, energy metabolism

2444 Evaluation of mammary gland plasma flow during dietary crude protein oscillations.

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Oscillating dietary crude protein (CP) content in 48-h phases has little effect on milk yield compared with static CP feeding. Mammary gland metabolism to maintain milk production under these conditions has not been described. We characterized mammary plasma flow (MPF) and metabolite uptake in 12 Holstein-Friesian cows (172 ± 39 DIM) fed diets oscillating (OS) in CP content in 48-h phases [low (13.4%; L) and high (17.1%; H) CP] relative to a static (ST) diet (15.2% CP) for 28 d in a randomized complete block design. Arterial and venous blood was collected between milkings on d 2 of each OS phase. We estimated MPF by assuming mammary Phe+Tyr uptake = milk Phe+Tyr output (PT), or mammary energy uptake = 120% of milk energy output (ME). Metabolite uptakes were calculated as MPF × arteriovenous differences. The ANOVA included treatment as a fixed effect and block as a random effect. With PT, MPF increased during L-OS compared with H-OS (Table 1). Energy uptake (Eup)$_{\mathrm{PT}}$ increased during L-OS compared with H-OS, but energy output (Eout)$_{\mathrm{PT}}$ did not differ. The resulting energy balance (Ebal)$_{\mathrm{PT}}$ suggests that MPF was overestimated, particularly during L-OS, when calculated with PT. In contrast, MPF$_{\mathrm{ME}}$ and Ebal$_{\mathrm{ME}}$ did not differ. There was a shortage of AA uptake (AAup) to account for milk AA output (AAout) during L-OS with ME compared with PT. Preliminary data suggests that mammary glands derived Phe and Tyr from sources other than free AA, particularly during L-OS, and that an energy-based MPF calculation may be more suitable during non-steady states of CP supply.

Key Words: milk protein, amino acid, mammary metabolism

2445 The effects of a sustained intravenous β-hydroxybutyrate infusion in combination with a systemic immune challenge in lactating dairy cows.

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Table 1 (Abstr. 2444). Plasma flow, energy balance, and amino acid balance in mammary glands estimated using different assumptions[1]

Item	L-ST	H-ST	L-OS	H-OS	SEM	*P*-value
L/h						
MPF_{PT}	1,132^{ab}	1,089^{ab}	1,559^{a}	772^{b}	167.5	0.02
MPF_{ME}	1,033	1,128	1,003	1,003	104.7	0.73
kcal/h						
Eup_{PT}	1,579^{ab}	1,548^{ab}	2,158^{a}	1,222^{b}	214.1	0.04
Eout_{PT}	1,210	1,314	1,231	1,225	99.1	0.72
Ebal_{PT}	369^{ab}	232^{ab}	927^{a}	−4^{b}	201.3	0.02
Eup_{ME}	1,452	1,577	1,477	1,470	118.9	0.72
Eout_{ME}	1,210	1,314	1,231	1,225	99.1	0.72
Ebal_{ME}	242	263	246	245	19.8	0.72
mmol/h						
AAup_{PT}	369	447	372	335	86.6	0.84
AAout_{PT}	441^{ab}	471^{a}	396^{b}	436^{ab}	24.1	0.07
AAbal_{PT}	−72	−26	−24	−100	84.6	0.90
AAup_{ME}	353	489	209	433	72.4	0.07
AAout_{ME}	441^{ab}	471^{a}	396^{b}	436^{ab}	24.1	0.07
AAbal_{ME}	−88	17	−187	−3	65.0	0.14

[a,b]Means in a row with no common superscripts differ ($P < 0.05$).

[1]L-ST = low CP phase of oscillating treatment; H-ST = high CP phase of oscillating treatment; L-OS = phase of static treatment corresponding to the low CP phase of the oscillating treatment; H-OS = phase of static treatment corresponding to the high CP phase of the oscillating treatment; PT = mammary plasma flow estimation assuming mammary Phe+Tyr uptake = milk Phe+Tyr output; ME = mammary plasma flow estimation assuming mammary energy uptake = 120% of milk energy output; MPF = mammary plasma flow; Eup = mammary energy uptake; Eout = milk energy output; Ebal = energy balance; AAup = mammary amino acid uptake; AAout = milk amino acid output; AAbal = amino acid balance.

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Although an elevation in blood β-hydroxybutyrate (BHB) is typically understood as a predictor of poor lactation performance and higher risk of disease, contradicting evidence suggests the nature of this relationship is misunderstood. Furthermore, BHB has known pleiotropic effects that include the attenuation of inflammation and oxidative stress. We aimed to evaluate the effects of sustained hyperketonemia via an intravenous (i.v.) BHB infusion. Eight multiparous Holstein (parity = 2.75 ± 0.89) lactating dairy cows (140 ± 48 DIM), were enrolled in a study with a 2 × 2 Latin square design. Cows were randomly assigned to i.v. infusion of a 2.5 m*M* Na-BHB solution to achieve BHB >1.2 m*M* (KET), or 2.5 m*M* NaCl control (CON) over two 72-h periods. A lipopolysaccharide challenge (*Escherichia coli* 055:B5; 0.085 mg/kg BW; LPSC) was i.v. administered at h 60 from infusion start. Rectal temperature (RT), respiration rates (RR), and pain scores (PS) were measured every 6 h on d 0, 1, and 2, and every hour post-LPSC. Continuous data were analyzed under a mixed model with the random effect of cow and fixed effects of time, treatment, its interactions, covariate, and optimal covariance structure. Categorical data were evaluated using PROC GLIMIX with the random effect of cow and a fixed effect of time, treatment, and their interactions. Cows sustained hyperketonemia throughout the 72-h experimental period (1.4 BHB m*M* vs. 0.72 BHB m*M* in KET vs. CON, respectively; $P < 0.001$). Although DMI and MY were not affected by KET, relative to CON+LPSC, KET+LPSC resulted in reductions of 22.3% and 44.3% in DMI and MY, respectively ($P < 0.05$). Milk fat tended to be reduced ($P < 0.1$), whereas lactose content increased ($P < 0.05$) following LPSC. Respiration rates tended to be reduced ($P < 0.1$), but a higher RT ($P < 0.05$) was observed in BHB cows; however, no differences in RT were detected during LPSC. No pain score differences were detected between treatments. Our results indicate that cofactors other than ketones may be necessary for the development of negative trajectories of health and performance in lactating dairy cows.

Key Words: ketosis, immune challenge, dairy cow

2446 Quarter-level milk yield variation pre- and post-separation among cow-calf contact cows.

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The interest in keeping dairy cows and calves together in cow-calf contact (CCC) systems is increasing among consumers, farmers and other stakeholders in countries with industrialized dairy farming. However, research on such management systems as well as consequences of the system on milk production, animal welfare and sustainability is scarce. Farmers repeatedly report milk ejection difficulties in CCC cows and also low milk yield after separation. The aim of this study was to investigate patterns in quarter-level milk yield before and after separation, among cows kept in a CCC system with automatic milking. In total 35 cows were enrolled in the trial and assigned to CCC (n = 17) until the calves were 127 ± 6.6 d old, or control (n = 18) that were separated from their calves within 12 h after calving. All cows were milked in an AMS and individual quarter-level records were obtained from each milking during whole lactations. ANOVA was performed on the data using the mixed model function in SAS Studio. Milk yield delivered was higher in control cows both per quarter and per milking than in CCC cows pre-separation ($P < 0.001$) but not post-separation ($P = 0.92$). Further analysis of quarter-level milk yields revealed higher overall variation in quarter milk yields pre- as well as post-separation in CCC cows (CV = 69 vs 40% pre- and 50 vs 42% post-separation, for CCC and control cows respectively). Within-cow variation displayed a similar pattern; CV = 58 vs 29% pre- and 38 vs 31% post-separation for CCC and control cows respectively. Within-quarter variation was similar between quarters, but higher pre- compared with post-separation in CCC cows (CV 60, 58, 57 and 58% for the LF, RF, LR and RR quarters respectively pre-separation, and 36, 36, 38 and 40% for the LF, RF, LR and RR quarters respectively post-separation). Collectively, these results show a higher between-milking variation in CCC cows compared with control cows pre- and post-separation, on group as well as cow

level. This may indicate persistent issues with milk ejection or irregular milking patterns after separation. Variation displayed no specific pattern indicating a preferred quarter for suckling.

Key Words: dam-rearing, AMS

2447 Maternal heat stress abatement improves daughter's whole-body and mammary growth post-weaning through pubertal development. S. L. Field*[1], B. D. Davidson[1], B. Dado-Senn[1], A. D. Beard[1], K. A. Riesgraf[1], P. L. J. Monteiro[1], M. C. Wiltbank[1], G. E. Dahl[2], and J. Laporta[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [2]Department of Animal Sciences, University of Florida, Gainesville, FL.*

Late-gestation heat stress reduces calf mammary gland (MG) size and weight with profound negative effects on epithelial ductal development and cell proliferation at birth and weaning. Herein, the objective was to characterize heifer MG growth from weaning to puberty and parenchyma architecture at puberty. Heifers were in utero heat stressed (IUHT, shade, n = 19) or in utero cooled (IUCL, shade, fans and soakers, n = 21) for the last 54 ± 5 d of gestation. Body weight (BW), hip height (HH), and front and rear teat length (FTL, RTL) were measured at 120, 240, 300, and 360 d of age, and MG length at 360 d of age. Puberty attainment occurred when circulating progesterone (P_4) concentrations reached >1 ng/mL. At 310 ± 15 d of age heifers were assigned to an estrus synchronizing protocol: d-9: GnRH$^+$CIDR, d-2: $PGF_{2\alpha}$, d-1: $PGF_{2\alpha}$+CIDR removal. On d 0 (~24 h pre-estrus) and d 12, blood was collected to capture expected high estradiol (E_2) and low P_4 and low E_2 and high P_4, respectively. On d 0 and d 12, MG tissue biopsies were performed on a subset of heifers (IUHT, n = 7; IUCL, n = 6) for histological analysis (H&E stain). Alveolar and ductal area were measured by tracing outside the epithelial cells and tracing the inner lumen within the parenchyma in ImageJ. Data were analyzed in SAS. Compared with IUHT, IUCL heifers had greater BW and HH at 120 and 240 d ($P < 0.05$). The FTL and RTL was greater in IUCL heifers on d 120, 240 and 360 ($P < 0.05$). Mammary gland length at 360 d was greater in IUCL, relative to IUHT (34.02 vs. 30.92 ± 0.51 cm, $P = 0.001$). On d 0, P_4 concentrations were similar between treatments (0.40 vs. 0.44 ng/mL ± 0.07, $P = 0.68$), however E_2 concentrations were greater in IUCL heifers, relative to IUHT (5.1 vs. 3.8 pg/mL ± 0.40, $P = 0.03$). On d 12, P_4 (8.8 vs. 8.4 ng/mL ± 0.39, $P = 0.55$) and E_2 concentrations (0.40 vs. 0.44 pg/mL ± 0.04, $P = 0.64$) were similar between treatments. Alveolar and ductal inner lumen and outer area were similar between treatments ($P > 0.31$). Prenatal heat abatement increased MG teat length and gland size during development leading to puberty, increased E_2 near estrus without major microstructural differences in mammary tissue.

Key Words: puberty

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2448 Evaluation of the use of beef semen on dairy operations: A survey of Idaho dairies. P. S. Smith*[1], J. B. Glaze Jr.[1], H. Tejeda[1], R. J. Collier[2], and M. Chahine[1], *[1]University of Idaho, Twin Falls, ID, [2]University of Idaho, Moscow, ID.*

Idaho is the third-largest milk producing state in the US. Over the last decade, the utilization of beef semen in dairy operations has substantially grown. The objective of this study was to survey current Idaho dairies on their use of beef semen and gain insights into the criteria and decisions made on farm. A mail-in survey was sent to all registered Idaho dairies (n = 369). The survey consisted of 40 questions that were a combination of multiple choice, ranking, and open answer. The survey included questions such as, "Are you breeding part of your herd to beef semen?" and "How do you decide which cows to breed to beef?" The survey was distributed following the Dillman (1978) method. The survey was mailed on August 8, 2022, a reminder postcard was mailed on October 8, 2022, to dairies that had not returned the initial survey, and then the survey was mailed a second time on November 5, 2022, to all the dairies that had not responded. We had a total response rate of 14.1%, and the responses were categorized by dairy size and region within Idaho. Data were analyzed using procedures FREQ and GENMOD of SAS 9.4. Of the dairies that responded, 78.8% breed a portion of their herd to beef semen. The use of beef semen was significantly affected by dairy size ($P < 0.05$). Only 8% of the dairies breeding to beef were using some sexed male beef semen and 29.4% were considering the use of sexed male beef semen. "Failure to conceive" was ranked highest when deciding which cows were bred to beef followed by "milk production." In addition, "dairy replacement inventory numbers" was selected the most when determining the percentage of animals bred to beef semen. Black Angus was the most chosen breed of sire followed by Charolais. Dairies ranked the "breeding service picks the bulls" the highest when selecting beef sires, followed by "calving ease" and "semen cost." Only 26.8% of dairies raise their own dairy × beef calves and 69.4% sell the dairy × beef calves as day-olds. The dairy × beef calves (41.9%) were sold primarily through private treaty. These results among others, will further guide our research of these dairy × beef animals.

Key Words: dairy, beef on dairy

2449 Survey of California dairy nutritionists on byproduct usage. J. Heguy*[1], E. DePeters[2], R. B. Lopes[2], and N. Silva-del-Rio[3], *[1]University of California Agriculture and Natural Resources, Modesto, CA, [2]University of California, Davis, Davis, CA, [3]University of California, Davis, Tulare, CA.*

The objective of the study was to determine byproduct feeding trends and opportunities in California. In March 2022, an invitation letter and a byproduct feeding and management survey (Qualtrics, Provo, UT) were sent electronically to known nutritionists in California (n = 61). Unanswered questions and unreasonable responses were not included in the data analysis. Descriptive statistics were performed with R (version 4.0.4, R Foundation for Statistical Computing) using the 'Rcmdr' package. Response rate was 43% (26/61). Nutritionists (n = 25) reported servicing 498 dairy farms and represented approximately 936,700 milking cows; one nutritionist serviced 30 dairies but did not provide the number of cows serviced. Respondents represented cows housed in San Joaquin Valley herds (87.5%), with some cows housed in the Northern (5.5%) and Southern California (7.0%) regions. The reasons for byproduct inclusion in rations were value (30.7%), value and availability (30.7%), value and price (3.8%), or the combination of availability, price, and value (34.6%). Most respondents (57.7%) utilized a commercial laboratory to determine the nutritional composition of all byproducts, whereas 42.3% analyzed only some byproducts. Average dietary byproduct inclusion rate ranged from 10 to 80% DM for lactating cows (mean = 39.8%), 5 to 80% DM for dry cows (mean = 35.4%) and from 5 to 80% DM for heifers (mean = 36.0%). Based on respondents' answers, a weighted average inclusion rate of byproducts was estimated at 40.9% DM in lactating cow diets. Respondents reported replacing forages (96.1%) and concentrates (96.0%) with byproducts. In lactating dairy cow diets, the 3 byproducts most frequently used to replace forages were almond hulls (40.6%), citrus (9.4%) and soybean hulls (7.8%) whereas citrus (13.7%), whey (12.1%), and millrun (10.3%) were most reported as a concentrate replacement. Byproduct feeding contributes to the sustainability efforts of the California dairy industry. Future water availability and regulation in California may increase the already important role byproducts play dairy rations.

Key Words: byproducts, California

2450 How benchmarking motivates improved calf care: A realistic evaluation. D. J. Wilson[1], S. M. Roche[1,2], J. A. Pempek[3], G. Habing[4], K. L. Proudfoot[5], and D. L. Renaud*[1], *[1]University of Guelph, Guelph, Ontario, Canada, [2]Acer Consulting, Guelph, Ontario, Canada, [3]Department of Animal Sciences, Columbus, OH, [4]Department of Veterinary Preventive Medicine, Columbus, OH, [5]Department of Health Management, Charlottetown PE, Canada.*

The objective of this study was to determine how and in which circumstances providing benchmark data can motivate improvements in neonatal calf care practices on dairy farms, particularly for surplus calves. Dairy producers from British Columbia, Canada were recruited through 2 veterinary clinics that offered benchmarking of calf data as part of their herd health services. For 8 weeks, blood samples were collected to evaluate calf serum total protein concentrations as an indicator of the effectiveness of colostrum management. The results were presented by the herd veterinarian in a one-on-one meeting where the farm's results were benchmarked against other participating herds. Following each meeting, interviews were conducted separately with the producers (n = 21) and the veterinarians (n = 7; one interview conducted after each meeting) to determine their perspectives on this benchmarking strategy. The resulting 42 interviews were analyzed qualitatively using realistic evaluation methodology to determine which contexts and mechanisms contributed to the success or failure of the meeting at motivating producers to improve their calf care. Four important contexts were identified that influenced the outcome of the benchmark meetings: (1) farm resources, (2) management strategies, (3) the producer's perception of their calf performance, and (4) the producer's personal values. Depending on these contexts, the illustrative data and veterinary advice provided in the benchmark meeting motivated change by influencing producer decision making, which depended on the value they saw in the data and the value they placed on their surplus and replacement calves. Regardless of their current colostrum management strategy for surplus calves, producers who valued their surplus and replacement heifer calves equally were more influenced by the benchmark data. Recommendations for future implementation of benchmarking include targeting producers who are motivated to improve, have engaged calf care personnel, and prefer data-driven decision making. This study supports the impactful role benchmarking and consultancy can play in motivating improved calf care practices.

Key Words: qualitative, colostrum, veterinarian

2451 Effect of different dry cow vaccination schedules on immunity and performance of dairy cows. J. Gao*, C. Guzi Savegnago, T. N. Marins, A. M. Roper, and S. Tao, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

Giving vaccines close to or on dry-off (DO) is common but induces inflammation. We aimed to study the effect of different dry cow vaccination schedules on immunity after DO and cow performance in the next lactation in a commercial dairy farm. Lactating cows were milked 3×/d. Farm standard operating procedure (SOP) included vaccination at 2 d before DO (Enviracor J-5, Scourguard 4KC, Somubac, and Ultrabac 7, Zoetis). Cows were then raised on pasture, fed only hay and milked 1×/d until DO. Dry cows were raised on pasture and moved to a close-up barn 3 wk before calving. Multiparous cows were assigned to 2 groups based on last digit (even/odd) of her name tag: 1. Vaccination at 2 d before DO (SOP, n = 164); 2. Vaccination at 11 d before DO (EV, n = 139). The SOP and EV cows had similar ($P \geq 0.11$) parity and test day milk yield (MY) and SCC before DO. Mastitis and daily MY were recorded up to 60 d of lactation. Blood was drawn at 3 d after DO to isolate peripheral blood mononuclear cells (PBMC). Cells were stimulated by concanavalin A (ConA) and lipopolysaccharide (LPS) to assess proliferation and cytokine production. Mastitis incidence was analyzed using logistic regression, and MY was analyzed as repeated measures data using mixed model of SAS. Giving vaccines earlier reduced MY before DO, but did not affect ($P \geq 0.33$) MY and mastitis incidence in early lactation. The SOP cows tended to have mastitis earlier than EV cows (26 vs. 42 d, $P = 0.06$). The PBMC collected from SOP cows tended to have greater proliferation to ConA ($P = 0.06$) but had similar proliferation to LPS ($P = 0.82$) than EV. When stimulated by ConA, PBMC from SOP cows had greater ($P \leq 0.01$) production of tumor necrosis factor (TNF)-α and interferon (IFN)-γ, and tended ($P = 0.06$) to produce more interleukin (IL)-1β than EV. When stimulated by LPS, PBMC from SOP cows had greater ($P = 0.03$) production of TNF-α and IL-1β, and tended ($P = 0.07$) to produce more IL-1α and macrophage inflammatory protein-1β than EV. In conclusion, giving vaccines 11 d before DO has minimal effects on performance in the following early lactation but minimized PBMC proliferation and cytokine production in early dry period.

Key Words: vaccines, dry-off, immunity

2452 Effect of drinking water salinity on feed and water intake, rumen physiology, and milk production of lactating cows. I. Adi[1,2] and B.-M. Yehoshav*[1], [1]*Department of Ruminant Science, Institute of Animal Science, Agricultural Research Organization (ARO)–Volcani Center, Rishon Lezion, Israel,* [2]*Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot, Israel.*

Increased use of desalinated seawater in arid and semi-arid regions resulted in drinking water low in salinity and minerals content. Although this water considered safe for drinking, low-salinity water may affect lactating cows' performance. Therefore, the aim of this study was to evaluate the effect of offering drinking water salinity, within an electrical conductivity (EC) range of 400 to 1,000 μS/cm, on lactating cows. After 2 weeks of adaptation to individual feeders and troughs, 4 Israeli Holstein multiparous lactating cows were randomly offered drinking water with EC levels of 400, 600, 800, or 1,000 μS/cm by adding concentrated NaCl solutions for 4 periods of 21 d each in a Latin square design. Each period included 5-d washout, 13 d for recording of data, and 3 d for sampling of urine and feces. Feces collected by grab sampling and urine collected by stimulating the bladder. Water EC in the troughs, feed and water intake, milk and milk component yields, and behavior (activity and recumbence time) were measured daily. The statistical model included treatment and period as fixed effects and cow as a random effect. The daily measurement served as a repeated measure factor modeled as AR1. A dose response was tested for linear and quadratic contrasts. The measured EC in the troughs were (average ± SD) 418 ± 38, 624 ± 51, 811 ± 53, and 1016 ± 71 μS/cm. Water EC affected feed intake and energy-corrected milk yields that were 27.5, 28.3, 29.6, and 29.7 kg DM/d (SEM = 0.53, $P < 0.01$) and 40.4, 41.9, 41.6, and 42.4 kg ECM/d (SEM = 3.38, $P < 0.01$), respectively, with the greatest response of yield (1.5 kg) for increasing water EC from 400 to 600 μS/cm. Water EC did not affect water intake, which was (average ± SD) 142 ± 35 L/d. Whole-tract NDF digestibility was highest when the cows were offered 600 μS/cm water (45.9 vs 43.8, 40.9, and 39.8% for EC of 400, 800, and 1000 μS/cm, $P = 0.048$). Although trial parameters (i.e., low roughage feed, summertime, 4-cow group, etc.) limit the overall conclusion for optimal water EC, this study indicated that drinking water salinity might affect intake and yield.

Key Words: drinking water, desalinated seawater, water salinity

2453 Recycled flush and lagoon water as a reservoir for antibiotic residues and resistant bacteria on California dairies. E. Okello*[1,2], E. Abdelfattah[1,2], P. K. Pandey[1], P. Ekong[1], T. Lehenbauer[1,2], and S. Aly[1,2], [1]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA,* [2]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare, CA.*

Freestall alleys on dairies are commonly flushing with recycled lagoon water which could facilitate the spread of antimicrobial drug (AMD) residues and antimicrobial resistant bacteria on the farm environment. Our research aimed to estimate the concentration of AMD residues in lagoon samples and determine the association between AMD residues and resistance of bacteria from the samples. Lagoon samples were collected monthly from 9 California (CA) dairies between October 2018 and August 2019. Study dairies were spread across Northern CA (NCA), North San Joaquin Valley (NSJV) and Greater Southern CA (GSCA). Presence of AMD residues in the lagoon samples were analyzed by indirect competitive ELISA. A total of 158 *Escherichia coli* and 173 *Enterococcus* spp. isolates from lagoon samples were tested for susceptibility against a panel of 19 AMD using microbroth dilution method. Four AMD residues were detected in lagoon water samples: penicillin (0.03–8.76 ppb), tetracycline (1.5–2.7 ppb), florfenicol (0.04–0.78 ppb), and tilmicosin (0.36–7.99 ppb). Tetracycline concentrations were higher in samples from NSJV (1.7 ± 0.85 ppb), compared with GSCA (1.32 ± 0.84 ppb) and NCA (1.14 ± 0.91 ppb), while tilmicosin were higher in lagoon samples from dairies in NCA (2.07 ± 1.75 ppb) compared with samples from GSCA (0.83 ± 0.47 ppb) and NSJV (0.86 ± 0.39 ppb). Most of the *E. coli* and *Enterococcus* spp. isolates (>40%) were resistant to florfenicol, tilmicosin, and tiamulin; these drugs are not approved for use in adult dairy cows. However, a lower percentage of *E. coli* and *Enterococcus* spp. isolates (<10%) were resistant to ceftiofur and ampicillin. Detection of florfenicol and tilmicosin in lagoon water from pens of dairy cows could be due to excretion by calves and heifers treated with said drugs, which were subsequently spread to adult cow pens through flush with recycled lagoon water or use of dried solid waste as a freestall bedding. Lagoon management practices should be explored to reduce the antibiotic residues in recycled flush water.

Key Words: lagoon, residues, antibiotics

2454 Survey of mastitis-causing organisms in dairy bedding types from across the United States.

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Mastitis is a frequently observed challenge throughout the dairy industry. Stall bedding is a key source of environmental mastitis as cows lie down 12 to 14 h a day, and their teats are in direct contact with bedding material. Different bedding types and environments can offer favorable or unfavorable bacterial growth conditions. The objective of this survey was to quantify the levels of mastitis-causing organisms in different bedding types collected from across the United States. From February of 2019 to January of 2023, 951 bedding samples were collected from 46 US dairy farms. Four different types of bedding were collected: recycled manure solids (RMS, n = 389), composted RMS (COMP, n = 433), digested RMS (DIG, n = 91), and sand (n = 38). Bedding samples were collected from unused bedding before going into the stalls (Pile, n = 115) and used bedding which had been laid on (Stall, n = 821). Eight farms were sampled before (Pre-Pile, n = 15) and after (Pile, n = 19) heating or composting in addition to Stall (n = 173). Samples were enumerated for *Escherichia coli*, coliforms, group D streptococci, and presumptive *Klebsiella*, and comparisons of bacterial levels were made using a one-way ANOVA. *Escherichia coli,* coliform, and presumptive *Klebsiella* levels were higher in Stall RMS ($P < 0.05$) compared with all other Stall bedding types. Group D streptococci were lower ($P < 0.05$) in sand Stall samples compared with all other Stall bedding types. All target bacteria levels increased ($P < 0.05$) in the Stall compared with the Pile for all bedding types. Heating and composting reduced bacterial levels ($P < 0.05$), comparing Pre-Pile samples to Pile, but levels of all targeted bacterial groups increased ($P < 0.05$) in the Stall. Stall samples also had significantly higher levels ($P < 0.05$) of *E. coli*, coliforms and group D streptococci compared with the Pre-Pile samples. This survey work provides a better understanding of mastitis-causing organisms in different bedding types which can be used in future research for mastitis control.

Key Words: mastitis, bedding, recycled manure solids

2455 Thermo-physiology and microclimate of calves in outdoor hutches with or without continuous ventilation in summer.

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Dairy calves exposed to heat stress have impaired growth and welfare. Herein, we characterized the thermo-physiological and microclimate effects of continuous ventilation on hutch-housed dairy calves in a continental summer. At 3 d of age, heifers (n = 32) were enrolled into active (ACT; solar-powered fan attached to hutch rear) and passive (PASS; hutch rear window open) ventilation groups. Fans were on for 28 d, then turned off until weaning (56 d). Calves were restricted 1 h outside (unshaded) and inside of the hutch thrice weekly. After each restriction, calf respiration rate (RR), skin (ST) and rectal (RT) temperature were recorded. Air speed (AS) and ammonia levels inside the hutch were recorded. Calf body weight (BW) was recorded weekly. On d 28, hutch air-born bacteria were quantified on sheep blood agar plates (BAP). Lung ultrasounds and health scores were performed thrice weekly (7–35 d), every other day (35–43 d), and twice weekly (44–56 d). Data were analyzed by PROC MIXED and GLM in SAS. Relative to PASS, ACT hutches had greater AS (1.11 vs. 0.07 m/s, $P = 0.001$), fewer colony-forming units (cfu, 16 vs. 121, $P < 0.05$) and tended to have lower ammonia levels ($P = 0.09$). Differences between outside and inside hutch restriction were evaluated for ST, RR, and RT. Once given access to the hutch, ST was reduced by 4.23°C in ACT calves compared with 1.2°C in PASS ($P = 0.01$). Additionally, ACT calves' RT was reduced by 0.21°C, compared with 0.14°C in PASS ($P = 0.05$). While enrolled in ventilation treatments (3–28 d), PASS and ACT calves had similar ADG ($P = 0.87$). After removed from treatment (28–56 d), PASS calves gained 0.29 kg more than ACT calves ($P = 0.04$). There was no treatment effect for ultrasonographic pneumonia ($P = 0.22$). However, for calves with below median plasma IgG, the ACT AUC was greater compared with PASS indicating that lung consolidation was greater in ACT calves with lower plasma IgG (ACT: 13.5 ± 0.9, PASS: 10.6 ± 0.8; $P = 0.04$). Provision of active ventilation to hutch-housed calves improved microclimate and promoted effective thermoregulation, without major impacts on growth and health.

Key Words: fans, heat abatement, pneumonia

2456 Preparing for the future: Assessing the risk of heat stress for dairy cows under different scenarios of climate change.

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With climate change, temperature and humidity are expected to increase globally, posing a challenge to dairy cows' productivity, health, and welfare due to heat stress. The objective of this study was to evaluate the risk of heat stress in dairy cows based on different projected climate change scenarios in Minas Gerais state, Brazil. Climate projections were obtained for this state from 20 Earth system models in 4 combined Shared Social Pathway (SSP) and Representative Concentration Pathway (RCP) scenarios. The scenarios covered a spectrum of greenhouse gas emission levels and mitigation difficulties, with the lowest levels represented by the SSP1–2.6, the highest levels by SSP5–8.5, and 2 intermediate scenarios (SSP2–4.5 and SSP3–7.0). The risk of heat stress was estimated using the Temperature and Humidity Index (THI), calculated based on the monthly average air temperature (°C) and relative humidity (%) extracted from each projection for 2040, 2070, and 2100, representing the short, medium, and long term, respectively. The THI values were interpolated to a homogeneous grid size of 15 × 15 km over the Minas Gerais state and combined per season and year by calculating the weighted average and standard deviation. The overall THI average was 70.5 (SD = 3.32), 71.7 (SD = 3.37), and 73.2 (SD = 3.80) for 2040, 2070, and 2100, respectively. Overall, no risk of heat stress (THI <68) was found only during the winter of 2040 and 2070 for SSP2–4.5, and during the fall of 2040 for SSP1–2.6, and SSP3–7.0. The risk of moderate heat stress ($72 \leq \text{THI} < 80$) was observed during summer and spring, regardless of the scenario and year. The northwest and Triângulo regions showed the highest THI values, with a risk of mild to severe heat stress, dependent on the scenario and year. Conversely, the southern region had the lowest THI values, although there was a risk of mild-to-moderate heat stress depending on the scenario and year. These findings provide important insights for dairy farmers and the industry to develop adaptation strategies to mitigate the impact of climate change on dairy cows.

Key Words: ambience, thermal stress, temperature and humidity index

Table 1 (Abstr. 2458). Mycotoxin contamination in US corn silage (dry matter basis)[1]

	Year						
Parameter	2018	2019	2020	2021	2022	SEM	*P*-value
N	94	68	81	86	73		
B-Trich							
Prevalence, %	89	97	99	88	93	124.3	0.039
Mean, ppb	3,336^{a}	3,468^{a}	2,990^{a}	2,802^{b}	2,835^{ab}		
ZEN							
Prevalence, %	56	43	31	67	48	124.3	0.002
Mean, ppb	1,010^{a}	478^{b}	368^{b}	555^{b}	783^{ab}		

^{a,b}Values across rows are different $P < 0.05$.

[1] SEM and *P*-values generated using the GLIMMIX procedure of SAS to test the effect of harvest year on mean contamination level of each mycotoxin group when samples were above each mycotoxin group limit of detection. Other toxin groups are not presented due to limited number of positive samples. B-Trich = type B trichothecenes; ZEN = zearalenone.

2457 Rectal temperature, heart rate, respiration rate, morphometric measurements and health scores in slick and wild-type Holstein calves during the milk feeding phase. M. D. Torres-Rivera*, C. R. Perdomo-García, M. Ruiz-Cortés, A. Rodríguez-Cruz, and G. Ortiz-Colón, *University of Puerto Rico, Alfonso Valdés, Mayagüez, Puerto Rico.*

Bovines with the slick (SL) gene tolerate heat stress better than wild-type (WT) cattle, and therefore, exhibit better production traits under high ambient temperatures. Because previous research has focused on mature cows, in our study we compared the rectal temperature (RT), morphometric measurements, heart rate (HR), respiratory rate (RR) and health score (HS) in SL and WT calves during the milk feeding phase in Puerto Rico (18.0246147° N, −67.0757998° W). Holstein calves (SL n = 16; WT n = 16) were provided high-quality colostrum within 2 h after birth and individually housed (50 cm × 152 cm). All calves were fed fresh whole milk. An accelerated growth milk feeding protocol was followed starting with an offer of 4 L of milk per day the first week and increasing gradually to 10 L of milk per day on wk 6. On wk 7, a stepdown weaning protocol was started and weaning was completed by wk 10. Calf starter (22% CP) and water were offered ad libitum. Growth data (hip height [HH], withers height [WH], body length [BL], body weight [BW]); RT, body condition scores (BCS); HR; RR and HS; was collected weekly from birth until weaning. Data were analyzed as repeated measures using the GLIMMIX procedure of SAS. Slick calves showed lower RT than WT calves (SL 38.8°C ± 0.04 vs. WT 38.9°C ± 0.04; $P = 0.0122$). On the other hand, SL calves showed higher HR (beats/min) than WT calves (SL 122.48 ± 1.77 vs. WT 115.48 ± 0.04; $P = 0.0087$). However, at weaning no difference ($P = 0.1273$) between SL and WT Holstein calves was documented in HH (SL 84.76 cm ± 0.89 vs. WT 86.21 cm ± 0.89); WH (SL 79.72 cm ± 0.82 vs. WT 81.08 cm ± 0.82), BL (SL 66.25 cm ± 0.69 vs. WT 67.2 cm ± 0.69), BW (SL 59.38 kg ± 1.76 vs. WT 63.28 ± 1.76), BCS (SL 1.94 ± 0.69 vs. WT 1.97 ± 0.69), RR (SL 45.2 ± 2.17 vs. WT 49.1 ± 2.15) and HS (SL 3.56 ± 0.178 vs. WT 3.71 ± 0.177). Although some physiological differences were documented between the genotypes, SL and WT Holstein calves grew similarly during the milking feeding phase in our study.

Key Words: heat stress, climate change, slick cattle

2458 Mycotoxin contamination trends in US corn silage: 2018–2022. P. N. Gott*, E. F. Schwandt, L. Zheng, and A. W. Levy, *DSM Nutritional Products, Parsippany, NJ.*

Mycotoxins are fungal metabolites which occur in feedstuffs and may affect animal health and performance. Mycotoxin occurrence and contamination levels in US corn silage were monitored in this study. Sample pools differ each year but were mainly sourced from Midwest and Northeast regions. Samples were analyzed via LC-MS/MS method for 6 major mycotoxin groups: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins, zearalenone (ZEN), and ochratoxin A. The presence of clinical health or performance concerns on-farm was noted for each sample. Effect of clinical concern (yes/no) and harvest year on contamination levels were analyzed using LSMeans statement with Tukey's adjustment in the GLIMMIX procedure (SAS 9.4, SAS Institute Inc.). There were differences between clinical concern for B-Trich; therefore, samples noted with clinical signs were excluded and harvest year was used as main effect. Initial results for 73 samples of 2022 corn silage identified B-Trich (93%) and ZEN (48%) most frequently. Mean contamination levels (parts per billion, ppb) are presented on dry matter basis in Table 1. Across the study period, the highest mean concentration of B-Trich was detected in 2018–2019 and the 2022 levels were intermediate across the 5 years. The highest mean concentration of ZEN was detected in 2018 (1,010 ppb) and did not differ from 2022 (783 ppb). Initial survey results indicate B-Trich and ZEN continue to occur frequently in US corn silage.

Key Words: corn silage, mycotoxins

2459 Effects of autolyzed yeast supplementation on the humoral response and gut health of calves. K. G. Primel[1], A. C. A. Abreu[1], B. Milla[1], S. N. de Oliveira[1], H. G. Bertagnon[1], M. A. Bonato[2], and W. L. S. dos Reis*[2], *[1]UNICENTRO, Guarapuava, Paraná, Brazil, [2]ICC, São Paulo, São Paulo, Brazil.*

Diarrhea and bovine respiratory complex impact morbidity and mortality in calves. In this trial, calves face challenges since the farm had an outbreak of eimeriosis. Yeast derivatives are known as gut health boosters and immunomodulators due to the action of mannans and β-glucans. Thus, we evaluate the effects of autolyzed yeast (RumenYeast) on humoral response and gut health. Twenty female dairy calves positive for eimeriosis (Willis–Malloy test) were randomly assigned to one of 2 groups: Control Group (CG, n = 10); and Autolyzed Yeast Group (AY, n = 10). AY, 10 g/animal/day, was supplemented in the milk fed to calves from 15 to 51 d old (trial length, D15 to D51). On D15, CG and AY were medicated for eimeriosis (Toltrazuril). On D30, CG and AY were vaccinated against BRC (Inforce) intranasal route. Blood samples were collected at D15, D33, D36, and D51 to measure immunoglobulins (IgA, IgG ELISA test), and neutrophil oxidative metabolism (nitroblue blood test, NBT). Dehydration greater than 5% (daily calf gum exam) was treated with an oral hydration protocol. Weight/ADG was estimated by chest girth. The data were analyzed with the GraphPad and considered significant ($P < 0.05$) or trend ($0{,}05 \leq P < 0.10$). We found that AY enhanced ($P < 0.05$) serum IgA over time. On D51 (3 weeks after vaccination), IgA and IgG were greater ($P < 0.05$, and $P < 0.1$) in AY than in CG. The number and duration of diarrhea episodes decreased ($P < 0.05$) with AY supplementation. No AY calf presented oocysts of *Eimeria* spp. after 2 weeks, while it persisted in 3 calves in CG. The CG received 51 hydration protocols while AY received 9. After vaccination (D31), calves (CG and AY) presented minor alterations in the auscultation of the respiratory system (side-effects of intranasal vaccine). This alteration evolved differently in the groups; in CG, 4 calves evolved to pneumonia, while in the AY group, just 1. In AY fewer pneumonia may be explained by NBT results. On D33 (after vaccine), NBT was superior ($P < 0.05$, 39% vs. 17%) in AY than in CG. Calves with pneumonia received antibiotic. Extra health care in CG did not avoid loss of performance (CG 539 vs. AY 650 g/calf/day). Autolyzed yeast (RumenYeast) seems to enhance mucosal immunity and vaccine efficacy reducing diarrhea and respiratory disorders.

Key Words: immunomodulation, immunoglobulins, autolysed yeast

Joint Reproduction, Physiology and Endocrinology, and Ruminant Nutrition Symposium: Mechanisms Linking Transition Health, Nutrition, and Fertility of Dairy Cattle

2460 Nutritional strategies to improve the health and fertility of dairy cows. B. J. Bradford*, *Michigan State University, East Lansing, MI.*

The importance of nutrition in supporting health and fertility has been recognized at least since the discovery of essential nutrients in the early 1900s. However, the optimal nutritional program for dairy cattle to enable a healthy transition to lactation, return to cyclicity, conception, and successful implantation of the conceptus remains imprecise at best. Large retrospective studies have provided strong evidence that transition disorders—not only infections but also metabolic diseases—are associated with poor subsequent fertility. Likewise, cows that lose substantial body condition in early lactation have poor reproductive efficiency, perhaps in part due to diseases underlying the loss in condition, but likely also due to endocrine and metabolite impacts of rapid tissue mobilization. These associations suggest that dietary strategies which reduce transition disorders and promote dry matter intake in early lactation should contribute to greater reproductive success. Both prepartum and postpartum dietary strategies and feed supplements have been evaluated for impacts on reproduction, and several meta-analyses have attempted to integrate these studies to generate stronger evidence for impacts of specific nutrients. Compelling results in the literature include the positive impacts of controlled energy diets in the weeks leading up to parturition and beneficial associations between early lactation protein balance and fatty acid intake with reproductive efficiency (reduced time to pregnancy). More specifically, omega-3 fatty acids fed around the time of breeding may have beneficial impacts on conceptus viability as well as maternal tolerance of the conceptus. To more clearly define impacts of specific nutrients (especially micronutrients) on longevity and reproductive outcomes, appropriately powered studies are needed; both automated feeding systems for individual animal treatment and very large pen-level studies provide opportunities to address this gap. Nutritional strategies that optimize health and reproductive outcomes can substantially impact dairy profitability and these topics warrant more research focus.

Key Words: reproduction, longevity, transition

2461 Direct and indirect effects of trace mineral nutrition on health and fertility of dairy cows. E. S. Ribeiro*, B. Mion, L. Ogilvie, B. Van Winters, G. Madureira, and J. F. W. Sprícigo, *Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.*

Trace minerals (TM) serve as cofactors of metalloenzymes required for several biological processes such as gene expression, protein synthesis, and oxidative balance. Because of insufficient concentration in most common feedstuff, dietary supplementation of Co, Cu, Mn, Se, and Zn is recommended to optimize health and performance in dairy cows. Multiple sources of TM are available for dietary supplementation and are mainly classified into 3 classes: inorganic salts of TM (STM), inorganic hydroxy TM (HTM), and organic TM (OTM). They differ in their chemical composition and bonding, stability and solubility in the digesta, microbial utilization and toxicity, and bioavailability for gut absorption, which can cause both pre- and post-absorptive effects. A supplementation method that improves rumen fermentation and digestibility can improve energy and nutrient intake and indirectly benefit health, especially during transition when feed intake is reduced. Moreover, greater bioavailability can result in enhanced TM status and directly benefit immunity, antioxidant capacity, and overall health. Improvements in transition health benefits reproduction indirectly. Nonetheless, the role of TM on biology of cells in the ovary, uterus, and developing conceptus allows direct effects of TM supplementation on fertility. We recently examined the effects of complete replacement of STM (Co, Cu, Mn, and Zn sulfates and Na selenite) by OTM (Co, Cu, Mn, and Zn sulfates and Na selenite) in both pre- and postpartum diets of 273 individually fed cows. The replacement strategy changed the systemic distribution of Co and Se, increased prepartum feed intake, reduced ruminal activity and enhanced neutrophil function during transition. In multiparous cows, OTM increased postpartum feed intake. In primiparous cows, it reduced milk yield, increased postpartum BCS, and hastened resumption of ovarian cyclicity. In addition, OTM reduced incidence of lameness and metabolic problems, and improved development of preimplantation conceptuses. No differences in incidence of other transition health problem and in reproductive performance were observed.

Key Words: micronutrient, transition period, reproduction

2462 Impact of dry matter intake and rumen-protected amino acids during the transition period to optimize uterine health and fertility. F. C. Cardoso* and A. R. Guadagnin, *University of Illinois at Urbana-Champaign, Urbana, IL.*

The depth and duration of negative energy balance (NEB) and negative protein balance (NPB) during the transition period are highly related to dry matter intake (DMI). Formulating and delivering appropriate diets that limit total energy intake to requirements but also provide proper intakes of all other nutrients [including the indispensable amino acids (IAA) Met and Lys] before calving can help lessen the extent of NEB and NPB after calving. Supplementation of rumen-protected methionine (RPM) during the transition period improved uterine immune function through improved glandular morphology, increased neutrophil infiltration after calving, and discovery of neutrophil extracellular trap formation in bovine endometrial tissue. Cows supplemented with rumen-protected lysine (RPL) had decreased liver oxidative stress (*SOD1*, superoxide dismutase 1) and acute phase response (*SAA3*, serum amyloid A3) and reduced gene expression of *NFKB1* (nuclear factor kappa B1) leading to increased proinflammatory *IL1B* postpartum, likely indicating improved immune activation when cows consumed RPL, a necessary component of early postpartum health. Prepartum supply of RPL altered uteroplacental metabolism and glucose transport. Additionally, we reported on the association of pre- and postpartum DMI, body weight (BW), body condition score (BCS), milk yield and milk composition, and days to first ovulation with cytological endometritis at 15 (CYT15) and 30 DIM (CYT30). A second objective was to understand the association of vaginal discharge with CYT15 and CYT30 and performance. Simple regression analyses demonstrated linear associations of increased DMI, particularly postpartum, with decreased uterine PMN percentage and lower vaginal discharge score. Additionally, increased units of vaginal discharge score and increased percentage units of uterine PMN were linearly associated with decreased milk yield. CYT15 was associated with lower DMI from 4 wk before calving until 4 wk postpartum and was associated with lower milk yield. In conclusion, the association of vaginal discharge with cytological endometritis was variable and dependent on the day of evaluation. Providing RPM and RPL during the transition period impacts dairy cows' health and uterine environment.

Key Words: methionine, lysine, dry matter intake PMN

2463 Effects of omega-3 fatty acid supplementation on the reproduction system in cows and bulls.

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Mammals can synthesize all of the essential fatty acids (FA), except those belonging to the omega-3 (n-3) and omega-6 (n-6) families, which should be supplied in the diet. Common feedstuffs are rich in n-6 FA, whereas the supply of n-3 FA in the intensive dairy industry is mainly limited to flaxseed and fish oils. The n-3 FA are involved in many biological processes, such as the reproductive and immune systems, and therefore their dietary supplementation in dairy cattle is of special interest. In a series of studies, we investigated the rate and total infiltration of n-3 fatty acids into the ovarian compartments in cows, and sperm in bulls, and consequently their physiological effects. Interestingly, selective-uptake mechanism of the n-3 FA has been demonstrated in the ovary compartments, as well as in bull sperm. For example, the content of eicosapentaenoic (EPAn-3), which originated from fish oil, was high in oocytes, but was not found in the follicular fluid. On the other hand, docosapentaenoic acid (DPAn-3), which is synthesized de novo from shorter n-3 FA, accumulated only in oocytes. We also found that a specific time period is required for the infiltration of dietary n-3 FA into the target tissues; this was particularly salient in bulls, but also applies to cows. The incorporation of these unique FA into the reproductive system exerts some positive effects on fertility. The intensity, duration, timing, and pattern of hormones' secretion around estrus were influenced by n-3 supplementation. In addition, we performed ovum pick-up, in vitro maturation, and oocyte fertilization, and found a higher cleavage rate in cows supplemented with n-3 FA. In bulls, the enrichment of sperm with n-3 FA improved the survival and quality of fresh and freeze–thawed semen. Furthermore, we found that the beneficial effects obtained for the reproductive system in cows and bulls from feeding n-3 FA can be achieved with the supplementation of α-linolenic acid (ALA) from flaxseed. In conclusion, dietary n-3 FA are incorporated into the compartments of the reproductive system in cows and bulls, and exert several beneficial effects.

Key Words: omega-3 fatty acids, reproductive system

2464 Reevaluating transition cow dogmas.

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Correlation analysis, retrospective classification, and epidemiology fueled the theory that adipose tissue released NEFA, the resulting hepatic derived ketones, and subclinical hypocalcemia (SCH) lead to inappetence, immune suppression, and ultimately morbidity and infertility. The tenet has evolved into a dogma that therapeutically treating subclinical hyperketonemia (SHK) and SCH improves cow health and productivity. But according to recent NAHMS reports, cow health is not improving, so it is appropriate to question whether we are medicating the causes or simply treating the symptoms. Adipose mobilization and ketogenesis are in fact necessary for high production in the transition and experimentally blunting them directly reduces milk yield. Furthermore, acute and transient SCH is normal in high-producing cows. These observations suggest there are alternative explanations to periparturient failures other than SHK and SCH. Recently, it has become firmly established that immune activation and the ensuing inflammatory response are a normal component of transition cow biology. However, if inflammation is persistent it both reduces feed intake and causes SCH. During established lactation, immune activation causes an immediate decrease in both milk yield and feed intake but in early transition the evolutionary drive to synthesize milk continues in spite of inadequate feed intake. The simultaneous requirements of immune activation and milk synthesis create an energetic and mineral imbalance characterized by SHK and SCH, a metabolic profile that is similar to a healthy high-producing transition cow. Our hypothesis is that immune activation and its induction of hypophagia are responsible for some cows with SHK and SCH and this explains why they are occasionally correlated with poor health, production, and infertility. We argue that changes in circulating NEFA, ketones, and calcium are simply reflective of either: 1) normal homeorhetic adjustments healthy high-producing cows use to prioritize milk synthesis or 2) the consequence of immune activation. Research to understand and ameliorate causes of excessive inflammation should be prioritized over efforts to reduce SHK and SCH which are non-specific symptoms of the underlying problem.

Key Words: ketosis, hypocalcemia, infertility

2465 Consequences of uterine inflammation on oocyte quality.

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Infections of the reproductive tract by gram-negative bacteria disrupts ovarian function, follicular growth, and fertility of cattle. Reproductive tract infections, including metritis and endometritis, occur in up to 40% of postpartum dairy cows and cost the United States dairy industry approximately $900 million annually. The cause of infection related subfertility likely involves receptivity of the endometrium, ovarian function and oocyte quality. We hypothesized that reproductive tract infections alter the quality of the finite reserve of oocytes needed for embryonic development. Reproductive tract infection results in the accumulation of bacterial lipopolysaccharide in follicular fluid of growing follicles that stimulates an innate inflammatory response by granulosa cells, therefore altering the developmental environmental of the growing oocyte. Induced reproductive tract infection using a combination of gram-negative *Escherichia coli* and gram-positive *Trueperella pyogenes* altered the abundance of 452 transcripts in oocytes collected 4 d after infection, and 539 transcripts in oocytes collected 60 d after infection. This suggests that oocytes at different stages of development are susceptible to the negative effects of infection as oocyte collected at d 4 would have resided in antral follicles at the time of infection, while oocytes collected at d 60 would have been present in secondary stage follicles at the time of infection. Using the same model of induced infection, we were able to show that post-infection oocytes collected by ultrasound guided follicle aspiration had a 14.3 percentage point reduction in the capacity to develop to the morula stage of development following in vitro fertilization (30.7 ± 3.0 vs. 45.0 ± 1.1%, $P \leq 0.05$). While it is unclear how long the negative effects of infection persist in the pool of oocytes, exposure of cortical ovarian explants to lipopolysaccharide results in the inappropriate activation of the primordial follicle pool which could decrease the follicle reserve in animals with infection. Collectively, these data suggest that reproductive tract infection in the absence of other clinical confounders compromises oocytes quality and may contribute to subfertility of cows after infection.

Key Words: oocyte, infection, inflammation

2466 Uterine and granulosa cell signaling via extracellular vesicles in response to non-esterified fatty acids. O. Bogado Pascottini*, *Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

Negative energy balance in transition dairy cows is characterized by upregulated adipose tissue modeling, insulin resistance, and systemic inflammation. However, maladaptation to the transition period and in consequence, excessive negative energy balance can be toxic for reproductive tissues, including granulosa cells and endometrium. Cumulus oocyte complexes matured under pathophysiological concentrations of different types of nonesterified fatty acids display impaired mitochondrial activity due to oxidative stress, which upon fertilization results in lower oocyte development and embryo quality. Adiposity and dyslipidemia have also been reported to change the intrauterine environment, shifting the histotroph composition which results in delayed embryo development and early embryonic death. The multi-directional communication among reproductive tissues is carried up via gap junctions or paracrine and autocrine secretion of molecules. Furthermore, recent advancements in the discovery and characterization of extracellular vesicles released from various cell types have provided an additional layer into the existing and well-known mechanism of cell-to-cell communication. The term "extracellular vesicle" is used to characterize several different vesicle types, such as exosomes, microvesicles, and apoptotic bodies, released by cells constitutively or in response to specific stimuli or cell stressors. These structures are capable of protecting their cargo molecules (e.g., miRNA) from enzymatic degradation during their transit through extracellular environments thanks to their bilayer lipidic membrane nature. This capability allows researchers to study acute signaling of reproductive tissues under distinct lipotoxic conditions to understand mechanisms that link metabolic stress with infertility.

Key Words: metabolic stress, fertility, exosome

Ruminant Nutrition 4: Calves and Heifers

2467 Hepatic metabolic flux responses to feeding milk fat, flaxseed oil, or soy oil to pre-ruminating calves. L. M. Beckett*[1], V. M. R. Malacco[1], K. Gouveia[1], A. Mann[1], C. Andolino[1], K. Harlow[1], N. E. Sunny[2], R. C. Neves[1], J. R. Burgess[1], J. P. Boerman[1], T. M. Casey[1], and S. S. Donkin[1,3], *[1]Purdue University, West Lafayette, IN, [2]University of Maryland, College Park, MD, [3]Oregon State University, Corvallis, OR.*

Fatty acids are potential regulators of glucose and energy metabolism. We hypothesized that shifts in dietary fatty acids in growing calves would alter circulating fatty acids, hepatic gene expression, and carbon flux in the TCA cycle. The objective of this experiment was to determine the impact of feeding flaxseed oil (~50% C18:3n3), high oleic soy oil (~70% C18:1n9), or milk fat (~50% C16:0) on hepatic *PC, PCK1*, and *PCK2* expression and carbon flux. Male Holstein calves ($n = 40$) were randomly assigned to a base diet of skim milk (as-fed basis) with addition of either: 3% milk fat (MF), 3% flaxseed oil (Flax), 3% soy oil (Soy), 1.5% MF + 1.5% soy oil (MF-Soy), or 1.5% MF + 1.5% flax oil (MF-Flax) with daily treatment diet intake at 12.5% of body weight, and no grain or hay provided. Calves received the treatment diets from d 14 postnatal (PN) to d 21 PN. Liver biopsy was taken on d 21 PN for mRNA transcript and separate liver explants were incubated in [U-^{13}C] propionate and [U-^{13}C] pyruvate for 2 h. Mixed model analysis indicated plasma C18:3n3 was 10 times higher in calves that received Flax (26.8 ± 0.6%) or MF-Flax (23.3 ± 0.6%) compared with other treatments ($P < 0.05$), plasma C16:0 was highest in MF (22.7 ± 0.74%; $P < 0.05$), and plasma C18:1n9 was similar between Soy (22.4 ± 0.62%), MF-Soy (21.1 ± 0.62%, and MF (20.2 ± 0.62%), but 3 times higher than MF-Flax (7.24 ± 0.62%) and Flax (7.36 ± 0.62%; $P < 0.05$). There was no treatment effect on liver *PC*, *PCK1*, or *PCK2* expression, but Soy (−0.47 ± 0.40 arbitrary units) numerically decreased ($P = 0.13$) *PC* expression compared with Flax (0.53 ± 0.43 arbitrary units). Pearson correlation analysis showed that *PC* expression tended to positively correlate ($r = 0.31$; $P = 0.08$) with the molar proportion of C18:3n3, but negatively correlated ($r = -0.39$; $P = 0.03$) with C18:1n9. *PCK2* expression correlated ($r = 0.43$; $P = 0.02$) with the molar proportion of C16:0, and *PCK1* expression tended to correlate ($r = 0.31$; $P = 0.10$) with proportion of C16:0. Treatment did not affect carbon flux of [U-^{13}C] pyruvate nor [U-^{13}C] propionate. The positive association of *PC* with C18:3n3 and PCK1 and PCK2 with C16:0 indicates that circulating fatty acids alter key genes in liver that regulate energy and glucose metabolism in growing calves.

Key Words: pre-ruminating calves, flaxseed oil, TCA cycle flux

2468 Sodium percarbonate as a potential preservative in waste milk fed to dairy calves. D. J. Wilson*[1], G. M. Goodell[2], R. Dumm[3], T. Kelly[2], and M. Bethard[2], *[1]Utah State University, Logan, UT, [2]The Dairy Authority, Greeley, CO, [3]Dairy Tech, LLC, Loveland, CO.*

The objective was evaluation of sodium percarbonate (SP), a preservative used in milk and drinking water, as an inhibitor of bacterial growth in pasteurized waste milk to be fed to dairy calves. Bacteria standard plate counts (SPC) in cfu/mL were performed using Standard Methods for the Examination of Dairy Products procedures. After pasteurization at 63°C for 30 min, dairy farm waste milk was incubated at 32°C (100°F) in a 0.4-m^3 (14.3-ft^2) incubator. Standard plate counts were calculated for 296 aliquots from 7.6-L (2-gallon) batches of pasteurized milk at times 0 (as soon as milk was cooled to 49° C for safe handling) and at 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after pasteurization. Concentrations of SP added to milk were 0 (untreated control), 200 mg/L and 400 mg/L. Statistical significance between SPC for the SP concentrations within each time point was tested using ANOVA. The SPC means at times following pasteurization for the 3 concentrations of SP are shown in Table 1. We found that SP added to pasteurized milk at 200 mg/L or 400 mg/L was associated with SPC remaining significantly lower according to ANOVA, usually <8,000 cfu/mL, all <22,000 cfu/mL, in comparison with marked increases in SPC beginning 4 h after pasteurization in untreated milk at 32° C. We conclude that SP has potential as a preservative for milk fed to calves; further studies are being conducted.

Key Words: calves, milk, sodium percarbonate

2469 Superiority of wheat straw over alfalfa hay for young Holstein calves. A. Nikkhah*[1], M. Alimirzaei[2], and H. Kazemi[2], *[1]National Elites Foundation, Tehran, Iran, [2]Behroozi Dairy Co., Tehran, Iran.*

The objective of this study was to determine effects of feeding starter diets containing either chopped wheat straw (WS), chopped alfalfa hay (ALF) or their mixture (WA) on performance of Holstein calves. Sixty newborn calves were randomly assigned to 1 of 4 dietary treatments, including feeding dry starter diets with 1) no forage (CO), 2) chopped wheat straw (WS), 3) chopped alfalfa hay (ALF), or 4) a 50:50 mixture of wheat straw and alfalfa hay (WA). The DM-based dietary forage inclusion rate was 10%. Diets were fed as total mixed rations. The experimental design was a randomized complete block. The data were analyzed with Mixed Procedures of the SAS program. The calves fed WS, and not ALF and WA, had greater ($P < 0.05$) total DMI (1546 vs. 1122 g/d) and starter intakes of DM (1327 vs. 902 g/d), ME (3.47 vs. 2.39 Mcal/d), starch (573 vs. 411 g/d), and NDF (233 vs. 98 g/d) than did calves fed CO. All forage-fed calves had greater ADF intake than did CO-fed calves ($P < 0.01$). The total (100 vs. 76 and 73 kg) and post-weaning (63 vs. 43 and 42 kg) starter DMI were greater ($P < 0.05$) for WS than for ALF and CO, respectively. The average daily gain (ADG, 727 vs. 633 g/d) and average BW (65 vs. 60 kg) tended to be greater for

Table 1 (Abstr. 2468). Mean bacteria standard plate counts (SPC; cfu/mL) in post-pasteurized waste milk over time at 32°C, comparing different concentrations of sodium percarbonate added to milk

Time post-pasteurization (h)	Sodium percarbonate concentration[1]		
	0	200 mg/L	400 mg/L
0	4,499	3,702	4,062
1	4,634^{a}	2,814^{b}	1,720^{b}
2	5,485^{c}	428^{d}	845^{d}
3	3,344^{e}	857^{f}	1,714^{f}
4	**85,282**^{g}	21,706^{h}	4,008^{h}
5	**58,424**^{i}	5,036^{j}	5,085^{j}
6	**88,663**^{k}	2,465^{l}	7,533^{l}
7	245,050	4,188	3,722
8	**476,679**^{m,2}	4,127^{n}	2,738^{n}
24[3]	**192,743,750**	**444,000**	15,324

$^{a–n}$Mean SPC within the same time (row) with different letters were significantly different at $P < 0.05$ (ANOVA).

[1] All values are SPC bacteria counts, cfu/mL.

[2] At 8 h, 0 sodium percarbonate mean was significantly different at $P = 0.10$.

[3] At 24 h, all means were significantly different at $P < 0.0001$.

WS than for others ($P < 0.10$). The average final BW (97.7 vs. 85.3 kg) and total post-weaning weight gain (13.4 vs. 9.5 kg) were greater for WS than for ALF ($P < 0.05$). Rumen pH tended to be greater ($P = 0.09$) for WS (6.28) and WA (6.36) than for CO (5.61) and ALF (5.88). Water intake increased as calves aged and was greater for forage-fed calves than for CO calves at wk 8. Fecal pH at weaning tended to be lower ($P < 0.10$) for WS (6.3) than for CO (6.8), ALF (7.2), and WA (6.8). Weaning body depth was greater ($P = 0.01$) for WS (120 cm) than for CO (111 cm) and ALF (112 cm). Blood urea N concentrations at weaning tended to be lower ($P = 0.08$) for WS (10.8 mg/dL) than for ALF (13.2 mg/dL). A tendency for decrease in non-nutritive oral behaviors was found for WS (21 min/d) than for CO (59 min/d), suggesting improved welfare in the WS-fed calves. Findings strongly support feeding forage in calf starter diets, with chopped WS being superior to ALF.

Key Words: alfalfa hay, calf growth, dry matter intake

2470 Effect of a phytogenic compound, a functional mineral compound, and yeast cell wall extract fed in calf starter on growth and health. S. E. Schuling*[1], T. L. Harris[2], and D. E. Schimek[1], *[1]NutriQuest, Mason City, IA, [2]Elanco Animal Health, Greenfield, IN.*

Female Holstein × Jersey (n = 390) and Holstein (n = 60) calves were used in a completely randomized block design to determine the effect of 2 levels of a combination of a phytogenic compound (PC), a functional mineral compound (FV), and yeast cell wall extract (YCW) on growth and health. Calves were blocked by breed and initial BW. Calves were randomly assigned to one of three 19.25% CP calf starters (CS): 1) control (CON), 2) CON + 100 mg PC+ 3 g FV+ 2 g YCW (PFY1), and 3) CON + 200 mg PC+ 6 g FV+ 4 g YCW (PFY2). Calves were fed whole milk reconstituted to a 14% solids level with a 26/20 milk replacer 2×/d from d 1–51 and 1×/d from d 52–54. Water and CS were offered ad libitum. Calves were housed singly in hutches (d 1–70) and in group pens (d 71–84; n = 12/pen). In group pens, calves were fed an 18% CP pellet and 454 g/calf of respective CS. From d 1–84, BW were measured bi-weekly. Starter intake (SI) was measured thrice weekly from d 1–70. Plasma and fecal samples were collected from the first 25 calves/treatment on d 1, 28, 52, and 84 (plasma only) and analyzed for plasma TNF-α and fecal myeloperoxidase (MPO). Fecal scores (FS) were determined biweekly from d 1–28. Scour and pneumonia events were recorded. Performance and health data were analyzed using PROC GLIMMIX and PROC LOGSITICS of SAS, respectively. There was no effect of treatment on BW, ADG, or health events. Compared with CON, SI was greater ($P < 0.01$) for calves fed PFY1 and PFY2 during wk 5–8, and SI was 1.02, 1.09, and 1.11 ± 0.06 kg, respectively. Compared with CON, FS were lower ($P < 0.01$) for calves fed PFY1 and PFY2 and were 0.60, 0.51, and 0.51 ± 0.03, respectively. Fecal MPO concentrations tended ($P = 0.07$) to be lower for PFY1 and PFY2 compared with CON, and were 0.113, 0.287, and 0.560 ± 0.17, respectively. On d 84, TNF-α was lower ($P < 0.01$) for PFY1 and PFY2 compared with CON and was 0.06, 0.06, and 0.44 ± 0.01 ng/mL, respectively. Feeding PFY1 and PFY2 increased SI before weaning, tended to decrease MPO, and decreased FS, and TNF-α on d 84, but did not increase BW at weaning or impact scour events.

Key Words: dairy calves, phytogenic compounds, yeast cell wall

2471 Evaluation of galacto-oligosaccharide for neonatal calves. K. Ike[1], D. Casper*[2], U. Anele[3], M. Scott[4], and W. Hansen[4], *[1]College of Science and Technology, North Carolina Agricultural and Technical State University, Greensboro, NC, [2]Casper's Calf Ranch, Freeport, IL, [3]College of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, NC, [4]Milk Specialties Global, Eden Prairie, MN.*

Galacto-oligosaccharide (GOS) is a prebiotic produced and isolated from whey. This study evaluated the optimal inclusion rate for improving growth and health performance of neonatal calves. Eighty-eight 2- to 5-d-old neonatal Holstein bull calves were blocked by initial body weight and randomly allocated to 1 of 4 treatments using a randomized complete block design. Treatments comprised a 22:20 (crude protein:fat) amino acid balanced milk replacer (MR) with GOS added at the rate of 0 g/d (control; 0), 2 g/d (2), 4 g/d (4), and 8 g/d (8). Calves received 0.283 kg MR in 1.9 L MR fed 2×/d for the first 14 d, then increased to 0.43 kg in 2.84 L fed 2×/d though d 35, followed by 0.43 kg MR in 2.84 L fed 1×/d through d 42, followed by weaning. The GOS feeding rate remained constant as milk amounts increased. Growth performance was determined by body weight and body frame measurements. Data were analyzed as a randomized complete block design using SAS Proc Mixed procedure with block, treatment and week as fixed effects. Calves fed GOS at 2, 4, and 8 g/d demonstrated lower growth performance variation compared with calves fed 0 g/d (control). Calves fed GOS at 4 g/d had a carryover effect into post-weaning resulting in a significant increase ($P < 0.05$) in final body weight (82.5, 83.0, 85.3, and 83.1 kg for 0, 2, 4, and 8 g/d, respectively) and a tendency ($P < 0.08$) for increase in BW gains (37.8, 38.2, 41.3 and 38.6 kg), and average daily gain (ADG; 687.5, 695.7 751.4, and 701.3 g/d). The ADG was increased ($P < 0.05$) by 9.3% when feeding 4 g/d GOS compared with calves fed 0 g/d GOS. Calf starter intake was greater ($P < 0.05$) at 7 (1.73,1.86, 1.95, 1.83 kg/d) and 8 (2.34, 2.50, 2.60, 2.49 kg/d) weeks of age for calves fed 4 g/d compared with calves fed 0 g/d with remaining treatments being intermediate and similar. Feed conversion (0.552, 0.529, 0.563, 0.545 kg/kg) was greater ($P < 0.05$) for calves fed 0 and 4 g/d compared with calves fed 2 g/d and 8 g/d being intermediate and similar. Body frame measurements were similar for calves fed all GOS inclusion rates. A treatment by week interaction at wk 2 indicated that calves fed 2 g/d GOS demonstrated greater fecal score = 0 than calves fed the remaining treatments, indicating less scours. In conclusion, supplementing GOS to a milk replacer at 4 g/d fed to neonatal calves improved final body weight, body weight gains and average daily gain compared with the control.

Key Words: calves, galacto-oligosaccharide, growth

2472 Prepartum CLA supplementation modulates dams' colostrum composition and calves' performance. C. L. Cardoso[1], E. Raffrenato[2,3], F. Righi[4], and G. Esposito*[2,4], *[1]Department of Production Animal, Faculty of Veterinary Science, University of Pretoria, Onderstepoort-Pretoria, South Africa, [2]Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa, [3]Department of Comparative Biomedicine and Nutrition, University of Padova, Padova, Italy, [4]Department of Veterinary Medicine, University of Parma, Parma, Italy.*

Conjugated linoleic acid (CLA) supplementation reportedly increase immunoglobulins (Ig) production in rats and circulating IGF-I levels in cows. Therefore, the objective of the study was to evaluate the effect of CLA dietary supplementation of the dam on colostrum and transition milk (CT milk) quality, and calves' IgG levels and growth. Forty Holstein cows blocked by parity (3 ± 1), body weight and body condition score were randomly assigned to 2 groups: control (CTL, 100 g/cow/day of Ca salts + 100 g of molasses) and CLA (100 g/cow/day of CLA+ 10 g of molasses). Individual top-dressed supplementation started 20 ± 7 d before calving until 5 d in milk. Calves were individually fed their mother's CT milk from the day of birth until 4 d of life. Colostrum yield was recorded, and samples were collected for analyses on colostrum composition, fatty acid (FA) profile and IgG quantification. Calves were bled every other day from 0 to 15 d for IgG and total protein (TP) levels. Weekly, from d 7 to 35, calves body measurements and weight were recorded for growth rate. The CT milk yield, composition, and FA profile, growth rate, average daily gain (ADG), blood and colostrum parameters were analyzed by analyses of variance (ANOVA) for repeated measures using GLM procedure. A trend for a lower fat percentage and a higher lactose content was observed in the colostrum from the CLA group ($P = 0.1$ and $P = 0.09$). Treatment increased the concentration of C15:0 and CLA, whereas it decreased the concentration of C18:3, total n6 FA and n6:n3 ratio ($P > 0.05$) in the CT milk. No differences between groups were observed for blood and colostrum IgG levels. However, an increased trend of TP levels was observed in the CLA group (treatment × age: $P = 0.079$). In CLA calves, a trend in increased ADG was observed ($P = 0.1$), while growth rate was higher (females: $P < 0.05$; males: $P = 0.1$; treatment × DIM: $P < 0.05$) until d 35 compared with the CTL. In conclusion, CT milk from CLA supplemented dams proved to have a time-dependent effect on calf growth; however, it failed to alter IgG levels. Further research is needed to understand the mechanism of action of the CLA in modulating calves' growth.

Key Words: CLA, colostrum fatty acid profile, IgG

2473 Zinc, copper, manganese, and iron balance in dairy calves fed a milk replacer or whole milk at two feeding allowances.

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Trace minerals inclusion in milk replacer (MR) largely exceed natural mineral occurrence in whole milk (WM). Our objective was to quantify trace mineral balance in calves fed either WM or MR at 2 feeding allowances (L: 4.5 L/d; and H: 9.0 L/d) during their first 13 weeks of life. Forty-eight newborn Holstein-Friesian calves (2 ± 1.0 d of age; 45.0 ± 4.37 kg body weight) were enrolled after receiving a standardized colostrum administration. Upon arrival at the facility, calves were blocked by age and arrival date and randomly assigned to 1 of the 4 treatments. The MR was fed at 169 g/L of final product to provide the same amount of metabolizable energy as WM (3.4 MJ/L). The concentrations of zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe; mg/L) in MR and WM were: 10.14 vs 4.64, 1.82 vs 0.07, 4.95 vs 0.03, and 12.19 vs 0.25, respectively. Starter feed and straw were introduced at wk 6, and calves were gradually weaned from wk 6 to 10 and studied up to wk 13. Complete 24-h collection of urine and feces were performed on wk 2, 4, 5, 7, 9, 11 and 13. All data were analyzed using PROC MIXED (SAS 9.4). Preweaning, Cu, Mn and Fe, balances were higher ($P < 0.01$) for MR-fed calves than WM-fed calves. Increasing milk allowance increased Cu and Fe balances for calves fed MR but not for calves fed WM ($P < 0.01$). For Zn, increasing milk allowance increased Zn balance for WM calves but decreased Zn balance for MR calves. During weaning, feeding higher milk allowance resulted in lower balances of Zn, Cu and Mn ($P < 0.01$), and Fe ($P = 0.08$). During that time, Cu balance was further reduced for WM-fed calves ($P = 0.04$), whereas Zn balance was increased by feeding WM ($P < 0.01$) as compared with feeding MR. Postweaning, greater Cu ($P < 0.01$) and Fe ($P = 0.04$) balances were observed for WM vs MR-fed calves. Although trace mineral balance was lower in WM-fed calves during preweaning, no impacts on scours occurrence or therapeutic interventions were observed ($P > 0.10$). These results highlight the opportunity for further research to review mineral inclusion in MR.

Key Words: calves, mineral retention, milk sources

2474 Effect of the interaction of SCFA concentration and pH on health and hematology in cannulated Holstein dairy calves.

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Optimizing rumen development is key for preparing calves for weaning; however, the impact of the physiological development of the rumen on calf health and stress is unclear. This study investigated the effects of ruminal short-chain fatty acid (SCFA) concentration and pH and their interaction on health parameters in dairy calves. Holstein calves (n = 32), housed individually on rubber mats, were fed milk replacer (26% CP, 18% fat; up to 900 g/d) twice daily, and calf starter (18% CP) and water ad libitum. At 10 ± 3 d of life, calves were ruminally cannulated. One week after surgery, calves were blocked by initial body weight and randomly assigned in a 2 × 2 factorial arrangement of treatments of SCFA concentration (10 vs. 285 m*M*) and pH (5.2 vs. 6.2), yielding 4 treatment groups: low pH, low SCFA (LL), low pH, high SCFA (LH), high pH, low SCFA (HL), and high pH, high SCFA (HH). On wk 3, 5, and 7, calves underwent a 4-h washed reticulorumen procedure with a physiological buffer containing one of the 4 treatments. The ruminal buffers were added to the rumen at the beginning of the 4-h period and ruminal pH was measured hourly. Daily intakes of milk replacer and calf starter were recorded. Body weight, clinical health measures, blood hematology, and fecal scores were obtained weekly. Data were analyzed in R with repeated measures and SCFA and pH as fixed effects. Grain intake and ADG both increased as the calf aged regardless of treatment ($P < 0.01$). Fecal score decreased as the calf aged ($P < 0.01$). Low rumen pH increased respiration by 4.7 bpm ($P = 0.02$). No difference was detected in body core temperature by treatment over time. From wk 5 to wk 7, white blood cell counts decreased in the LL group ($P = 0.04$) but increased in the HL group ($P = 0.04$). No differences were detected in calf red blood cell counts, platelet counts, cortisol levels and β-hydroxybutyrate by treatment or age. Hemoglobin ($P = 0.05$) and hematocrit percent ($P < 0.01$) increased in the HL groups during wk 5 and 7. Haptoglobin decreased by 0.2 mg/mL every 2 weeks regardless of treatment ($P < 0.01$). Overall, rumen environment does not impact clinical health but does impact hematological markers.

Key Words: calf health, short-chain fatty acids, ruminal pH

2475 Changes in microbial community and host transcriptome in the duodenum in newborn calves.

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Human and mouse studies indicated the critical roles the small intestine (SI) microbiome in the development and maturity of the immune system early in life. However, few studies investigated the microbiome in the SI in neonatal calves. In this study, we employed whole transcriptome sequencing to interrogate changes in the microbial community and associated host transcriptome changes in the duodenum during the first 2 d after birth. Eight calves were enrolled in this study. Pasteurized colostrum was offered starting at birth. Four of the calves were euthanized within 12 h of birth (12h), and the rest of the calves were euthanized at 2 d of age (2d). Duodenum tissues were collected and subjected to RNA sequencing. Between 12h and 2d, 282 genes were differentially expressed (fold-change >2; *P*-value < 0.1). Genes upregulated at 2d (170 genes) were enriched in cell cycle and cell division related molecular pathways (MP). Genes downregulated at 2d (112 genes) were enriched in transcripts encoding asparagine-rich proteins and glycoproteins, both of which had reported roles in barrier function and intestine development. A total 13 genera showed significant abundance changes (fold-change >2; *P*-value < 0.1). Genera increased at 2d were previously reported in cow teat or milk, indicating a significant contribution of colostrum feeding in promoting microbial colonization in the young calf small intestine. Genera decreased at 2d are commonly found in rumen contents. Most strikingly, *Curtobacterium* and *Jeotgalibaca* showed significant association with expression changes in 243 and 157 genes, respectively. Genes associated with *Curtobacterium* were exclusively enriched in cell cycle and cell division related MP. Genes associated with *Jeotgalibaca* were primarily enriched in those related to glycoprotein synthesis. These findings indicated that colostrum feeding may be an effective way to target assembly and establishment of the microbiome in the small intestine. In turn, microbes in the small intestine interact with the host extensively to drive host hind gut development and barrier function maturation.

Key Words: neonatal calves, colostrum feeding, small intestine

2476 Impact of milk replacer feeding program on growth and efficiency of Angus × Holstein calves.

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Dairy beef (DB) crossbreeding has become prevalent in the dairy industry to help manage heifer inventories and increase calf values; however, minimal information is available on pre-weaning nutrition for DB crossbred calves. Our objective was to determine if a high-protein milk replacer (MR) fed at 0.78 kg/d dry matter (DM) would promote faster gains than a traditional MR fed at 0.57 kg/d DM. Angus × Holstein crossbred calves (n = 96; 48 heifers and 48 bulls) were blocked by expected birthdate and sex into 4 blocks (24 animals; 12 heifers and 12 bulls). Calves were then randomly assigned to 1 of 2 MR feeding treatments, being either 1) a 28% CP and 14% fat MR with weaning at 35 d old (28/14) or 2) a 22% CP and 20% fat MR with weaning at 42 d old (22/20). Liquid MR was fed twice daily until one week before weaning, then reduced to one feeding until weaning. Calf starter (18% CP) was offered free choice at one day of age with intakes monitored through 49 d of age. Water was offered ad libitum after each milk feeding. Data were analyzed (SAS v9.4) as a randomized complete block design with calf considered the experimental unit and MR treatment and calf sex included as fixed effects. Intake of MR was affected by treatment ($P < 0.01$) with calves fed 28/14 having higher total MR intake (24.5 kg DM) compared with 22/20 (21.9 kg DM). Total starter intake was not affected by MR treatment or calf sex ($P > 0.48$) with a mean of 46.6 kg starter DM consumed from birth to 7 weeks of age. Daily gain from birth to weaning was not affected by MR treatment ($P = 0.74$) with calves fed 28/14 gaining 0.62 kg/d for 35 d compared with calves fed 22/20 gaining 0.63 kg/d across 42 d of MR feeding. Daily gains from birth to 7 weeks of age were also not affected by MR or sex ($P > 0.59$) with a mean of 0.75 kg gain/d. Gain efficiency from birth to 7 weeks of age was not affected by MR treatment or sex ($P > 0.49$) with a mean efficiency of 0.54 kg gain/kg DM intake. Overall, MR programs with varying composition, feeding amounts, and weaning age had little effect on DB crossbred calf growth, starter intake, or gain efficiencies.

Key Words: dairy beef cross, calf, milk replacer

2477 Effects of ororuminal forced feeding in severely dehydrated calves.

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Diarrhea is the most common disease in bovine neonates. Intravenous fluid therapy (IV) and oral electrolytes may solve some clinical signs in diarrheic calves (e.g., dehydration and acidosis). This study aims to better understand the effects of different combinations of IV fluid therapy and force-fed ororuminal milk and electrolytes on acid-base balance to help determine an effective treatment for diarrheic calves. We hypothesized that severely dehydrated calves treated with IV combined with oral electrolytes would recover their electrolyte and acid-base balance faster than dehydrated calves treated with IV combined with milk replacer. Between May and July 2022, calves (n = 80) ages 5–14 d old were randomly enrolled at diagnosis of mild or severe dehydration by commercial farm personnel into one of the 4 groups: healthy control (HC), IV only (Mixture of Ringer Lactate 1 L, vitamin B_{12} 10,000 IU, and $NaHCO_3$ 14 g), IV plus oral force-fed electrolyte (2.0 L water with 80 g of Calf Gold Lyte electrolyte mix; Bio Vet; ELE), and IV plus oral force-fed milk replacer (2.0 L of Genuine Lawley's Product milk replacer; MILK). A dehydrated calf was defined as having one of the following clinical signs: enophthalmos, depression, lack of suckling reflex, and reduced appetite. Mixed linear models were used to analyze differences in some serum metabolites concentration. We did not observe a difference in sodium blood concentration between the groups ($P = 0.32$). All treatment groups returned to normal bicarbonate levels following treatment. The only statistical difference was between HC and other treatment groups at enrollment ($P = 0.01$). Therefore, 14-g $NaHCO_3$ may be an effective treatment for metabolic acidosis. Moreover, the anion gap in MILK was the highest following treatment at 20.8 mEq/L (16.2, 20.2) compared with the HC, IV only, and ELE, indicating that this treatment may be detrimental to acid-base balance.

Key Words: dehydrated calves, ororuminal, treatment

2478 Long-term impacts of in utero heat stress on heifer feed efficiency and enteric gas emissions.

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As global climate changes, producers may face the consequences of increased heat stress events during the dry period on performance and efficiency of the cow and her unborn calf as evidenced by previous research. Studies regarding the feed efficiency of lactating cows are readily available, but data regarding the feed efficiency of growing heifers are limited. Our objective was to evaluate the long-term effects of in utero heat stress on subsequent heifer performance and greenhouse gas emissions. A total of 38 heifers had been subjected to heat stress (HT; n = 17) or artificial cooling (CL; n = 21) in-utero (last 56 d of gestation) and were enrolled in a 63-d study at 18–20 mo old. Heifers were blocked by weight and randomly assigned to 3 pens with Calan gates and access to a GreenFeed machine (GF; C-Lock) for 8 ± 1 d to measure CH_4 and CO_2 gas fluxes. Body weights (BW) were measured on days −2, −1, 0 and 61, 62, 63 and used to calculate average daily gain (ADG). Hip height (HH), hip width (HW), and chest girth (CG) were recorded on d 0 and 63. All heifers were fed the same TMR consisting of 46.6% oatlage, 44.6% grass/alfalfa haylage, 7.7% corn silage, 0.3% urea, and 0.8% mineral (DM basis). Statistical analyses were performed using R version 4.1.1 (R Core Team, 2021) with packages lme4, and emmeans. Residual feed intake (RFI) was calculated by subtracting predicted DMI from observed DMI. The HT and CL heifers did not differ in initial (lsmean ± SE; 551 ± 8.6 kg, 543 ± 7.8 kg; $P = 0.47$, respectively) or final BW (615 ± 10.1 kg, 607 ± 9.1 kg; $P = 0.57$, respectively). There were no treatment effects ($P > 0.1$) on DMI (HT: 12.0 ± 0.46 kg, CL: 12.0 ± 0.46 kg DM) or ADG (HT: 1.00 ± 0.04 kg/d, CL: 1.02 ± 0.03 kg/d). We found that RFI was similar between HT (−0.009 ± 0.1) and CL

(0.007 ± 0.1, $P = 0.90$). Methane production, CO_2 production, and visits to the GF did not differ between treatment ($P > 0.5$). Despite previously reported reductions in growth and feed intake of in utero heat-stressed heifers during the pre-weaning phase, it does not seem to have long-term effects on growth, feed efficiency, or methane emissions later in life.

Key Words: efficiency, methane

Ruminant Nutrition 5: Gut Physiology, Fermentation, and Digestion

2479 Effects of a *Bacillus*-based direct-fed microbial on production and metabolism during feed restriction in mid-lactation Holstein cows. B. M. Goetz*[1], M. A. Abeyta[1], S. Rodriguez-Jimenez[1], J. Opgenorth[1], A. D. Freestone[1], T. A. Flemming[1], S. R. Fensterseifer[2], R. P. Arias[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*United Animal Health Inc., Sheridan, IN.*

Objectives were to evaluate the effects of a *Bacillus*-based direct-fed microbial (DFM; Strateris ECL; United Animal Health) on production and metabolism during feed restriction (FR) in mid-lactation Holstein cows. Thirty-six cows were randomly assigned to 1 of 3 dietary treatments (TRT): 1) control (CON; 7.5 g/d rice hulls; n = 12), 2) DFM10 (10 g/d *Bacillus* [*B. subtilis* and *B. pumilus* blend] DFM, 4.9×10^9 cfu/d; n = 12) or 3) DFM15 (15 g/d *Bacillus* DFM, 7.4×10^9 cfu/d; n = 12). Before study initiation, cows were fed their respective TRT for 32 d. Cows continued to receive TRT during the trial consisting of 3 experimental periods (P): P1 (5 d) served as baseline for P2 (5 d), during which all cows were restricted to 40% of P1 feed intake, and P3 (5 d), a "recovery" where cows were fed ad libitum. Effects of TRT, time, TRT × time, replicate, and parity were assessed using PROC MIXED of SAS. Feed restriction decreased dry matter intake (DMI), milk yield (MY), and energy-corrected milk (60, 32, and 29%, respectively; $P < 0.01$). Overall, P2 MY tended to be ($P = 0.07$) and P3 MY and DMI were quadratically increased in DFM10 cows relative to CON and DFM15 ($P \leq 0.02$). During P3, milk protein yield tended to decrease in DFM15 relative to CON and DFM10 (7%; $P = 0.07$). Further, lactose yield was increased in DFM10 relative to CON and DFM15 during P3 (9%; $P = 0.02$). All cows lost body weight (BW) during FR (57 kg; $P < 0.01$) and during P3 all cows regained BW, but a quadratic treatment tendency was observed where DFM10 cows gained more BW than CON and DFM15 cows ($P = 0.09$). Regardless of treatment, FR decreased circulating insulin, glucagon, and L-lactate (62, 40, and 24%, respectively; $P < 0.01$) and increased NEFA and BHB (3.4-fold and 8%, respectively; $P < 0.01$) during P2 relative to P1. Additionally, a quadratic effect was observed where DFM10 cows had increased circulating glucose relative to CON and DFM15 during P2 and P3 (6 and 9%, respectively; $P \leq 0.06$). Circulating glucagon tended to decrease in DFM15 cows relative to CON during P3 ($P = 0.06$). In summary, FR altered metabolism and feeding DFM10 increased key production metrics.

Key Words: probiotic

2480 The effects of supplementing cactus (*Opuntia ficus-indica*) powder on dairy calves' health and growth performance. P. M. Moshidi*[1], A. S. Sindane[1], S. Washaya[2], and M. C. Muya[3], [1]*University of South Africa, Johannesburg, South Africa,* [2]*Université Officielle de Mbujimayi, Kinshasa, Democratic Republic of the Congo,* [3]*Great Zimbabwe University, Masvingo, Zimabwe.*

The study aimed to evaluate the use of cactus (*Opuntia ficus-indica*) powder and *Lactobacillus* as a supplement on calf fecal pathogens and growth within 42 d of life. The study was conducted at the Agriculture Research Council, approximately 20 km south of Pretoria in the Gauteng Province of South Africa. It is situated at latitude 25.91° and longitude 28.21° with an altitude of 1 526 m above sea level. Cactus leaves were harvested, sun-dried for 14 d and thereafter oven-dried at 105°C, before grinding into a powder. Twenty-seven Holstein dairy calves, (39.03 ± 0.75 kg live weight) were randomly assigned to each of the 4 treatments: T1; control group (no supplementation); T2, *Lactobacillus* (LACT), 5 g/d (positive control); T3, 2.5 g/d cactus powder (CACP); and T4, 5 g/d CACP. Treatments were applied from d 4 until weaning. Calf starter feed and clean water were provided ad libitum for the entire study period. Data for feed intake and growth were measured daily and reduced to weekly means before analysis. Least squares means was presented by using the procedure PROC MIXED of SAS (2009) and significance was declared at $P < 0.05$. The treatments had an effect ($P < 0.05$) of weaning weight, average daily gain (ADG) and feed conversion ratio (FCR) compared with the control group. The result of the current study shows that supplementing *Lactobacillus* and cactus powder does reduce fecal pathogens and improve feed intake and body weight during the first 42 d of life.

Key Words: cactus powder, *Lactobacillus*, pathogens

2481 Effects of slow-release urea on lactation performance, plasma metabolites, rumen fermentation parameters of mid-to-late lactation cows under heat stress. M. Jiang*, X. Zhang, K. Wang, K. Zhan, and G. Zhao, *College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China.*

Slow-release urea (SRU) is under development to alleviate the increasing shortage of high-quality raw materials in China. The objective of this study was to evaluate the effects of supplementing SRU on body temperature indices, respiratory rate, plasma metabolites, and rumen fermentation parameters during heat stress (HS). Thirty multiparous lactating Holstein cows (body weight = 641.79 ± 56.17 kg; days in milk = 191.14 ± 27.24; and parity = 2.5 ± 0.1) were used in an experiment conducted in 2 groups (15 cows/group). Cows were randomly assigned to 1 of 2 dietary treatments: control diet (SM; basal diet + 500 g/d soybean meal, n = 15) or the basal diet supplemented with 100 g/d of slow-release urea (n = 15; Uridan, Menon). The trial period consisted of 5 d for adaptation and 30 d for sampling. Cows were milked 3 times a day, DMI and milk yield were measured daily, and milk components, body weight, and body condition were measured weekly. Blood was sampled weekly and plasma analyzed for concentrations of glucose, amino acid, immunoglobulin, SOD, CAT, TAOC, GSH-PX, MDA, HSP-70 and HSP-90α. Ruminal fluid was collected on 2 consecutive d 4 h after the morning feeding for measurements of pH, concentrations of short chain fatty acids, and NH_3-N. Data were analyzed using *t*-test, and $P < 0.05$ was considered significant. Rectal temperature and respiration rate were obtained 3 times a day (0800, 1400, and 2000 h). The milk yield and milk urea nitrogen significant increased with supplemental SRU in diet ($P < 0.05$). In the current study, supplementation of SRU significantly increased the concentrations of acetic acid in rumen fluid ($P < 0.05$). Supplementation of SRU significantly increased plasma IgA and decreased HSP-70 and HSP-90α levels in plasma ($P < 0.05$). Furthermore, the results showed that the supplementation of SRU in diet significantly increased the concentration of AA in plasma and milk ($P < 0.05$). Here, SRU efficiently improved the activity of plasma antioxidant enzymes. Overall, feeding 100 g of SRU/d to mid-to-late lactation cows under HS increased activity of antioxidant enzymes, increased the concentrations of acetic acid in rumen fluid, and decreased HSP-70 and HSP-90α levels in plasma.

Key Words: heat stress, slow-release urea, plasma metabolites

2482 Enteric gas emissions and rumen fermentation in dairy cows fed sucrose- or ethanol-rich diets. E. M. V. Hvas*[1], M. R.

Weisbjerg[1], M. Hanigan[2], J. C. Saunders[2], A. L. F. Hellwing[1], and M. Larsen[1], [1]*Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Aarhus University, Tjele, Denmark,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA, United States.*

The aim was to examine enteric gas emissions and rumen fermentation in dairy cows fed sucrose- or ethanol-rich diets by including either fresh or ensiled sugar beets in the diets. Sugar beets were washed, crushed, and stored either fresh (frozen at −20°C) or ensiled (barrels) in daily portions. Four rumen cannulated Danish Holstein cows were used in a replicated (one complete and one incomplete) 3×3 Latin square design with 3 treatments and three 21-d periods. The control diet (CON) was based on grass/clover silage, corn silage, barley, soybean meal, dried beet pulp, urea, and minerals. In the sucrose- (SUC) and ethanol- (ETH) rich diets, 20% of DM from CON was replaced with either fresh or ensiled beets. The diets were fed ad libitum as total mixed rations. Each period comprised 10-d adaptation, 7-d collection, and 4-d in respiration chambers. Data were analyzed using linear mixed models with treatment, time, period, and treatment × time interaction as fixed effects, cow as random effect, and considering time as repeated measure. The ethanol content in diets averaged (±SD) 2.51 ± 0.63, 2.11 ± 0.53, and 15.6 ± 2.38 g/kg DM for CON, SUC, and ETH, respectively. The DMI ($P = 0.37$; mean ± SEM; 22.3 ± 0.99 kg/d) and ECM yields ($P = 0.13$; 29.2 ± 2.90 kg/d) were unaffected by diet. Daily hydrogen emissions were greater for SUC and ETH than for CON ($P < 0.01$; 1.27 vs. 0.89 ± 0.09 g/d). Daily methane emissions ($P = 0.38$; 442 ± 24.5 g/d) and methane emissions per kg DMI ($P = 0.15$) were unaffected by diet. Rumen pH tended to be lower for ETH than for CON ($P = 0.09$; 6.29 vs. 6.38 ± 0.11). Molar proportions of propionate were unaffected by diet ($P = 0.33$), where molar proportions of acetate were greater for CON than for ETH ($P = 0.03$; 59.4 vs. 57.0 ± 0.89 mol/100 moles) and molar proportions of butyrate were greater for SUC than for CON ($P = 0.02$; 16.8 vs. 13.3 ± 0.78 mol/100 moles). Ruminal ethanol was higher for ETH than for CON and SUC ($P < 0.01$; 2.75 vs. 0.55 ± 0.14 mmol/L). In conclusion, feeding sucrose or ethanol rich diets altered ruminal fermentation and increased enteric hydrogen emissions but did not alter enteric methane emissions.

Key Words: methane, hydrogen, beets

2483 Effect of monensin and live-cell yeast supplementation on the lactation performance, apparent total-tract digestibility, and feeding behavior of dairy cows. E. C. Diepersloot*, M. R. Pupo, C. Heinzen Jr., B. A. Saylor, and L. F. Ferraretto, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objective of this experiment was to determine the effect of dietary supplementation with monensin and live-cell yeasts on lactation performance, apparent total-tract digestibility, and feeding behavior of dairy cows. Sixty-four multiparous Holstein cows were randomly assigned to 1 of 4 treatments with a 2×2 factorial arrangement for a 10-week treatment period, after a 2-week acclimation period and 2-week covariate period. Treatments consisted of both (MON-LCY) monensin (18 g/ton) and live-cell yeast (*Saccharomyces cerevisiae*; 1×10^{10} cfu/hd/d), only monensin (MON-CON; 18 g/ton), only live-cell yeast (CON-LCY; *S. cerevisiae*; 1×10^{10} cfu/hd/d), or neither (CON-CON). Data were analyzed as a completely randomized design with data from the covariate period as a covariate using a mixed model with week of treatment as repeated measures. The model included MON, LCY, week, and their 2- and 3-way interactions as fixed effects, while cow (treatment) was a random effect. Three-way interactions were observed for dry matter intake ($P = 0.001$) and feed efficiency ($P = 0.01$). Dry matter intake was 1 to 2 kg greater for CON-CON than MON-LCY during wk 4, 5, 8, 9, and 10 of the treatment period, while feed efficiency was approximately 0.1 unit lower for CON-CON than MON-LCY for wk 2, 5, and 9. An interaction between MON and LCY was observed for dry matter and organic matter digestibility ($P = 0.02$ and $P = 0.02$, respectively) with both being approximately 3%-units lower for CON-CON than other treatments. Milk yield (51.8 kg/d, on average) and concentrations of milk fat (3.59%, on average), protein (3.19%, on average), and lactose (4.74%, on average) were unaffected by treatments ($P > 0.10$), whereas a tendency ($P = 0.08$) for LCY to reduce milk fat yield by 0.06 kg/d was observed. These results suggest that feeding a combination of MON and LCY can reduce intake while improving feed efficiency by dairy cows without impairing milk components.

Key Words: direct-fed microbial, ionophore, feed efficiency

2484 Effects of fibrolytic enzyme additive on in vitro rumen fermentation. D. Djouvinov, E. Bungenstab*, and G. Gomes, *AB Vista, Plantation, FL.*

The study aimed to investigate the effect of a fibrolytic enzyme additive (VPT, VistaPre-T, AB Vista) on in vitro rumen fermentation of palm kernel meal (PKM) alone or combined with grass silage in a total mixed ration (TMR). The treatments (TRT; Table 1) were run with 5 replicate vessels with 40 mL of total volume each. Rumen fluid from a fistulated cow fed grass silage ad libitum and 8 kg/day concentrate. A total of 38 mL of buffer solution plus the test compounds were added to vessels under the oxygen-free CO_2 flow, 2 mL of strained rumen fluid was added, then sealed with rubber stoppers. The incubation continued for 10 h at 38°C. After incubation total gas production in each bottle was measured and the simulation vessels were analyzed for individual volatile fatty acids (VFA). The experimental data were subjected to ANOVA and means separated using Student's *t*-test, differences deemed significant at $P < 0.05$. When TMR was added, concentrate added at (400 mg/vessel), while PKM was added at 100 mg/vessel and silage was either 500 or 600 mg, depending on the inclusion of PKM. The VPT inclusion was 7.5 mg/vessel. Adding PKM significantly ($P < 0.01$) reduced microbial gas production from 113 mL in TRT 1 to 109 mL when added to TMR (TRT 2). The VPT in combination with TMR (TRT 3) improved total gas production to 114.0 mL and to 110.0 mL in PKM (TRT 4), mitigating the negative effect of PKM. The suppressive effect ($P < 0.01$) of PKM (TRT 2) in TMR samples (TRT 1) was confirmed by reduced concentrations of total SCFA (91.5 mmol/L vs 95.6 mmol/L), acetic (41.96 mmol/L vs 44.01 mmol/L), propionic (40.53 mmol/L vs 42.25 mmol/L) and butyric (6.34 mmol/L vs 6.45 mmol/L) acids. The VPT did not significantly affect either SCFA or acetic and propionic acids contents. However, butyric acid was increased ($P < 0.05$) from 6.45 mmol/L in TMR samples (TRT 1) to 6.53 mmol/L when incubated with VPT (TRT 3). The PKM negatively impacted in vitro fermentation, but VPT improved total gas

Table 1 (Abstr. 2484). Treatment (TRT) design[1]

TRT	TMR	PKM	VPT
1	+	—	—
2	+	+	—
3	+	—	+
4	+	+	+
5	—	+	—
6	—	+	+

[1] PKM = palm kernel meal; VPT = fibrolytic enzyme additive, VistaPre-T, AB Vista. + indicates treatment contains component; — indicates treatment does not contain component.

and butyric acid production (TMR or PKM alone). We find that VPT can be used to ameliorate the detrimental fermentation effects of PKM, improving total gas and butyric acid concentration.

Key Words: total gas, VFA, palm kernel meal

2485 Effects of supplementing native rumen microbes on rumen fermentation and bacterial abundance in transition and mid-lactation Holstein cows. M. Bulnes*[1], J. Lefler[2], C. Marotz[2], J. Halfen[3], T. Fernandes[3], M. Embree[2], J. Osorio[3,1], and M. E. Uddin[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Native Microbials Inc., San Diego, CA,* [3]*School of Animal Sciences, Virginia Tech, Blacksburg, VA.*

Our objective was to evaluate the effects of a rumen derived microbial product composed of 4 microbial species: *Clostridium beijerinckii* at 2 × 10^6 cfu/g, *Pichia kudriavzevii* at 2 × 10^7 cfu/g, *Ruminococcus bovis* at 2 × 10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2 × 10^7 cfu/g [Galaxis Frontier (GF); Native Microbials Inc.] on rumen fermentation and bacterial abundance in transition and lactating cows. Fifty-six Holstein cows were enrolled in a randomized complete block design from −22 to 100 d in milk (DIM). Cows were blocked based on expected calving date, parity, and previous lactation milk yield for multiparous or genetic merit for primiparous cows. At −21 DIM, cows were randomly assigned to a control (CON, basal diet + 150 g/d of ground corn; n = 29) or treatment group (GF, basal diet + 150 g/d of ground corn + 5g/d of GF; n = 27), top-dressed once a day. All cows received the same basal close-up diet until calving (1.29 Mcal/kg DM and 10.8% CP) and lactation diet from calving to 100 DIM (1.67 Mcal/kg DM and 15.3% CP). Rumen fluid samples were collected for ammonia, volatile fatty acids and bacterial abundance (quantified via RT qPCR) from a subset of multiparous cows (n = 12/treatment) at −22, −14, −7, 1, 7, 14, 21, 70 and 100 DIM. Statistical model included the random effects of block and cow; and the fixed effects of treatment, time, parity and their interactions. Compared with CON, GF cows had higher ruminal butyrate ($P = 0.04$) and tended ($P = 0.06$) to have higher valerate and lower ($P = 0.10$) acetate. These effects were coupled with alterations in rumen microbiota, where lactate-utilizing bacteria (*Megasphaera elsdenii*) tended to be more abundant at 14 DIM whereas cellulolytic bacteria (*Fibrobacter succinogenes*) tended to be less abundant at 1 and 21 DIM in GF than CON cows ($P = 0.07$). Abundance of *Prevotella albensis*, associated with butyrate production, tended to be higher ($P = 0.09$) in GF than CON cows. Supplementation of GF during transition period altered rumen bacterial abundance along with ruminal butyrate, valerate and acetate proportions suggesting potential benefits on rumen environment.

Key Words: feed additive, rumen fermentation, rumen bacteria

2486 Metabolomic profiling of rumen fluid reveals modulation upon *Fucus* spp. dietary supplementation in dairy cows. M. V. Curtasu*[1], E. Chasse[1], M. Thorsteinsson[1,3], M. Battelli[2], A. Bruhn[3,4], and M. O. Nielsen[1,3], [1]*Department of Animal and Veterinary Sciences, AU-Viborg, Research Centre Foulum, Aarhus University, Tjele, Denmark,* [2]*Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy,* [3]*Center for Circular Bioeconomy, Aarhus University, Foulum, Tjele, Denmark,* [4]*Department of Ecoscience, Aarhus University, Aarhus, Denmark.*

Enteric methane (CH_4) emission from ruminants is a major contributor to global greenhouse gas emissions. Seaweeds, particularly 2 *Fucus* species, have previously been shown in vitro to reduce CH_4 emission from rumen fermentation. However, the effects of *Fucus* spp. on the rumen fluid metabolome are unknown, and therefore we aimed to gain insights into the underlying mechanisms of the anti-methanogenic properties of *Fucus* spp. in an in vivo trial where CH_4 emissions and other parameters were assessed. A study was conducted using 4 multi-cannulated Danish Holstein dairy cows in 2 repeated (one complete and one incomplete) 3 × 3 Latin square design experiments, with 3-wk periods. Each period lasted 21 d with an adaptation period of 14 d. Cows were fed 3 different total mixed rations (TMR) including a control TMR without seaweed (CON) and 2 rations with 4% on dry matter basis of either *Fucus serratus* (SER) or *Fucus vesiculosus* (VES) diluting the control TMR. Rumen sample was collected at 8 separate time points to account for diurnal variation in feed intake. Untargeted metabolomics of rumen fluid was performed using liquid-chromatography mass-spectrometry on a Shimadzu LCMS-9030 system. Data were collected in positive/negative ionization modes on a C18 column. Multivariate analysis on 691 variables was conducted to identify differences in metabolome between control (n = 32) and *Fucus* groups (SER, n = 32; VES, n = 32). A supervised partial least squares (PLS) analysis was performed where metabolite abundance data were used as predictor variables and the experimental diets as response variables. The PLS analysis revealed that CON group separates from SER and VES groups in the first 2 principal components of score plots. However, no separation was observed between the metabolic profiles of SER and VES. Important variables contributing to the separation between CON and SER/VES, such as sinapyl alcohol and sinapine, were putatively identified and found to have higher intensity in CON fed cows. Findings from this study suggest that addition of the 2 *Fucus* spp. to diets of dairy cows has similar impacts on the rumen fluid metabolome without affecting methane emission. Further analysis will explore the metabolic profile of *Fucus* spp. responsible for the anti-methanogenic actions in vitro.

Key Words: macroalgae, methane emission, rumen fluid

2487 The effects of supplementing a seaweed *Asparagopsis taxiformis* on the rumen microbiome in dairy cows. N. Indug*[1], H. A. Stefenoni[2], M. Hennessy[1], B. Vecchiarelli[1], J. Bender[1], R. Shah[3], S. Garapati[4], C. Yarish[5], S. Welchez[2], S. E. Räisänen[2], D. Wasson[2], C. Lage[2], A. Melgar[2], A. Hristov[2], D. Pitta[1], [1]*University of Pennsylvania, New Bolton Center, PA,* [2]*The Pennsylvania State University, State College, PA,* [3]*University of Pennsylvania, Philadelphia, PA,* [4]*Drexel University, Philadelphia, PA,* [5]*University of Connecticut, Stamford, CT.*

Among the most effective methane mitigation strategies, *Asparagopsis taxiformis* (AT) has been shown to reduce methane emissions by 80%. Understanding the effect of AT on rumen microbiota may shed light on the mechanism of action of AT. We aimed to investigate the effect of AT on the rumen microbiome of dairy cows. Twenty Holstein cows were randomly assigned to 4 [basal diet (control), or basal diet supplemented with (dry matter basis) 0.25% AT, 0.5% AT and Oregano] treatments in a replicated 4 × 4 Latin square design with four 28-d periods. Enteric gas emissions were measured using the GreenFeed system. Toward the end of each period, ruminal samples were collected using stomach tube, solid fraction was separated, extracted for genomic DNA and processed for 16s rRNA, metagenomics (metaG) and metatranscriptomics (metaT). The 16s rRNA analysis performed in QIIME2 and metaG and metaT reads analyzed using HUMAaN3 pipeline followed by statistical analysis using R with PERMANOVA test and generalized linear models. In 16s rRNA analysis both bacterial and archaeal communities were influenced by treatment ($P < 0.05$). We found that AT inhibited methane emissions by 60% in period 1 and this was accompanied by near elimination of *Methanosphaera* whereas *Methanobrevibacter* remained unaltered. As the effect of AT decreased from period 2, *Methanosphaera* increased in

AT compared with other treatments and then stabilized and were similar to other treatments in period 3 and 4. Among bacterial taxa, the butyrate producing bacteria such as *Butyrivibrio*, *Moryella* and *Eubacterium* were increased in AT treatments compared with control throughout the experiment, In metaG and metaT, we also found the lower abundance of *Methanosphaera* and higher abundance of butyrate producing bacteria; these were also accompanied by a decreased abundance ($P < 0.05$) of genes/transcripts encoding for Methyl-coenzyme M reductase (EC 2.8.4.1) and for butyryl-coA dehydrogenase (EC 1.3.8.1) in AT compared with other treatments. These findings indicate that AT reduced CH4 emissions and selectively inhibited methanol utilizing methanogens such as Methanosphaera which may have a greater share in total methane formation in the rumen. However, further investigation is required to ascertain the role of AT on individual methanogens.

Key Words: alternate pathways, methylotrophic methanogens, ruminal methanogenesis

2488 Effects of feeding *Bacillus subtilis* and *Clostridium beijerinckii* to Holstein cows on ruminal degradability of alfalfa haylage and corn silage. L. Garcia*[1], F. F. Cardoso[1], J. S. Thompson[2], M. N. De Jesus[2], A. H. Smith[2], T. G. Rehberger[2], M. R. Murphy[1], and F. C. Cardoso[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*Arm and Hammer Animal and Food Production, Waukesha, WI.*

Our objective was to determine the ruminal degradability of alfalfa haylage (AH) and corn silage (CS) in response to feeding 2 strains of *Bacillus subtilis* (2.0×10^9 cfu/daily); 6 strains of environmental *Clostridium beijerinckii* (4×10^6 cfu/daily), or both in lactating Holstein cows. Treatments were: TMR top-dressed with *B. subtilis* (CER; Certillus, Arm and Hammer and Food Production; 20 g/cow/day), *C. beijerinckii* (BJE; 0.001% of dietary DM), both (CBJ), and neither (CON). Cows were fed from −21 d relative to expected calving until 70 d in milk on cow's performance. The chemical composition of the postpartum diet was 44.5% DM, 16.1% CP, 22.9% ADF, 33.3% NDF, 3.48% lignin, 23.8% starch, 5.2% crude fat, and 7.7% ash. Samples (8 g) of dried and ground AH and CS were placed into (4 replicates/feed) polyester bags on d 65 and incubated for 0, 2, 4, 12, 24, or 48 h in 3 rumen-cannulated cows per treatment (n = 3). Nutrient disappearance data from the bags was used to fit a nonlinear function to model digestion. A data set was created for results including soluble (S), digestible (D), and indigestible (I) fractions of each nutrient, fractional rate of digestion (K_d), and effective degradability (ED) were estimated. Statistical analysis was preformed using the MIXED procedure of SAS. Digestible fraction of DM for AH was higher ($P < 0.02$) for cows in CON (0.54%) than CER (0.38%) and BJE (0.35%). Indigestible fraction of DM for AH was lower ($P < 0.03$) for cows in CON (0.14%) than BJE (0.20%). Indigestible fraction of DM for CS tended to be higher ($P = 0.10$) for cows in CON (0.26%) than BJE (0.14%). Dry matter K_d for CS was higher ($P < 0.01$) for cows in CON (0.08 h^{-1}) than CER (0.03 h^{-1}) and BJE (0.04 h^{-1}). Soluble fraction of DM for AH was lower ($P < 0.05$) for cows in CON (0.31%) than CER (0.46%) and BJE (0.44%). Soluble fraction of DM for CS was lower ($P < 0.01$) for cows in CON (0.32%) than CER (0.48%) and BJE (0.47%). In conclusion, feeding *B. subtilis* and *C. beijerinckii* altered CS and AH degradation rate differently resulting in increased nutrient indigestible fractions for AH but not CS.

Key Words: degradability, *Bacillus*, *Clostridium*

2489 Effects of feeding *Bacillus subtilis* and *Clostridium beijerinckii* on total mixed ration apparent digestibility of lactating Holstein cows. L. Garcia*[1], F. F. Cardoso[1], J. S. Thompson[2], M. N. De Jesus[2], A. H. Smith[2], T. G. Rehberger[2], M. R. Murphy[1], and F. C. Cardoso[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*Arm and Hammer Animal and Food Production, Waukesha, WI.*

Nutrient restriction due to limited dry matter intake (DMI) during early lactation in dairy cows may be a limiting factor for farm profitability. Improvement of feed digestibility can help alleviate challenges faced during this period. Therefore, our objective was to determine the apparent digestibility (AD) of total-mixed ration (TMR) in response to feeding 2 strains of *Bacillus subtilis* (2.0×10^9 cfu/daily); 6 strains of environmental *Clostridium beijerinckii* (4×10^6 cfu/daily), or both in lactating Holstein cows. Forty multiparous Holstein cows, blocked by parity, previous 305-d milk production, and expected calving date were assigned to 1 of 4 dietary treatments in a randomized, complete block design. Treatments were: TMR top-dressed with *B. subtilis* (CER; Certillus, Arm and Hammer and Food Production; 20 g/cow/day), *C. beijerinckii* (BJE; 0.001% of dietary DM), both (CBJ), and neither (CON). Cows were fed from −21 d relative to expected calving until 70 d in milk on cow's performance. Cows were milked 2× per d and milk yield along with DMI were recorded daily. Samples of TMR were collected on d 63 to 65 and orts were collected on d 64 to 66 and analyzed for DM, NDF, ADF, and starch. The TMR was fed at 1400 h daily. Fecal samples (120 mL, wet weight) were collected directly from the cow's rectum at 8 time points across d 63 to 65. We used uNDF as an internal marker. Statistical analysis was performed using the MIXED procedure in SAS. There was no difference for DMI (CON = 22.9, BJE = 23.4, CER = 22.5, CBJ = 23.7 kg/d) and milk yield (CON = 43.8, BJE = 41.3, CER = 44.2, CBJ = 45.4 kg/d; $P > 0.10$). There were no treatment differences ($P > 0.11$) for AD of ADF. Cows in CON tended ($P < 0.10$) to have lower AD of DM (82.57%) than cows in CER (85.92%). Cows in CON tended ($P = 0.07$) to have lower AD of NDF (68.15%) than cows in CER (76.09%) and BJE (74.90%). Cows in CON had lower ($P = 0.04$) AD of starch (97.81%) than cows in CER (98.43%). In conclusion, feeding *B. subtilis* seems to have altered rumen microbial population, thus improving TMR AD of DM, NDF, and starch.

Key Words: apparent digestibility, *Clostridium beijerinckii*, *Bacillus subtilis*

2490 Adaptation of rumen bacteria to pH alters the membrane lipid composition. D. de Oliveira*, E. Mir, B. Barreta, and F. Batistel, *University of Florida, Gainesville, FL.*

The rumen pH of high-producing dairy cows varies substantially throughout the day because of the high concentration of fermentable carbohydrates. Although it is well established that non-rumen bacteria alter the proportions of saturated and unsaturated fatty acids in the cell membrane to survive environmental changes in pH, limited information is available about rumen bacteria. We hypothesized that rumen bacteria alter the fatty acid profile of the cell membranes under different pH conditions in a similar form. Our objective is to characterize and compare the fatty acid profile of the cell membrane of rumen bacteria under normal and slightly acidic pH. Four pure cultures of rumen bacteria were anaerobically cultivated under 2 different pH (6.5 and 5.8) in a completely randomized design. Cultures were terminated after reaching half of the exponential and stationary phases and stored for further analysis. Lipids were extracted from microbial cultures and phospholipids were separated using aminopropyl-bonded phase columns. Fatty acids were verified by gas chromatography. Compared with the pH of 6.5, the pH of 5.8 decreased growth and production of short-chain fatty acid ($P < 0.01$) for all bacteria analyzed. Compared with the pH of 6.5, *Fibrobacter succinogenes S85* decreased the concentration of C9:0 ($P = 0.08$) and C17:0 ($P < 0.01$), and increased *iso*-C17:0 ($P = 0.01$) and *cis*-9 C18:1 ($P = 0.06$) in the cell membrane under the pH of 5.8. The pH of 5.8 increased the concentration of *cis*-9 C16:1 ($P < 0.01$) and *cis*-7 C18:1 ($P = 0.06$), and decreased C19:0 ($P = 0.01$) in the cell membrane of *Lachnospira multipara* D32. *Prevotella ruminicola* increased the concentration of *iso*-C16:0 ($P < 0.01$) and *cis*-9 C16:1 ($P < 0.01$), and decreased *cis*-7 C18–1 ($P = 0.02$) in the cell membrane compared with the pH of 6.5. Under the pH of 5.8, *Pseudobutyrivibrio sp. B1A1* increased the membrane concentration of *anteiso* C13:0 ($P = 0.07$) and *cis*-9 C16:1 ($P < 0.01$), while decreased *iso* C16:0 ($P < 0.01$) and C17:0 ($P < 0.01$). Our preliminary results indicate that rumen bacteria change the membrane fatty acid profile to maintain homeostasis when challenged by acidic conditions.

Key Words: fatty acid, cell membrane, acidic condition

Dairy Foods: Milk Protein and Enzymes Committee Symposium: High Milk Protein Foods Innovation Opportunities

2491 High milk protein foods, sensory and consumer insights. M. A. Drake*[1] and D. M. Barbano[2], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Protein-rich foods, including beverages and bars, continue to enjoy high consumer demand. The perceived importance of milk protein versus other types of protein has decreased with consumers in recent years. There is increased competition from plant and animal free protein sources. However, milk proteins have unique flavor, functional and nutritional properties that align with consumer desires and provide opportunities for innovation. Consumers desire convenient and credible sources to know more about milk protein and need effective messaging to reduce their vulnerability to incomplete information and misinformation. Qualitative and quantitative studies of consumer insights will be discussed.

Key Words: consumer insights, milk protein, beverages

2492 High-protein ice cream: Processing and chemistry. S. VanWees, S. Rankin*, and R. Hartel, *University of Wisconsin–Madison, Madison, WI.*

Preference for better-for-you desserts has expanded the ice cream category to include reduced-calorie, low-sugar, and high-protein alternatives. Fortification of ice cream with protein-rich ingredients presents several challenges in manufacturing unique frozen desserts with acceptable functional attributes. Commercially available high-protein frozen desserts are being manufactured to provide intended nutritional benefits, yet are routinely plagued with poor texture, flavor character, and storage stability. Work within this realm demonstrates that each step of the manufacturing process affects the structure, thus functional behavior of finished frozen desserts. While proteins are known to provide several key benefits, such as mix viscosity, emulsifying capacity and improved foam structure, more recent work demonstrates that proteins, especially at higher levels can negatively affect the multiphase structure of ice cream. Research suggests that the source of protein, the hydration state and history of the protein ingredient, and the intrinsic properties of the protein influence the development of fat, ice, and air structures, as well as the stability of these dispersed phases during distribution and storage. Our work proposes a novel paradigm for frozen dessert structure based on assessments from temperature-cycled storage on ice crystal size and foamability/foam stability as affected by proteins. Dispersions of milk protein were affected by rehydration, solubilized solids and particle size distributions, and the dilatational viscoelasticity of the protein-stabilized air interface may help predict air cell stability during abusive storage. Frozen desserts made with globular whey proteins may be more resistant to foam destabilization caused by storage abuse compared with those made with caseins due to the increased dilatational viscoelasticity. Investigations on composition, microstructure, and interfacial properties on stability of frozen foams also inform the causes and mechanisms of shrinkage in frozen desserts; therefore, it is imperative to utilize the physicochemical attributes of protein to facilitate the design of high-quality, better-for-you frozen desserts.

Key Words: ice cream, protein, structure

2493 Innovations in high-protein food bars and preventing unwanted hardening. D. J. McMahon*, *Utah State University, Logan, Utah.*

High-protein food bars are often manufactured using combinations of whey protein isolate (WPI), partially hydrolyzed WPI, and/or milk protein isolate as the major dairy protein source. Other functional ingredients depending on the target market include solid vegetable oil, various sugar alcohols, glycerin, fiber, hydrocolloids and emulsifiers. This presentation covers changes that occur during storage of high protein food bars, mechanisms proposed as the cause of hardening. An understanding of how proteins interaction with the surrounding water and sugar alcohol cosolvents can be used to prevent unwanted bar hardening.

Key Words: protein, bars, hardening

2494 Muscle protein synthesis in response to dairy protein fractions, whole protein-dense foods, and resistance exercise. N. M. M. P. de Hart*, *The University of Utah, Salt Lake City, UT.*

Stimulation of muscle protein synthesis is necessary for maintaining muscle mass. Protein from different dairy sources affects the magnitude of protein synthesis and further enhanced when combined with resistance exercise. The interplay between dietary protein and exercise has emerged over the years with a specific focus on protein fractions such as whey and casein. On the contrary, whole protein-dense foods are less understood but investigating these foods are necessary to advance practical approaches to promote maintenance of muscle mass since most people consume mixed macronutrient protein-dense whole foods and not solely isolated protein fractions. An overview of the current understanding of muscle protein synthesis in response to protein feeding and in combination with resistance exercise will be discussed.

Key Words: muscle protein synthesis, exercise, whole protein-dense foods

Animal Behavior and Well-Being 1

2495 Validation of tri-axial accelerometers for classifying feeding and postural behaviors in lactating dairy cows on pasture.

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The objective was to validate the HOBO tri-axial accelerometers against visual observations for feeding (grazing, ruminating) and postural (lying, standing, walking) behavior classification in lactating dairy cows on pasture, by using machine learning techniques. In spring, 12 Holstein multiparous dairy cows (3.4 ± 0.9 parity; 154.4 ± 30.5 DIM; 27.0 ± 3.7 kg/d milk) were selected from the Veterinary Faculty Experimental herd. Cows were milked 2×/day, fed concentrate in the milking parlor, a mixture of concentrate and silage in feeders post milking and grazed pasture in daily strips between milking (0730–1400 h and 1730–2000 h). Throughout the study, cows were managed together with the rest of the herd (158 cows). On day −5, cows were fitted with 2 tri-axial HOBO accelerometers (Hobo Pendant G Acceleration Data Logger, Onset Computer Corporation), one in a halter and other in the left hind leg, that were set up to register acceleration and tilt every 30 s from day 0 to 21. During the same period, scans samplings every 5 min were done by direct visual observations by 2 trained observers to record feeding and postural behaviors of each cow during the time they were at the pasture. Loggers were removed every 7 d and data downloaded. A total of 16,700 visual observations were registered and a data set was obtained in which observations for each cow were combined with the accelerometer data. Different features were computed from both accelerometers and the F-score and accuracy were computed to test the relation between the different features and each target behavior. In addition, 2 random forest algorithms were trained to classify each behavior. An accuracy of 0.84 was found for feeding behaviors, with F-score values of 0.92 and 0.83 for grazing and ruminating activities, respectively. Postural behaviors presented an accuracy of 0.92, with F-score values of 0.47 for walking and 0.94 for lying and standing behaviors. In conclusion, tri-axial HOBO accelerometers accurately classified both postural and feeding behavior, with greatest accuracy for lying, standing and grazing activities.

Key Words: algorithm, cattle, grazing

2496 Impact of dystocia on behavior of dairy cattle around calving.

S. J. Minard and N. Blackie*, *Royal Veterinary College, Hatfield, Hertfordshire, UK.*

Dystocia can be a costly issue within dairy production systems in terms of veterinary treatments costs, poor calf viability and potential reduction in future reproductive performance in the dam. Dystocia rates can be up to 15% on some dairy farms (Stafford, 2011). Being able to predict calving and more importantly a parturition in which dystocia is present is therefore, important to the dairy industry. This study aimed to investigate the impact of dystocia on motion index, lying times and number of lying/standing transitions on the day of and day before calving. A total of 44 Holstein dairy cows were recruited to the study retrospectively using calving records from 2021 and the first half of 2022. Of these cows, 37 had normal births and 7 had dystocia. All cows had data loggers (IceQube, IceRobotics) placed on a rear leg to assess their behavioral activity and were housed in an individual straw bedded calving pen. Daily lying time (LT; h/d); motion index (MI) and number of lying bouts (LB; n/d) were assessed 24 h before and 24 h after calving took place. Data were analyzed using GraphPad Prism with significance declared at $P \leq 0.05$. For unassisted births when comparing the day before partition (DBP) with the day of partition (DOP) there was no significant differences in MI (mean ± SD: 4,397 ± 2,204 vs 4,721 ± 2,812), respectively) or LT (11.30 ± 3.2 vs 10.75 ± 3.5) there was a significant increase in LB on DOP ($P \leq 0.0001$; 25.4 ± 13.6 vs 42.2 ± 14.1). Similarly, for cows with dystocia there was no significant differences between DBP and DOP for MI (3,102 ± 1,908 vs 4,052 ± 1,480) or LT (11.06 ± 2.9 vs 10.69 ± 2.5) there was a significant increase of LB on DOP ($P = 0.0017$; 26.2 ± 6.18 vs 50.5 ± 11.7). Results indicate that LB could be useful to predict calving. Cows with dystocia on the day of calving showed a 96% increase in transitions (LB) compared with the day of calving showing it may be a useful metric to detect calving in general or more so to flag cows with a potential dystocia.

Key Words: dystocia, activity monitoring, cattle behavior

2497 Milking parlor behavior, body measurements, and body condition scores of first-lactation cows raised in individual, pair, or group housing compared to dam-reared housing.

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The objective of this study was to determine milking parlor behavior, body measurements, and body condition scores of first lactation cows raised in individual (I), pair (P), group (G), or dam-raised (D) housing systems during the pre-weaning period. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN. Seventy-nine Holstein and crossbred heifer calves were assigned to housing treatments by birth order and were born from September to December 2020. The I, P, and G raised calves were placed in a housing system at 3 d and were fed 10 L of milk per d. Calves raised by their dam were isolated with the dam for 3 d and introduced to a larger group where they suckled ad libitum. All calves were weaned at 63 d. First lactation cows for the current study calved from September to November 2022. Behavior measurements were from the first 8 milkings after calving. Independent variables were the fixed effects of breed, treatment, milker, and cow and date of measurement were repeated effects. Dam-reared cows had 2.3 ± 0.3 times greater ($P < 0.01$) odds of being restless, very restless, or hostile during milking than I, P, and G cows. Furthermore, dam-reared cows had 2.3 ± 0.3 times greater ($P < 0.01$) odds of stomping, 4.3 ± 0.4 times greater ($P < 0.01$) odds of kicking off the milking unit, and 2.3 ± 0.3 times greater ($P < 0.05$) odds of kicking than I, P, and G raised cows. The G cows had larger ($P < 0.05$) heart girth than D cows (198.4 cm vs. 189.2 cm), but similar heart girth to I and P cows. The I, P, and G cows had larger ($P < 0.05$) rear udder width (5.2 cm, 4.8 cm, and 5.4 cm, respectively) than D cows (4.2 cm). Hip height, body length, pin width, thurl width, rear udder height, body condition score at calving, and body condition score 4 weeks post-calving were not different ($P > 0.05$) for I, P, G, and D cows. Results from this study indicate there may be behavioral advantages to raising calves individually, in pairs, or in groups versus raising them with their dam during the pre-weaning period.

Key Words: individual, pair, dam-raised

2498 Agreement between welfare and health assessment evaluation protocols used during auction sale of cull dairy cows. M. Puerto-Parada*[1], S. Buczinski[1], J. Dubuc[1], L. Blouin[2], and M. Villettaz-Robichaud[1], *[1]Université de Montréal, Saint-Hyacinthe, Québec, Canada, [2]Producteurs de bovins du Québec, Longueuil, Québec, Canada.*

Welfare of cull dairy animals has received more interest lately with studies reporting conditions of dairy cows at auction market sale rings. Because the duration of observation for each animal in a sale ring is very short (<1 min), the data collected from this evaluation may not be reflective of the true state of the animal. Our objective was to quantify the agreement between a quick visual evaluation done in the sale ring and the results of the same evaluation done outside of it without any time limitation. Cull dairy cows were evaluated during their actual sale at auction. After the sale, a second evaluation was performed at close-range in the post-sale pens, using the same standardized data collection procedures. General physical condition of each animal was evaluated by body condition score (BCS), locomotion, udder condition, lesions, and swollen areas assessment. The agreement between both evaluations was assessed using Gwet's AC1/AC2 agreement coefficient. Respectively, AC1 and AC2 using the predefined quadratic weight. A total of 136 cows were included. The general agreement between the 2 evaluations was between good and very good. Gwet's AC2/AC1 (95% confidence interval), was 0.95 (0.93–0.96) for BCS. It was 0.73 (0.62–0.85) for presence of lameness, 0.96 (0.86–0.97) for presence of head bob and 0.85 (0.77–0.93) for evidence of pain in one specific leg. For udder characteristics, 0.86 (0.78–0.94) for fill level, 0.95 (0.91–0.99) for presence of redness, and 0.78 (0.68–0.89) for swelling. When assessing lesions and swelling, it was 0.77 (0.71–0.84) for hock lesions, 0.85 (0.78–0.92) for knee lesions, 0.82 (0.75–0.88) for lesions in another location and 0.93 (0.89–0.96) for hock swelling, 0.96 (0.93–0.99) for knee swelling, and 0.97 (0.95–0.99) for swelling in another location. Visual assessment of health indicators on culled cows during the sale at the action market can be difficult because of the limited time to perform it. The high agreement between the 2 evaluations shows that such assessment can be trusted for most indicators observed.

Key Words: animal welfare, cull dairy cows, auction market

2499 Nociceptive thresholds associated with digital dermatitis stages. G. Fabbri*, A. Desrochers, H. L. M. Ruel, M. Rousseau, and M. Villettaz-Robichaud, *Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.*

Bovine digital dermatitis (DD) is the most common cause of infectious lameness in dairy cattle, with negative effects on production and welfare. Digital dermatitis is described according to a 5-stage classification (M0–M4.1). The degree of pain associated with each stage of DD is unknown. Objective assessment of pain could significantly improve animals' welfare, allowing for more effective pain management. Pressure algometry has been successfully used to measure mechanical nociceptive thresholds (MNT) in cattle. The objective was to compare MNTs between different stages of DD lesions in lactating dairy cows. Our hypothesis was that acute lesions (M1 and M2) would be more painful than chronic ones (M3 and M4). Sixty-four lactating Holstein cows from 2 farms in Québec, Canada, were examined, for a total of 128 pelvic limbs assessed. Locomotion scores (5-point scale) were determined for each cow while walking toward the trimming chute. The examination order of cows and limbs was randomized. Cows with other types of hoof lesions were excluded. Stages and size of DD lesions were recorded. Pressure was then applied perpendicularly to the lesion on its center, using a digital algometer. The pressure applied at the time of limb withdrawal (avoidance movement) was recorded. A linear mixed model and Bonferroni post-hoc analysis was performed. Tested groups were healthy (M0), chronic stages of DD (M3 and M4), acute stages (M1 and M2), and recurrent form (M4.1). The locomotion score of each animal was tested against the worst stage of digital dermatitis afflicting that animal by means of Kruskal-Wallis test. Locomotion scores were as follow: 1 = 36%, 2 = 44%, 3 = 17% and 4 = 3%. Mean MNT was 40.9 ± 37.7 N (range: 0.6 – 100 N). MNT recorded on healthy limbs was significantly higher than those measured on limbs affected by any stage of DD ($P < 0.001$). Acute stages of DD were associated with lower MNT than chronic stages ($P < 0.05$). There was no difference between the recurrent form (M4.1) and acute stages, nor between M4.1 and chronic ones. These results suggest that all stages of DD are painful. As acute stages elicited more pain than chronic ones, they could affect more cows' welfare.

Key Words: digital dermatitis, nociceptive thresholds

2500 Effect of post-transport oral electrolyte supplementation on behavior, health, and hydration in neonatal calves. J. Pempek[1], Z. England[2], G. Habing*[3], and A. Niehaus[4], *[1]USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN, [2]Department of Animal Sciences, The Ohio State University, Columbus, OH, [3]Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, [4]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.*

Most male dairy calves are sold soon after birth for veal or dairy beef production. This process may involve indirect marketing and prolonged fasting, contributing to calf dehydration and hypoglycemia. The objective of this study was to determine the impact of 0, 1, 2, or 3 d of oral electrolyte solutions (OES) on calf behavior, biochemical measures of hydration, and subsequent health. Two cohorts of 60 Holstein bull calves approximately 3–7 d of age were used in this experiment. Calves (n = 30/treatment) were randomized to 1 of 4 treatments on arrival at a calf-raising facility: 1) 1-d OES; 2) 2-d OES; 3) 3-d OES; or 4) no OES (control). Calves were evaluated for signs of arthritis, depression, dehydration, diarrhea, fever, navel inflammation, and respiratory disease on arrival (d 0) and 1, 2, 3, and 7 d after arrival. Blood was obtained following each examination to assess biochemical measures of hydration, including serum electrolytes, glucose, hematocrit, lactate, pH, and serum total protein. Calves were also fitted with 3-dimensional accelerometers to continuously measure lying time. Linear regression models with Gaussian or Poisson link functions were used to test differences between treatments in blood parameters, disease frequency, and behavior. On arrival, 90% (95% CI: 84.6–95.4%) of calves had mild to moderate dehydration. The prevalence of failed transfer of passive immunity and hypoglycemia was 26.7% (18.6–34.7%) and 76.5% (68.7–84.2%), respectively. The frequency of moderate dehydration was lower for calves that received 2-d OES compared with the control group (rate ratio: 0.58; 0.37–0.92%; $P = 0.02$). Duration of OES did not impact blood biochemical measures of hydration. Calves spent the most time lying immediately post-transport (d 0); however, there was no effect of OES treatment on lying time. These results underline the need for more research on rehydration strategies to help inform calf processing protocols. Future research is also needed on the dairy farm and during marketing to mitigate dehydration and hypoglycemia before arrival at calf-raising facilities.

Key Words: calf behavior, dehydration, calf health

2501 Veterinarians' and veterinary students' attitudes toward dairy cows' experiences and the role of veterinarians in the promotion of positive welfare.

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Improving the lives of animals in agricultural systems has primarily focused on minimizing negative experiences. Research is needed on the promotion of positive experiences (pleasure, enjoyment, play, choice, happiness) for animals and the role of veterinarians in their promotion. Our aims were to 1) describe how Canadian bovine veterinarians and veterinary students perceive the role of a veterinarian in positive vs. negative experiences for dairy cows and 2) describe the attitude, professional values, and perceived ability of veterinarians to promote positive experiences for dairy cows. Canadian practitioners (n = 78) and veterinary student (n = 148) were asked, on a 7-point scale, how important the role of a veterinarian is to promote practices that influence the experience of dairy cows. Practices to minimize negative experiences was most important (mean ± SE; 6.8 ± 0.03), a balance of positive and negative experiences was less important (6.4 ± 0.05), and encouragement of positive experiences scored lower (6.0 ± 0.06), indicating modest differences in valuing avoidance of negative vs. promotion of positive welfare. There were no differences between veterinarians and veterinary students. Participants had favorable attitudes toward providing positive experiences for dairy cows (6.3 ± 0.04), prompting positive experiences was less important than decreasing negative experiences (5.9 ± 0.09), positive experiences for dairy cows is valued by the veterinary profession (5.4 ± 0.05), and veterinarians could confidently suggest positive experiences to clients (6.1 ± 0.06). These results indicated favorable views toward positive welfare opportunities for dairy cows. However, participants neither agreed nor disagreed that the decision to suggest positive experiences to producers was within a veterinarian's control (4.3 ± 0.11) and on farm implementation was out of the veterinarian's control (2.1 ± 0.07). We conclude that veterinarians are favorably disposed to positive aspects of welfare for dairy cows but may be more focused on avoidance of negative aspects of welfare and uncertain of their ability to influence change to current practices.

Key Words: barriers, control, relative-importance

2502 Effect of repeated ACTH challenge on hair cortisol, growth, and behavior in preweaned dairy calves.

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The efficacy of utilizing hair cortisol as a non-invasive measurement to quantify chronic stress has been demonstrated in adult cattle, yet less research has explored this measure in calves. The study objective was to investigate the effects of repeated HPA axis stimulation using Cosyntropin (a synthetic adrenocorticotropin hormone) injections on hair cortisol concentrations, growth, and behavior in pre-weaned dairy calves. Twenty-seven Holstein calves were assigned to triads (based on sex and birth order) and randomly assigned to 1 of 3 treatments: 1) control (2 mL saline IM weekly); 2) moderate (alternating Cosyntropin [2 µg/kg body weight (BW)] and saline IM weekly); or 3) frequent (Cosyntropin [2 µg/kg BW] IM weekly). Calves received their first injection on d 0 (7 ± 1 d of age). An initial hair sample was collected from the tail switch between d −5 and −3, and subsequent samples were collected on d 21 and 49 of the study. Calves were fitted with 3-dimensional accelerometers to continuously monitor lying time before the first injection, and accelerometers were removed on d 49. Growth measures (BW, hip height, hip width) were recorded weekly. The temperature humidity index (THI) was averaged for the week before each injection. Data were analyzed using repeated measures ANOVA in SAS; models included the fixed effects of treatment, triad, study day, THI, and interaction between treatment and day. Body weight and average daily gain were similar across treatments. However, there was a negative association between BW and THI ($P = 0.02$). There was no effect of treatment on hair cortisol concentrations, but concentrations decreased from d 21 (1.34 ± 0.05 ng/mL) to 49 (0.94 ± 0.05 ng/mL; $P < 0.0001$). Treatment did not influence average daily lying time (control [18.5 ± 0.23 h/d], moderate [18.6 ± 0.23 h/d], and frequent [18.5 ± 0.23 h/d; $P = 0.97$]). These results indicate that repeated HPA axis stimulation, through Cosyntropin administration, did not influence hair cortisol concentration, growth, or behavior.

Key Words: hair cortisol, ACTH challenge, calf stress

2503 Cow-calf contact rearing systems in a pasture-based dairy system: Effects on cow health.

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In dairy production systems, consumers are concerned about cow-calf separation soon after birth and access to pasture. The objective of this study was to investigate the impact of prolonged cow-calf contact (CCC), from birth to weaning, on dairy cow (n = 50) health within a pasture-based, seasonal calving system in Ireland. We compared 3 dairy rearing systems operating on the same farm: 2 CCC systems [full-time access to calf (FT; 14 cows) and part-time access to calf (PT; 18 cows)] and no access [conventional system (control); 18 cows]. Control and FT cows grazed full-time and were milked twice daily (0800 and 1500 h). The PT cows were milked once a day (0800 h), grazed until mid-afternoon (1500 h) when they were brought inside and reunited with their calf. After 8 wk, FT and PT cow-calf pairs underwent a 7-d gradual weaning and separation process; after, all cows grazed full-time and had no calf access. Body weight (BW) and body condition score (BCS) were recorded 1×/wk until wk 12, then at lactation wk 35. The BW and BCS from wk 4, 8, 12, and 35 were analyzed using linear mixed model procedures. Injuries (hock, knee, neck; 1×/wk), locomotion (1×/wk), and clinical health (ocular and nasal discharge; 2×/wk) were scored until wk 11 and analyzed using logistic regression. The PT cows were heavier than the similar control and FT cows during wk 4 ($P < 0.01$) and 8 ($P < 0.01$), possibly due to a reduced milking frequency, but not during wk 12 ($P = 0.15$). The PT cows had a higher ($P < 0.05$) BCS than the control cows, but not the FT cows, at wk 4, 8, and 12. Cow-calf contact system did not affect locomotion ($P = 0.35$), ocular ($P = 0.72$) or nasal discharge ($P = 0.18$), or hock ($P = 0.75$) or knee ($P = 0.63$) injuries. The FT ($P < 0.001$) and PT ($P < 0.001$) cows were less likely to be dirty than the controls. We observed no negative impact of CCC on BW, BCS, clinical health, injury prevalence, or locomotion; however, other aspects of CCC systems, such as cow production, fertility, and calf health, need to be considered before CCC rearing systems are implemented on pasture-based, seasonal calving systems.

Key Words: cow-calf separation, cow health, pasture-based dairy system

2504 Taste aversion to assess hunger in dairy calves. D. W. Brown*[1], M. Fischer[1], S. Sigl[1], A. Clark[1], H. Olsen[1], J. H. C. Costa[2], and K. C. Creutzinger[1], *[1]University of Wisconsin-River Falls, River Falls, WI, [2]University of Kentucky, Lexington, KY.*

Dairy calf hunger is a common welfare challenge. Novel methods are needed to assess calf motivation to access feed. The objective of this study was to determine if taste aversion could be used as a motivation test to assess calf hunger. Holstein and dairy-beef calves (n = 25) were enrolled in the study at 21 ± 3 d of age. This study was comprised of 2 phases. In each phase, calves were randomly assigned to receive unaltered or "bitter" milk replacer. In phase one, calves were fed 4.0 kg unaltered (n = 13) or "bitter" milk replacer with 0.0, 0.2, 0.3, and 0.4 g/L of quinine added (n = 12). Quinine concentration order was randomized and calves were tested every third day. In phase 2, calves were fed 4.0 kg unaltered (n = 12) or "bitter" milk replacer with 0.35 g/L quinine added (n = 12) and liquid feed was withheld for 6, 12, and 16 h. All calves received all feed withhold times on separate days in a random order and were tested every third day. Milk replacer was removed 30 min after delivery and refusals were weighed. Feed consumption was assessed as percent feed consumed [100 − (fed weight − refusal weight) × 100)]. Multivariable mixed linear models were used for analysis. In phase 1, milk consumption was not different between 0.0 (77.8 ± 11.9% LSM ± SEM), 0.2 (78.4 ± 11.9%), and 0.3 (73.4 ± 11.9%) g/L of quinine ($P > 0.05$). Calves fed 0.4 g/L (38.0 ± 11.5%) consumed less milk than all other treatments ($P < 0.05$). In phase 2, there was an interaction between feed withholding time and treatment (bitter vs. altered milk; $P = 0.03$). Calves fed bitter milk replacer consumed more at 16 h (71.5 ± 11.6%) than 6 h (39.8 ± 11.6%) and 12 h (43.5 ± 11.7%) of feed withholding ($P < 0.05$). Calves fed unaltered milk drank more than the calves provided bitter milk at all time points ($P < 0.05$). Dairy beef calves consumed more milk than the Holstein calves (95.1 ± 13.4% vs. 62.3 ± 7.6%; $P = 0.02$). Additionally, milk consumption increased with age ($P = 0.03$). These results suggest that taste aversion may be effective to assess calf hunger.

Key Words: hunger, motivation, calves

2505 Associations between environmental conditions and dairy cow location in compost-bedded pack barns. T. I. Gordon*[1], A. M. Wilson[1], D. B. Haley[2], G. W. Price[3], T. C. Wright[4], D. F. Kelton[2], C. Wand[4], R. J. Gordon[5], and R. Bergeron[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [3]Department of Engineering, Faculty of Agriculture, Dalhousie University, Truro, NS, Canada, [4]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, [5]University of Windsor, Windsor, ON, Canada.*

The objective of this observational study was to explore associations between environmental conditions and the distribution of dairy cows on a compost-bedded pack (CBP) during the summer season. Four CBP barns in Ontario were visited once during the summer. Packs were measured and divided into 8–15 similar-sized sections. The position of high-volume low-speed fans relative to individual sections was noted. Environmental variables (air temperature, relative humidity, windspeed [WS], pack surface and 15 cm-depth temperatures, and pack moisture content [MC]) were collected from 2 spots per section at 2 time points (morning and afternoon periods). Video footage from 4 cameras overlooking the pack was used to determine the proportion of the herd in each section, the proportion of the herd lying in each section, and the proportion of the herd standing in each section. The 6 environmental variables were tested in univariable models and assessed for collinearity. Predictor variables with $P < 0.25$ in the univariable analysis were included in multivariable β regression models constructed using PROC GLIMMIX including period as a repeated measure with subject as section within farm. Farm was included as a random effect, and the area of the section was included as a covariate to control for variation in section sizes within and between farms. The proportion of the herd in each section was positively associated with WS ($P < 0.001$) and section MC ($P < 0.001$) and was negatively associated with section 15 cm-depth temperature ($P = 0.007$). The proportion of cows lying in each section was also positively associated with WS ($P < 0.001$) and MC ($P < 0.001$). The proportion of cows standing in each section was positively associated with WS ($P < 0.001$) and air temperature ($P = 0.049$) and was negatively associated with depth temperature ($P < 0.001$). Relative humidity and surface temperature were not associated with any outcome variables. Our results suggest that cow distribution on the pack may be associated with WS and MC. Uneven distribution of WS in CBPs may influence pack management in the summer.

Key Words: environmental conditions, housing, compost-bedded pack

2506 Understanding the market: Value chain stakeholder attitudes to surplus dairy calf management in Australia. S. E. Bolton*[1,2], B. Vandresen[1], and M. A. G. von Keyserlingk[1], *[1]The University of British Columbia, Vancouver, British Columbia, Canada, [2]The University of Melbourne, Melbourne, Victoria, Australia.*

The management of surplus dairy calves in Australia has traditionally been influenced by the economic viability of different practices. When beef prices are favorable, more surplus calves are raised for beef, and when beef prices are low, more calves are euthanized at birth or slaughtered at 5 to 30 d of age. It is now recognized that early life killing of surplus calves is out of step with public values and threatens the industry's social license to operate. The aim of this study was to describe the views of value chain stakeholders regarding the management of surplus calves. Representatives from 7 companies (beef and dairy processors, feedlots, genetics companies, and supermarkets) were interviewed as individuals or in groups from a single company. Using semi-structured interviews, participants were asked about their views on current practices, alternatives to early life killing and how best to implement change. Interviews were transcribed using a professional transcription service, and transcripts were subjected to inductive thematic analysis. Responses were organized into 3 themes: 1) Ethics of surplus calf management, including public perception of the issue, impact on industry stakeholders, and animal welfare impacts of different practices; 2) Economics of surplus calf management, including divergent perspectives between beef and dairy stakeholders, logistical and practical challenges of alternatives to early life killing, and opportunities to improve the profitability of dairy beef; and 3) Change toward solutions including attitudes to change, downstream benefits of dairy beef production such as positive environmental outcomes, and the role of leadership and collaboration in affecting change. We conclude that stakeholders widely recognized early life killing of surplus calves as a threat to the industry's social license. While technical solutions such as sexed semen and beef on dairy breeding programs were cited as important, participants emphasized that sustainable solutions addressing early life killing will require collaboration, leadership and commitment by all stakeholders along value chain.

Key Words: welfare, surplus calves, social license

Joint AAVI (American Association of Veterinary Immunologists) and ADSA Animal Health Symposium: Harnessing Novel Molecular Technologies to Address Challenges in Livestock Production

2507 Single-nuclei transcriptomics as a tool to address adipose tissue dysfunction in dairy cows. C. Strieder-Barboza*[1,2], [1]*Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX,* [2]*School of Veterinary Medicine, Texas Tech University, Amarillo, TX.*

A robust body of literature supports the central role of adipose tissue (AT) dysregulation in the development of metabolic disease in periparturient dairy cows. Yet, gaps of knowledge about mechanisms underlying AT dysfunction remain. Our group uses single-cell RNA sequencing analysis (scRNaseq) to identify distinct cell (sub)types involved in the subcutaneous (SAT) and visceral (VAT) AT dysfunction and metabolic disease pathogenesis in dairy cows. Single-cell-RNaseq allows an in-depth characterization of cellular diversity by providing the transcriptional profile of individual cells, including what genes are expressed, at what expression level, and how they vary across thousands of cells within a heterogeneous sample. A limitation of scRNaseq in AT is that mature adipocytes are missing in data sets as they are too large to be sorted by microfluidics. To overcome this, we developed methods to perform single-nuclei RNaseq (snRNaseq) from cryopreserved AT samples. Our novel snRNaseq work identified vast cellular diversity in SAT and VAT of dairy cows, including distinct subtypes of adipocytes (AD), adipocyte progenitor cells (APC), endothelial cells (EC), macrophages (MAC), and mesothelial cells (ME). We found that ME are a VAT-exclusive cell type, and that the abundance of APC, EC, and MAC varies between SAT and VAT in dairy cows. Our most recent snRNaseq data indicate that subclinical ketosis affects the transcriptome of APC, EC, and AD populations in a depot-dependent manner in VAT and SAT of early lactation dairy cows by altering the expression patterns of genes associated with extracellular matrix remodeling, anti- and pro-inflammatory responses, and adipogenic regulation. These findings point to a network of cell subpopulations regulating depot-specific AT function in metabolic disease. The discovery of novel molecular and cellular targets and a better understanding of the mechanisms linking depot-specific AT dysfunction to metabolic disease provided by snRNaseq can help with the development of potential depot- or cell-specific preventive and therapeutic interventions to improve health in periparturient dairy cows.

Key Words: transcriptome, adipocyte, metabolic disease

2508 Connecting the dots: Immune status understanding using single-cell sequencing approaches. J. E. Wiarda*[1,2], J. M. Trachsel[1], A. L. Shircliff[1], J. B. Stasko[1], S. K. Sivasankaran[1,3], J. D. Lippolis[1], E. J. Putz[1], M. R. Ackermann[1], C. K. Tuggle[3], and C. L. Loving[1], [1]*National Animal Disease Center, ARS, USDA, Ames, IA,* [2]*Oak Ridge Institute of Science and Education, Oak Ridge, TN,* [3]*Iowa State University, Ames, IA.*

Traditional “bulk” assessment of gene expression via RNA sequencing captures overall patterns of transcriptional activity but fails to define cellular or spatial dynamics that influence functional outcomes related to immune status. High-resolution transcriptomics have revolutionized understandings of livestock immune status by resolving gene expression to the level of individual cells via single-cell RNA sequencing (scRNA-seq) and small-area tissue space via spatial transcriptomics (STomics). Blood and milk are routinely obtained via minimally invasive procedures and are thus ideal for assessing livestock immune status. scRNA-seq was used to dissect cellular compositions of blood and milk from cattle with chronic mastitis, revealing an abundance of granulocyte clusters in milk and lymphocytes in blood that contributed to distinct immune landscapes. While scRNA-seq reveals immune status in cells isolated from blood and milk, delineating how immune cells function within an organized tissue landscape is complicated because spatial context of cells is lost via scRNA-seq. To establish spatial organization of cells recovered from immunologically complex tissues, scRNA-seq and STomics data sets recovered from pig intestinal tissues containing Peyer’s patches were integrated. Data set integration allowed prediction of cellular locations within transcriptionally and morphologically distinct intestinal regions, revealing 2 biologically-relevant routes of immune cell activation and differentiation that were highly organized according to spatial locations. However, as scRNA-seq and STomics become common practice in research, we must also be cognizant of and overcome obstacles related to both technology- and species-specific limitations, including transcript capture efficiency, capture granularity, genome annotation, sample-assay compatibility, sampling capacities, operating costs, data analysis, and interpretation of results. Nevertheless, scRNA-seq is useful for gaining insight into livestock immune status through study of underlying cellular dynamics, while integration of scRNA-seq and STomics creates even greater potential for understanding immune dynamics within organized tissue space.

Key Words: single-cell, scRNA-seq, mastitis

2509 A single-cell atlas of bovine skeletal muscle reveals mechanisms regulating intramuscular adipogenesis and fibrogenesis. L. Wang[1], P. Gao[1], C. Li[1], Q. Liu[1], Z. Yao[2], Y. Li[1], X. Zhang[1], J. Sun[2], C. Simintiras[1], M. Welborn[3], K. McMillin[1], S. Oprescu[4], S. Kuang[4], and X. Fu*[1], [1]*School of Animal Science, Louisiana State University Agricultural Center, Baton Rouge, LA,* [2]*Department of Computer Science, Old Dominion University, Norfolk, VA,* [3]*School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA,* [4]*Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Background: Intramuscular fat (IMF) and intramuscular connective tissue (IMC) are often seen in human myopathies and are central to beef quality. The mechanisms regulating their accumulation remain poorly understood. Here, we explored the possibility of using beef cattle as a novel model for mechanistic studies of intramuscular adipogenesis and fibrogenesis. Methods: Skeletal muscle single-cell RNaseq was performed on 3 cattle breeds, including Wagyu (high IMF), Brahman (abundant IMC but scarce IMF), and Wagyu/Brahman cross. Sophisticated bioinformatics analyses, including clustering analysis, gene set enrichment analyses, gene regulatory network construction, RNA velocity, pseudotime analysis, and cell-cell communication analysis, were performed to elucidate heterogeneities and differentiation processes of individual cell types and differences between cattle breeds. Experiments were conducted to validate the function and specificity of identified key regulatory and marker genes. Integrated analysis with multiple published human and non-human primate data sets was performed to identify common mechanisms. Results: A total of 32,708 cells and 21 clusters were identified, including fibro/adipogenic progenitor (FAP) and other resident and infiltrating cell types. We identified an endomysial adipogenic FAP subpopulation enriched for *COL4A1* and *CFD* (log2FC = 3.19 and 1.92, respectively; $P < 0.0001$) and a perimysial fibrogenic FAP subpopulation enriched for *COL1A1* and *POSTN* (log2FC = 1.83 and 0.87, respectively; $P < 0.0001$), both of which were likely derived from an unspecified subpopulation. Further analysis revealed more progressed adipogenic programming of Wagyu FAP and more advanced fibrogenic programming of Brahman FAP. Mechanistically, NAB2 drives *CFD* expression, which in turn promotes adipogenesis. *CFD* expression in FAP of young cattle before the onset of intramuscular adipogenesis was predictive of IMF contents in adulthood ($R^2 = 0.885$, $P < 0.01$). Similar adipogenic and fibrogenic FAP were identified in humans and monkeys. In aged humans with metabolic syndrome and progressed Duchenne muscular dystrophy (DMD) patients, increased *CFD* expression was observed ($P < 0.05$ and $P < 0.0001$, respectively), which was positively correlated with adipogenic marker expression, including *ADIPOQ* ($R^2 = 0.303$, $P < 0.01$; and $R^2 = 0.348$, $P < 0.01$, respectively). The specificity of *Postn/POSTN* as a fibrogenic FAP marker was validated using a lineage-tracing mouse line. *POSTN* expression was elevated in Brahman FAP ($P < 0.0001$) and DMD patients ($P < 0.01$) but not in aged humans. Strong interactions between vascular cells and FAP were also identified. Conclusions: Our study demonstrates the feasibility of beef cattle as a model for studying IMF and IMC. We illustrate the FAP programming during intramuscular adipogenesis and fibrogenesis and reveal the reliability of *CFD* as a predictor and biomarker of IMF accumulation in cattle and humans.

Key Words: adipogenesis, fibrogenesis, fibro/adipogenic progenitor, intramuscular adipose tissue, single-cell RNAseq

2510 Multi-OMICs integration opens a new bridge to knowledge gaps in regulatory mechanisms underlying bovine mastitis.

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Bovine mastitis, regarded as the most common disease of dairy animals, is multifaceted and multifactorial, involving the interaction of various factors and biological processes. Subclinical mastitis accounts for a larger share of the economic losses associated with mastitis, due to its higher incidence rate and persistence. Extensive single-OMICs studies have provided clues of the molecular mechanisms underlying mastitis through the identification of associated genomics alterations (SNPs, QTLs), differential transcriptomics (mRNAs, miRNAs, cirRNAs, lncRNAs), epigenomics (DNA methylation, histone modifications), proteomics and metabolomics, etc. These single-OMICs data sources only provide insights as to which biological processes they individually impact. However, they are limited in their ability to capture the interactions and relationships between different biological processes and thus, cannot provide a comprehensive view of the molecular mechanisms underlying mastitis. To address these limitations, the multi-OMICs approach has emerged as a promising tool for gaining a deeper understanding and elucidation of the complex biological systems and potential causative molecular mechanisms underlying various conditions. The multi-OMICs approach has been applied in various fields, including human disease, agriculture and environmental science. Applying multi-OMICs approaches to integrate a range of high dimensional data sets at multiple layers, including DNA methylation, mRNA and non-coding RNA (miRNA, snoRNA, lncRNA) transcriptomes and QTL data, we and others have revealed DNA methylation-ncRNA-gene networks and identified key regulatory pathways involved in mastitis. This approach has provided a more comprehensive view of the biological processes implicated in the host response to mastitis pathogens and has enhanced and extended our current understanding of the complex regulatory systems of subclinical mastitis, which can inform the development of new strategies for managing mastitis (e.g., breeding for mastitis resistance, new diagnostic and treatment methods).

Key Words: multi-OMICs approach, DNA methylation/ncRNA/mRNA transcriptome, regulatory networks of mastitis

Breeding and Genetics 1: Breeding for the Future—Efficiency, Sustainability, and Resilience

2511 Assessing genotype by temperature-humidity index interaction for milk production traits in Holsteins. I. L. de Campos*[1], C. Baes[1], F. Miglior[1,2], and F. Schenkel[1], [1]*University of Guelph, Guelph, Ontario, Canada,* [2]*Lactanet, Guelph, Ontario, Canada.*

Heat stress has been shown to impact the Canadian Holstein cattle production. Temperature-humidity index (THI) thresholds for the onset of heat stress have been determined for different regions in Canada. For further studies on genetic selection for heat tolerance, the investigation of possible genotype by environment (G×E) interaction is necessary. The objective of this study was to assess possible G×E interaction when regions are considered different environments. Test-day (TD) records for milk, fat, and protein yields from first-parity Holstein cows spanning a 7-year period (2015 to 2021) were provided by Lactanet Canada. For the analyses, cows were required to have a minimum of 5 records within the interval from 5 to 305 d in milk. Age at calving records were restricted to 18 to 40 mo. Herds were required to have records of at least 5 cows in a year. The final data set contained 4,426 herds across Canada and TD records for milk production traits from 606,561 cows. Meteorological data from the NASA POWER database were retrieved. The data were divided into 5 Canadian regions: Atlantic, West Coast, Prairie, Ontario and Quebec. An ANOVA was used to compare differences between regions with respect to potential heat stress conditions during the summer as characterized by the THI values. To assess potential G×E interaction, phenotypes recorded in different regions were considered distinct traits and were analyzed via bivariate repeatability test-day models. All pair-wise comparisons of the mean THI of the regions were significantly different, confirming the variability of average summer THI values across regions in Canada. However, the genetic correlation between traits recorded in different regions were high ranging from 0.83 (0.001) to 0.96 (0.002). The overall high genetic correlation found in this study suggest no sizeable G×E effect on milk production traits across the Canadian regions exist. Therefore, genetic studies using THI as an environmental descriptor could assume production traits as single traits across the different regions in Canada.

Key Words: dairy cow, heat stress, temperature-humidity index

2512 A five-generation study of the effect of heat stress during female ancestor pregnancy on milk production traits in Italian Simmental cattle. N. P. P. Macciotta*[1], L. Degano[2], D. Vicario[2], C. Dimauro[1], and A. Cesarani[1], [1]*Dipartimento di Agraria, Università di Sassari, Sassari, Italy,* [2]*ANAPRI, Udine, Italy.*

The effect of heat stress (HS) represents an issue for dairy cattle also in temperate climates. Heat stress exerts a direct negative effect on welfare, productive and reproductive performances of cows. Breeding to improve HS tolerance mostly relies on indirect measures based in the variation of animal performances in response to environmental challenge. However, the phenotypic expression of this trait is complicated by epigenetic mechanisms. The occurrence of HS during pregnancy of a dairy cow could affect also future generations. Previous studies have reported effects of calving months of ancestors on EBV for production and functional traits in dairy cattle farmed in different Countries. In the present work, the effect of calving month of ancestors on milk production performances of Italian Simmental cattle across 5 generations are investigated. Data were EBV for milk, fat and protein yields, and SCS of 45,818 Italian Simmental cows provided by the Italian Simmental Association. For each cow (F4), data from the dam (F3), granddam (F2), great granddam (F1) and great great granddam (F0) EBV and calving dates were available. Data were analyzed with a linear model. that included the fixed effects of cow birth month and F0 calving month, and the fixed covariables of F0 calving date (to account for the genetic trend) and F0 EBV for the considered trait. All the effects included in the model significantly affected EBV of the 3 production traits. F0 calving months that exhibited the largest lsmean were May for milk yield, June for fat and protein yields, respectively. Lowest values were observed for November for all the considered traits. No effects of F0 calving month was detected for SCS EBV. Results of the present study obtained across 5 generations confirm previous reports on transgenerational studies in cattle. In particular, great great granddam late (F0) pregnancies carried out in spring had a favorable effect on F4 performances whereas pregnancies that occurred in summer had a negative effect, respectively. These results support the hypothesis that epigenetic effects could be detected also after 5 generations.

Key Words: epigenetics, climate, milk production

2513 Genetic associations between behavioral and feed efficiency traits in US Holstein cows. B. M. Nascimento*[1], L. Cavani[1], M. J. Caputo[1], M. Borchers[2], R. L. Wallace[3], H. M. White[1], F. Peñagaricano[1], and K. A. Weigel[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Zoetis Inc., Kalamazoo, MI,* [3]*Zoetis Inc., McFarland, WI.*

The evaluation of dairy cow feed efficiency using residual feed intake accounts for known energy sinks; however, behavioral traits may also contribute to the variation in feed efficiency. Our objective was to estimate the heritability and repeatability of behavioral traits and their genetic correlations with feed efficiency traits in Holstein cows. Data consisted of 28,617 daily behavioral records collected using a sensor (Smartbow, Zoetis) from 620 cows between 2019 and 2023, and 13,536 weekly feed efficiency records collected from 1,617 cows between 2009 and 2022. Behavioral traits consisted of sensor-based rumination, lying, and activity, measured in minutes per day, while feed efficiency traits consisted of dry matter intake and residual feed intake. The statistical models included the fixed effect of lactation, linear and quadratic effects of days in milk, and the random effects of cohort, permanent environment, and animal. Heritability estimates for behavioral traits using daily records were 0.13 ± 0.04 for activity, 0.15 ± 0.06 for rumination, and 0.26 ± 0.06 for lying. Repeatability estimates for behavioral traits were 0.34 ± 0.02 for activity, 0.50 ± 0.03 for lying, and 0.51 ± 0.03 for rumination. Strong genetic correlations were found between activity and rumination (0.66 ± 0.09) and activity and lying (−0.67 ± 0.07), while a moderate genetic correlation was observed between rumination and lying (0.33 ± 0.20). Weekly behavioral and feed efficiency records were used to estimate genetic correlations between behavioral and feed efficiency traits. Both dry matter intake and residual feed intake were genetically correlated with rumination (0.40 ± 0.02 and 0.32 ± 0.02), lying (−0.39 ± 0.02 and −0.25 ± 0.02), and activity (0.20 ± 0.02 and 0.39 ± 0.02). These results indicate that more efficient cows tend to spend more time lying and less time active. Overall, sensor-based behavioral traits are heritable and genetically correlated with feed efficiency traits and, therefore, they can be used as indicators to identify feed efficient cows within the herd.

Key Words: genetic correlation, residual feed intake, rumination

2514 Consistency of daily dry matter intake as an indicator of resilience: Heritability estimates and associations with feed efficiency in Holstein cows. L. Cavani*[1], K. L. Parker Gaddis[2], R. L. Baldwin[3], J. E. P. Santos[4], J. E. Koltes[5], R. J. Tempelman[6], M. J. VandeHaar[6], H. M. White[1], F. Peñagaricano[1], and K. A. Weigel[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, [2]Council on Dairy Cattle Breeding, Bowie, MD, [3]Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD, [4]Department of Animal Sciences, University of Florida, Gainesville, FL, [5]Department of Animal Science, Iowa State University, Ames, IA, [6]Department of Animal Science, Michigan State University, East Lansing, MI.*

Resilience can be defined as the capacity to maintain performance or bounce back to normal functioning after a perturbation. Using high-frequency data to analyze fluctuations in daily feeding intake can be a promising tool for identifying resilient dairy cows. Our goal was to develop new phenotypes based on consistency of daily dry matter intake (DMI) and estimate heritabilities and genetic correlations with residual feed intake (RFI) in Holstein cows. Data consisted of 171k daily DMI records of 2,915 lactating Holstein cows collected from 2010 to 2022 in 5 research stations across the United States. Consistency phenotypes were calculated based on the deviations from expected daily DMI for each cow during the feeding trial (27 to 122 d). Expected values were derived from parametric and non-parametric models, including quadratic and cubic quantile regression using a 0.5 quantile, and Loess regression using span parameters ranging from 0.2 to 0.7. We then calculated the log of variance (logVar) of the deviations for each model. Genetic parameters were estimated using an animal model, including lactation and days in milk as fixed effects and cohort and animal as random effects. Estimates of heritability for logVar were around 0.05 ± 0.01 and did not differ across models. Spearman's rank correlation of sires' breeding values for logVar ranged from 0.80 to 0.98 across models. The estimated genetic correlation between logVar from cubic quantile regression and RFI was 0.18 ± 0.20, and between logVar from Loess regression with 0.7 span parameter and RFI was 0.18 ± 0.19. Overall, variance of deviations from expected daily DMI can be used as a phenotype for genetic analysis because it is heritable and appears to be genetically correlated with feed efficiency, indicating that cows with greater variation in daily DMI may be less efficient and less resilient.

Key Words: genetic correlation, log of variance, residual feed intake

2515 Host and microbiome contributions to feed efficiency traits in Holstein cows. G. Martinez Boggio*[1], H. F. Monteiro[2], F. S. Lima[2], C. C. Figueiredo[3], R. S. Bisinotto[3], J. E. P. Santos[3], B. Mion[4], F. S. Schenkel[4], E. S. Ribeiro[4], K. A. Weigel[1], and F. Peñagaricano[1], *[1]University of Wisconsin–Madison, Madison, WI, [2]University of California, Davis, CA, [3]University of Florida, Gainesville, FL, [4]University of Guelph, Guelph, Ontario, Canada.*

It is now widely accepted that dairy cow performance is influenced by both host genome and microbiome composition. The genomic contribution to the phenotype is quantified by the heritability, whereas the contribution of the microbiome to the phenotype is quantified by the microbiability. If both the host genome and the microbiome are included in the model, then the heritability reflects only the contribution of the direct genetic effects. The objectives of this study were to quantify the host and microbiome contributions to dry matter intake (DMI), net energy secreted in milk (NESec), and residual feed intake (RFI) in lactating Holstein cows. Data consisted of feed efficiency records, SNP genotype data (79k SNPs), and 16S rRNA rumen bacterial abundances from 434 mid-lactation Holstein cows from 2 research farms. Three alternative mixed models were fit to each trait: one with only the genomic effect (model G), one with only the microbiome effect (model M), and one with both the genomic and the microbiome effects (model G+M). For each trait, we estimated the heritability (h^2), microbiability (m^2), and direct heritability (h_d^2). Goodness of fit of the alternative models was evaluated using a likelihood ratio test. For DMI, h^2 was 0.39 ± 0.12, m^2 was 0.27 ± 0.10, and h_d^2 was 0.29 ± 0.11. For NESec, h^2 was 0.46 ± 0.12, m^2 was 0.30 ± 0.10, and h_d^2 was 0.32 ± 0.11. For RFI, h^2 was 0.08 ± 0.10, m^2 was 0.11 ± 0.07, and h_d^2 was 0.07 ± 0.09. The model G+M was significantly better ($P < 0.05$) than the model G for all the traits. These findings show a low to moderate contribution of rumen microbiome to feed efficiency traits, and the differences between h^2 and h_d^2 suggests that the microbiome mediates part of the host genetic effects.

Key Words: rumen microbiota, microbiability, feed efficiency

2516 Residual feed intake prediction from milk spectra in Italian Simmental cattle breed. A. Cesarani*[1], L. Degano[2], D. Vicario[2], C. Dimauro[1], and N. Macciotta[1], *[1]Dipartimento di Agraria, University of Sassari, Sassari, Italy, [2]Associazione Nazionale Allevatori Pezzata Rossa Italiana (ANAPRI), Udine, Italy.*

Residual feed intake (RFI) is one of the most adopted traits to evaluate the feed efficiency of livestock. However, it is hard to obtain this trait on a large scale because it needs the real individual dry matter intake for its computation. Recently, dry matter intake data are becoming available at farm level due to the availability of automatic feeding systems. These systems have been recently installed at the genetic center of the Italian Simmental Breeders Association (ANAPRI), where the best young males of the population are tested every year for the performance test. Therefore, RFI data are only available in the best males of this breed. The aim of this work was to evaluate the possibility to expand the RFI measured on bulls to their female relatives by predicting EBV for RFI from the milk spectra. A total of 643 genotyped bulls had RFI values and a ssGBLUP model allowed to propagate these phenotypes to the whole population by estimating breeding values (EBV). Thus, EBV were then available for 33,821 cows with milk spectra, from which principal components (PC) were extracted. This data set was divided in training (90% of the records) and validation (10% of the records) for 1,000 replicates. In each round the PC that explained at least 1% each were used to predict the EBV for RFI in a linear model, weighted for the reliability of the EBV. The model was evaluated through the correlation between the real and the estimated EBV. The first 2 PC extracted from the milk spectra explained the 31.97% and 8.73% of the total variance, respectively. A total of 18 PC explaining at least 1% were retained for the prediction model. The average correlation between the real and estimated EBV across the 1,000 replicates was 0.35 ± 0.01. These preliminary results, even if based on a small number of phenotypes, suggest that this strategy could be used to propagate the RFI information from the small subset of males with records to the whole female population, and to routinely predict RFI from milk spectra.

Key Words: multivariate, feed efficiency, estimated breeding value

2517 Using milk spectral data to predict dry matter intake based on different cross-validation schemes. A. Yilmaz Adkinson*[1,2], M. Abouhawwash[1], K. L. Parker Gaddis[3], F. Peñagaricano[4], H. M. White[4], K. A. Weigel[4], R. Baldwin[5], J. E. P. Santos[6], M. J. VandeHaar[1], J. E. Koltes[7], and R. J. Tempelman[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]Department of Animal Science, Erciyes University, Talas, Kayseri, Türkiye, [3]US Council on Dairy Cattle Breeding, Bowie, MD, [4]Department of Animal*

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Feed efficiency is important for improving sustainability of dairy farms; however, recording dry matter intakes (DMI) is costly. Our objective was to investigate whether milk spectral data could be used to predict DMI and residual feed intake (RFI) based on a comparison of different models involving a base model of energy sinks versus another based on energy sinks plus milk spectral data. Our data set was derived from 4 research stations contributing to the US Holstein database on DMI. Records were based on 28-d averages, leading to 2,989 records on 1,291 cows from 32 different experiments/cohorts. Energy sink predictors included body weight, body weight change, and milk component yields. All models also included parity and days in milk with all analyses based on the use of partial least squares. Cross-validation (CV) schemes were based on 10-fold cow-independent and experiment-independent validation, both done within each station separately and as a joint CV study involving all of the data. Herd-independent partitions of training and test sets was also conducted as a final CV study. In general, the addition of spectral data significantly ($P < 0.05$) lowered root mean square of prediction (RMSEP) relative to the base model including only energy sinks for cow independent CV within 3 of the 4 stations and in the joint analysis, suggesting that spectral data may be promising for predicting DMI and RFI. Average RMSEP dropped from 2.2 kg when including only energy sinks to 1.7 kg when additionally including spectral data in the joint cow-independent CV study. However, there was no advantage observed for using spectral data in experiment-independent and herd-independent CV as average RMSEP increased substantially to 2.3 and 3.4 kg, respectively, for the full model in the joint CV study. Hence, any broad scope usage of milk spectral data to predict DMI or RFI in herds or management systems having no training data (i.e., DMI) of their own may have limited merit. Consideration of alternative prediction algorithms, particularly those that can accommodate heterogeneous associations with energy sinks across environments, is needed.

Key Words: mid-infrared, residual feed intake

2518 Phenotypic variability of feed intake in Holstein heifers until 8 weeks of age.

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Feed efficiency is an important component of both profitability and sustainability on dairy farms. The objective of the current study was to determine the variability of feed intake in Holstein heifers until 8 weeks of age. Four feed intake traits were studied: dry matter intake (DMI), metabolizable energy intake (MEI), residual dry matter intake (rDMI), and residual metabolizable energy intake (rMEI). Calves (n = 4,534) from 3 commercial dairies were raised at the University of Minnesota Southern Research and Outreach Center in Waseca, Minnesota. Most calves were fed 0.57 kg/day of a 20% crude protein and 20% fat milk replacer. Calves were fed ad libitum an 18% crude protein complete texturized starter. Calves were weaned at 6 wk of age. Intake data were recorded daily and body weights biweekly until 8 weeks of age. Metabolizable energy intake was also calculated. Univariate mixed models were used for analyses. Dry matter intake and MEI summarized over 8 weeks were treated as dependent variables. Fixed effects included metabolic bodyweight midpoint, average daily gain over 8 weeks, herd-year of birth, and month of birth; treatment nested within trial was fit as a random effect. Residual DMI and rMEI were calculated as the residual values from their respective intake models. The mean DMI across the first 8 weeks was 68.1 kg (SD = 13.1 kg) and ranged from 28.4 kg to 122.9 kg. The mean MEI across the first 8 weeks was 253.6 Mcal (SD = 44.1 Mcal) and ranged from 125.6 to 432.9 Mcal. By definition, rDMI had a mean of 0.0 kg (SD = 4.2 kg) and ranged from −14.5 to 26.3 kg. Animals with lower rDMI were considered more efficient since they consumed less feed than predicted from regression. This equates to a 40.8-kg difference in residual intake between the most efficient and least efficient animal based on DMI. For rMEI, the mean was 0.00 Mcal (SD = 13.8 Mcal) and ranged from −45.2 to 86.7 Mcal. In conclusion, variation for intake and residual intake exists in preweaning Holstein heifers, and this variation could potentially be exploited for management and genetic selection decisions that result in more feed efficient growing dairy heifers.

Key Words: feed efficiency, calves

2519 Greenhouse emissions in pure and crossbreed Danish dairy cows.

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Methane emissions in dairy cattle have been investigated intensely in the last decade. However, most of the information on methane emissions comes from purebred animals, mainly Holstein cows. Though, there are other dairy breeds and crossbreeds of importance that need to be studied. In the Nordic countries, one breed that has significant presence is the Nordic Red Cattle (NRC) breed. Nowadays, several farms are using crossbred animals with Holstein, Nordic Red, or other breeds. To the authors knowledge, there are not reports on their methane emissions of any of these breeds/crossbreds. In this study, we aimed to estimate daily averages for 5 emission traits: methane concentration (MeC; CH_4 in ppm), carbon dioxide concentration (CDC; CO_2 in ppm), ratio methane-carbon dioxide, methane production (MeP; CH_4 in g/d) and methane intensity (MeI: g CH_4/kg milk) in 601 purebred and crossbred cows. Cows were from 2 pure cattle breeds: Holstein (HOL, n = 227), Nordic Red (NRC, n = 123) and their crossbreds (HOL_NRC n = 67, NRC_HOL n = 148). Data was collected in a commercial farm in Denmark, through 4 sniffers equipment placed in 8 automatic milking systems for 2 mo (from March to May) in 2021. A total of 17 million of methane records were analyzed to calculate daily averages per animal (n = 21,833) and subsequently averages per breed. These averages were adjusted by background level, date, robot, and parity. *t*-Tests were used to determine the significance between the means per trait per breed. Methane concentration and CDC averages were lower in crossbred than in purebred animals, whereas ratio average was lowest in purebred Holstein. Methane production average was lowest in NRC purebred and highest in RDC_HOL crossbred, whereas MeI average was lowest in HOL breed, which can be explained as these 2 breed/crossbreds were the highest in milk yield. Our results open the question about how different methane emissions among purebred and crossbred dairy cows are. Further investigation is needed in this topic to validate these results. The availability of this information would help to account properly for greenhouse emissions in crossbred animals, as more crossbred animals are currently being used in the dairy industry than before.

Key Words: methane emissions, crossbred dairy cows.

2520 Withdrawn.

2521 Correlations of methane production, intensity, and yield with residual feed intake throughout lactation in Holstein cows. S. Fresco*[1,2], D. Boichard[2], R. Lefebvre[2], S. Barbey[3], M. Gaborit[3], S. Fritz[1,2], and P. Martin[2], *[1]Eliance, Paris, France, [2]Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France, [3]INRAE UE326 Domaine Expérimental du Pin, Exmes, France.*

Various studies demonstrated the possibility of selecting against methane (CH_4) emissions. However, CH_4 production is biologically related to feed intake and digestive process and the relationship between CH_4 production and feed efficiency is not well known. This study aims to estimate the correlations between CH_4 emissions and residual feed intake (RFI) across lactation. Phenotypes were collected for 2 years from 107 1st and 2nd parity Holstein cows. Methane emissions, fat- and protein-corrected milk (FCPM), dry matter intake (DMI), and body weight (BW) were recorded daily and averaged per week, while body condition score (BCS) was recorded monthly. Methane emissions were measured using 2 GreenFeed devices and expressed in g/day (MeP), g/kg DMI (MeY), and g/kg FPCM (MeI). A 5-trait random regression model was developed for each CH_4 trait, using 2nd order Legendre polynomials to model the whole animal effect, in addition to the fixed effects of contemporary group, days in milk, and parity. The limited number of cows did not allow to disentangle genetic and permanent environmental effects. Correlations between CH_4 and RFI were computed from the variance-covariance component matrix, with RFI weekly defined as the difference between the observed DMI and DMI predicted from FPCM, BW, and BCS. Both MeP and MeI were positively correlated with RFI across the lactation, with correlations varying from 0.05 to 0.47, thus reducing CH_4 emissions would reduce RFI, i.e., increase feed efficiency. Regarding MeY, its correlations with RFI went from favorable to slightly unfavorable across the lactation, with correlations decreasing from 0.71 to −0.18 during the 24 first weeks of lactation and increasing to 0.12 thereafter. Those animal correlations need to be confirmed by genetic ones, but they provide initial insight into the different traits of interest for selection against CH_4 emission.

Key Words: methane, GreenFeed

2522 A comprehensive meta-analysis of heritability and genetic correlation estimates for resilience indicator and production efficiency traits in worldwide Holstein cattle. J. Maskal*, V. Pedrosa, H. R. de Oliveira, and L. Brito, *Purdue University, West Lafayette, IN.*

Selection for resilience indicator (RIND) traits in Holstein cattle is becoming a key breeding goal as the worldwide dairy population is being exposed to increased environmental stressors due to climate change and new industry standards, while requiring continued high production outputs. However, the genetic relationship between RIND and production efficiency (PEFF) traits is still not clear, although unfavorable correlations have been previously reported in the literature. Consequently, it is crucial to investigate these genetic interactions when incorporating novel traits into selection indices to consider the trade-offs between RIND and PEFF traits. To investigate these genetic interactions and provide valuable pooled summary estimates, a random effects meta-analysis was conducted for heritability and genetic correlation estimates for PEFF and RIND traits in Holstein cattle. In total, 973 heritability estimates for 9 PEFF traits and 28 RIND traits and 362 estimates of genetic correlation (PEFF × RIND) were collected from studies conducted on 6 continents. The RIND traits were grouped into: Metabolic Diseases, Hoof Health, Udder Health, Fertility, Heat Tolerance, and Other. Pooled estimates of heritability for PEFF traits ranged from 0.201 ± 0.05 (lactation energy-corrected milk) to 0.377 ± 0.06 (lactation protein percentage) while pooled estimates of heritability for RIND traits ranged from 0.032 ± 0.02 (incidence of lameness and incidence of milk fever) to 0.497 ± 0.05 (measures of body weight variability). Pooled estimates of genetic correlation ranged from −0.360 ± 0.25 (lactation protein percentage vs. milk acetone concentration) to 0.535 ± 0.72 (measures of fat-to-protein ratio vs. milk acetone concentration). Our results confirmed that PEFF and RIND traits are heritable and suitable for genetic improvement in Holstein cattle worldwide. Additionally, the variation among pooled genetic correlation estimates that were observed in this study indicates the need to incorporate both PEFF and RIND traits into selection indices and fully evaluate their interactions.

Key Words: meta-analysis, resilience indicators, Holstein

Dairy Foods 3: Chemistry

2523 Can bovine β-casein phenotype alter the structure and functional properties of skimmed milk yogurts? D. Daniloski*[1,2], T. Vasiljevic[1], and N. McCarthy[2], [1]*Victoria University, Melbourne, Victoria, Australia,* [2]*Teagasc Food Research Centre, Fermoy, Co. Cork, Ireland.*

Bovine β-casein is a common protein found in bovine milk and has several phenotypes, distinguished based on their amino acid sequence. Two genetic variants of β-casein that have acquired growing attention from both academia and industry are β-caseins A2 and A1. The difference between these proteins is the inclusion of either proline in β-casein A2 or histidine in β-casein A1 at position 67 in the peptide chain. There has been significant research on the health implications of consuming milk containing β-casein A1, but as off yet it is uncertain what role this protein has on any non-communicable diseases. However, the fact that milk is now segregated for certain markets based on β-casein phenotype means that from a milk functionality perspective there may be implications on product functionality. The aim of this study was to investigate if differences in β-casein polymorphic motif would affect the properties of A1/A1, A1/A2 and A2/A2 skim milk yogurts. Raw bovine milk samples were collected from Teagasc Moorepark research farm and upon receipt the 3 milk types (~100 L of each) were pasteurized using a tubular heat exchanger and subsequently skimmed at 60°C. For the yogurt production, the pasteurized skim milk samples were heated to 85°C for 10 min, cooled to 43°C and mixed with a yogurt starter culture. The inoculated milk was then distributed into containers and incubated at 43°C until a pH of 4.6 was obtained. Triplicate measurements were taken for each yogurt type. The onset of acid gelation occurred significantly faster in A1/A1 and A1/A2 milks compared with A2/A2 milk ($P < 0.001$). Furthermore, A2/A2 yogurt possessed marginally lower elastic modulus, water holding capacity, and increased syneresis compared with yogurts containing β-casein A1 ($P < 0.05$). Microscopy images also showed a more porous gel network in A2/A2 yogurt compared with the other systems. Overall, this study clearly showed that the onset of gelation takes longer for A2/A2 milk and that the resulting gel structure is much more open and porous. These physical properties may have significant consequences for set-style yogurt production and should be considered carefully when using milks separated based on β-casein phenotype.

Key Words: yogurt, β-casein phenotypes, structure and rheology

2524 Impact of protein and pH on sedimentation in high-protein sterilized milk. A. Schnell*[1], M. Molitor[2], and J. Lucey[1,2], [1]*University Of Wisconsin–Madison, Madison, WI,* [2]*Center for Dairy Research, Madison, WI.*

Worldwide there is an increasing demand for high-protein ultra-high temperature (UHT) milk because of its extended shelf life and stability at ambient temperatures. However, manufacturers of UHT dairy beverages are observing a common defect found during storage, sedimentation. Sedimentation is the formation of a protein-rich material at the bottom of the package that starts shortly after processing. A few theories have been proposed for this sedimentation including destabilized whey proteins, enzymatic reactions, and protein aggregation involving ionic calcium (Ca^{2+}) and pH. This study investigated the impact of protein concentration and pH on sediment, calcium equilibrium, sediment particle size, and microstructure of sterilized milk during storage. Milk was prepared using MPC80 in a split-split plot factorial design (n = 3) with protein levels of 4, 6, and 8% wt/wt, and pH was adjusted to 6.5, 6.7, and 6.9. Samples were thermally processed using an autoclave at 121°C for 5 min. Samples were stored at ambient temperatures for 4 wk and analyzed for sediment, Ca^{2+}, acid-base titrations, sediment particle size, and microstructure before heat treatment, and 1 d, 2 d, 1, 2, and 4 wk. Protein, pH, and storage time significantly impacted Ca^{2+} ($P < 0.05$). The Ca^{2+} values ranged from 2.4 to 4.9 m*M*. There was a trend that as protein increased and pH decreased, the Ca^{2+} increased. Results showed a slight decrease in Ca^{2+} by 1 d, but then slowly increased during storage. Storage time and pH had a significant impact on sedimentation ($P < 0.05$). The highest sediment level was found in the sample with 8% protein and pH 6.5 by 2 wk. Acid-base titrations indicated that there was a shift in the form of calcium phosphate in some milk samples during storage. Sediment analyzed under confocal microscopy showed that large protein particle aggregates were present in the sediment (>100 µm). These results suggest that the protein content, pH value, and an altered calcium equilibrium play a critical role in the development of sediment during storage. We observed some slow chemical reactions occurring in the milk during storage that could contribute to sediment formation.

Key Words: high protein, ionic calcium, sediment

2525 Composition-dependent techno-functional properties of E 472b in aerosol whipping cream. M. Blankart*[1], J. Hinrichs[1], C. Oellig[2], and K. Schuster[2], [1]*Department of Soft Matter Science and Dairy Technology, University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany,* [2]*Department of Food Chemistry and Analytical Chemistry, University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany.*

E 472b, emulsifiers that are applied to foamable emulsion based dairy products, are synthesized by the esterification of distilled monoacylglycerides (MAG) with lactic acid in position C1 or C2. Besides the monolactic acid esters, E 472b contain also polylactic acid esters, saturated MAG (sMAG), free lactic acid and glycerol. Each of these components has an individual hydrophilic-liphophilic-balance-value, interfacial activity, and surface orientation and structure. A previous work with high performance thin-layer chromatography with mass spectrometry (HPTLC-MS) demonstrated that the composition of E 472b was variable between suppliers and batches. The objective of this work was to correlate changes of E 472b composition with changes of the emulsion and foam properties of model aerosol whipping cream (AWC) processed with E 472b. Model AWC with a fat content of 30 $g \cdot 100\ g^{-1}$ was processed out of cream and skim milk and homogenized at 80°C with 6/1 MPa in the presence of $0.8 \cdot 100\ g^{-1}$ E 472b of modulated composition. The particle size (static light scattering) and viscosity (flow curves) of the AWC samples were determined and the foam properties were measured after foaming with an aerosol can gassed with nitrous oxide. A one-way ANOVA with subsequent Tukey test ($\alpha < 0.05$) was performed for all analyses. A higher proportion of MAG in the E 472b had no effect on the particle size or viscosity, but decreased the foam stability of model AWC significantly. A concurrence situation between the α-gel of the lactic acid ester and the Pickering stabilization of sMAG was postulated as destabilizing mechanism. A higher amount of free lactic acid increased the fat globule size which enhances creaming. Increased amounts of free lactic acid were found to destabilize the foam of model AWC. Therefore, the composition of different E 472b batches should be characterized before application to prevent undesired changes of the product properties.

Key Words: E 472b, by-products, monoacylglyceride content

2526 Ultrasound-induced changes in physicochemical, microstructural and antioxidative properties of whey protein concentrate encapsulated 3,3′-diindolylmethane nanoparticles.

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This study determined the impact of ultrasound duration on the encapsulation of 3,3′-diindolylmethane (DIM) using whey protein concentrate (WPC) nanoparticles. The WPC-based DIM nanoparticles were prepared and treated with different ultrasound times (0–20 min), 30% amplitude, 20 kHz at 4°C. The results showed that ultrasound treatment significantly decreased the mean particle size (from 265 nm to 218 nm) and PDI value (from 0.49 to 0.43); as well as zeta potential values were notably increased. The EE% increased with increasing sonication time (0, 5, 10, 15, 20 min) from 76% to 77, 79, 81, and 88%, respectively. The ultrasound treatment had a significant effect on the apparent viscosity, and decrease in the viscosity as a function of shear rate was observed with increasing sonication time. We found that TEM micrographs demonstrated that all the formulations treated with different sonication times had a smooth and uniform spherical shape and ultrasound treatment led to the reduction of particle size, especially for 20 min of ultrasound. The thermal stability of the WPC–DIM nanoparticles was enhanced with increasing sonication time by increasing peak denaturation temperature and enthalpy. The Fourier-transform infrared spectroscopy (FT-IR) spectra analysis revealed that ultrasound treatment had a remarkable effect on the secondary structure of WPC–DIM nanoparticles; electrostatic interactions and hydrogen bonds between DIM and whey protein were strengthened. Moreover, the length of ultrasound treatment exhibited a significant effect on the DPPH (2,2-diphenyl-2-picrylhydrazyl) scavenging activity (from 56% to 62%) and ABTS [2,2′-azinobis(2 ethylbenzothiazoline-6-sulfonate)] scavenging activity (from 47% to 68%). In conclusion, the ultrasound treatment successfully improved the physicochemical, microstructural, and anti-oxidative properties of WPC–DIM nanoparticles; therefore, it is considered an effective method to develop whey protein concentrate-based DIM nanoparticles for medical and nutritional applications.

Key Words: 3,3′-diindolylmethane, whey protein concentrate

2527 Verification of grass-fed milk by infrared spectroscopy and potential new biomarkers of its authenticity.

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The Canadian standard for grass-fed milk (GFM) requires producers to determine the ratio of linoleic acid to α-linolenic acid (LA:ALA) by gas chromatography (GC) at least 6 times per year for authentication purposes. This ratio must be ≤3 in GFM. There were 2 objectives for this project: 1) determine the best algorithm to authenticate GFM from milk infrared (IR) spectra, 2) evaluate the adequacy of LA:ALA to authenticate GFM. Monthly bulk tank milk samples were collected from 27 GFM and 13 conventional (CON) herds from 3 Canadian provinces in 2021 and 2022 (n = 388). For each sample, major milk components and 74 fatty acids were determined by IR and GC, respectively. Selected samples were analyzed by nuclear magnetic resonance to determine 60 metabolites. For the first objective, several regression and classification algorithms were evaluated for their ability to authenticate GFM from milk IR spectra. Partial least squares regression (PLS) proved to be the most robust algorithm in predicting LA:ALA (range 0.57–11.38; average LA:ALA ± SD: GFM 1.63 ± 0.597, CON 3.84 ± 1.545). For the test set, the root mean square error of prediction and R^2 were 0.57 and 0.73, respectively. For the second objective, LA:ALA values for 7 CON herds were frequently below 3 throughout the year. Biomarker analysis by receiver operating characteristic curve revealed that reducing LA:ALA threshold to 2.53 will increase its specificity to reject CON milk for not being GFM while maintaining the same sensitivity as the current threshold. It further revealed that ratios of orotic acid to hippurate, maltose to dimethylamine, and hippurate to maltose might be as powerful as LA:ALA in differentiating between GFM and CON milk (area under the curve >0.9). These ratios were significantly different between the 2 groups of milk samples ($P < 0.001$). To conclude, predicting LA:ALA from milk IR spectra by PLS is a viable option to monitor the authenticity of GFM throughout the year. Lowering the LA:ALA threshold to 2.53 will better differentiate between GFM and CON milk. Additional biomarkers should be investigated to improve the authentication of GFM.

Key Words: grass-fed milk, Fourier-transform infrared spectroscopy, biomarkers

2528 Preliminary results of the feasibility of near-infrared spectroscopy to predict ovine colostrum quality.

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Rapid methods are needed to estimate colostrum quality on farm. Colostrum differs from milk composition presenting greater protein content among other differences, therefore specific calibration models are needed. Moreover, colostrum IgG and insulin concentration are fundamental to ensure the acquisition of passive immunity and uptake of sugars, respectively. This preliminary study evaluated the feasibility of near-infrared spectroscopy to predict ovine colostrum quality. A total of 45 colostrum samples collected <6 h postpartum from Lacaune (n = 21) and Manchega (n = 24) ewes from an experimental farm in northeastern Spain were scanned in duplicate with a benchtop instrument (NIRSystem 5000, FOSS Electric A/S) every 2 nm from 1,100 to 2,500 nm wavelength. Absorbance was recorded as log(1/transflectance). Sample spectra were matched with the reference data, and the prediction models were developed applying modified partial least squares regressions using 5-fold cross-validation. The T-outliers were set at 2.5. Mean ± SD for fat, protein, true protein, casein, total solids, IgG, and insulin for the retained samples were 7.35 ± 3.45%, 15.84 ± 4.89%, 15.22 ± 4.73%, 5.81 ± 1.84%, 26.17 ± 7.16%, 25.10 ± 10.96 mg/mL, and 13.32 ± 6.12 µg/L, respectively. Excellent calibration models were obtained for total solids, total protein, and true protein with a coefficient of determination in cross-validation (R2cv) of 0.99, and a ratio of prediction to deviation (RPD, SD/SEcv) ranged from 9.40 (true protein) to 15.84 (total solids). A very good calibration model applicable for quality control was obtained for total fat (R2cv, 0.96; RPD, 3.58), and poor calibration applicable for a rough screening was achieved for casein (R2cv, 0.80; RPD, 2.29). However, very poor calibration models were obtained for IgG (R2c, 0.88; R2cv, 0.39; RPD, 1.27) and insulin (R2c, 0.68; R2cv, 0.26; RPD, 1.14). In conclusion, preliminary results revealed the potential of near-infrared spectroscopy to predict sheep colostrum gross composition, but a greater sample size may improve the accuracy of the prediction models for IgG and insulin.

Key Words: colostrum, insulin, IgG

2529 Designing pristine casein-based electrospun bead-free nanofibers.

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The biodegradability, biocompatibility, and hydrophilicity of caseins, promote their potential in designing new-age sustainable materials and devices. The fabrication of electrospun nanofibers by blending casein with electrospinnable polymers and/or additives is well reported, but the electrospinnablility of pure casein still needs to be explored. The present study aims at analyzing the influence of pH, ethanol content, ionic environment, and casein concentration on the solubility, solution viscosity, conductivity and electrospinnablility of casein solutions. Electrospinnable homogeneous casein solutions were obtained for solution mixtures with ~20 wt. % casein content, prepared using ~60% ethanol/water mixture at pH ~10. The solutions were prepared using sodium pyrophosphate tetrabasic (TSPP) ~2 wt. % of casein content and resulted in the formation of fibers with a minimum number of bead defects (BN ~$6 \times 10^{-3}/\mu m^2$), bead area (BA ~$8.7 \times 10^{-2}/\mu m^2$), average fiber diameter (FD ~424 nm) and porosity (~52%). Further, the bead-free fibers were obtained for electrospinning solutions with a concentration ~1.7–2.5 times the entanglement concentration (Ce). Thus, the study outlines the influence of pH, ionic strength, solution concentration and ethanol content on the preparation of electrospinnable casein solutions, to fabricate novel biodegradable, biocompatible and non-toxic casein-based nanostructured mats for various food, cosmetic, packaging, and biomedical applications.

Key Words: electrospinning, casein, biodegradable

2530 Functional and bioactive properties of camel whey protein concentrate as influenced by spray drying and ultrasonication processing.

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Camel milk is gaining importance mainly due to its positive impact on the health which is linked mainly with the bioactive proteins and peptides present in whey fraction of camel milk. Whey proteins (WP) in general are attracting more attention as a functional ingredient due to their nutritional, techno-functional and bioactive properties. Here whey fraction was separated from skimmed camel milk by isoelectric precipitation, then either spray-dried (SPD) at 170, 185 and 200°C, or treated by ultrasonication (US; 20 kHz) for 5, 10 and 15 min followed by freeze-drying to obtain WPC powders. Both SPD and US WP were prepared in 3 batches to serve as 3 replicates which were statistically analyzed using ANOVA by Duncan's multiple range. The study aimed at investigating the effect of SPD and US on functional and bioactive properties of camel WP, with untreated camel WP serving as control. The characterization of WP powders by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reverse-phase ultra performance liquid chromatography (RP-UPLC) showed that the US treatment degraded the proteins in camel whey more than SPD. The morphology, particle size, and surface charge of WP samples were further studied using scanning electron microscopy (SEM) and Zetasizer, and the lowest particle size of 215.1 nm with surface charge of −21.6 mv was achieved with SPD-185 powder. Moreover, SPD samples revealed whiter color compared to the US-treated samples which were slightly yellowish in color. The US-15 sample exhibited high protein solubility (100%), whereas the camel WP SPD at a temperature of 200°C (SPD-200) showed slightly reduced solubility (92.67%; $P < 0.05$). Significant improvement in the emulsifying properties of WPs powders was observed after SPD and US. Overall, SPD and US treatments enhanced antioxidant activities of the samples and US-10 sample exhibited the highest α-amylase (IC50: 81.18 μg/mL), α-glucosidase (IC50: 130.1 μg/mL), and dipeptidyl-peptidase-IV (IC50: 67.92 μg/mL) inhibitory activities among all samples ($P < 0.05$). Thus, SPD and US processes were found to improve the techno-functional and bioactive properties of camel WP, and thus can be utilized as a promising strategy to preserve and enhance techno-functional and bioactive properties of camel WP.

Key Words: camel whey protein, ultrasonication, spray-drying

2531 Soft matter strategy for creating novel food texturizer: Electrostatic-driven gelation of Pickering emulsion stabilized by colloid whey protein assemblies.

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The rough texture of whey protein gel hinders its application in diversified food products. Incorporating oil droplets in protein gel may decrease the friction property, the resultant system is termed as "emulsion-filled gel (EFG)." However, the knowledge about whey protein (WP) based EFG in terms of gelation method, phase stability, and texture is scarce. This work aims to establish an optimized cold-set gelation process for manufacturing WP-EFG and to investigate textural nature of WP-EFG. In this work, fractal assemblies (FA) of whey protein (133.16 ± 3.59 nm) were made by heating whey protein isolate (WPI) solution (90 g/L) at 80°C (pH 7.0) for 24 h. Fractal morphology of FA was confirmed by transmission electron microscopy. Two bulk emulsion samples were manufactured using WPI and FA particles respectively. Both emulsion systems (pH 7.0) showed similar mean droplet size (~1 μm; $P > 0.05$). Cold-set emulsion gels (2% wt/vol protein, 5% vol/vol oil) were fabricated from the mentioned 2 bulk emulsions by manipulating 2 electrostatic gelation factors pH (3 levels: 4.6, 5.8 and 7.0) and $CaCl_2$ (3 levels: 0, 5 and 10 m*M*) yielding 18 emulsion gels in total ($18 = 2 \times 3 \times 3$). None of the 9 WPI emulsions resulted in stable gel. In contrast, 5 out of 9 FA-EFG samples (labeled as pH-Ca level: FA5.8-0, FA5.8-5, FA5.8-10, FA7.0-5, and FA7.0-10) showed no visible serum separation after 24-h storage. The syneresis rate of 5 FA emulsion gels as induced by centrifugation (101 × *g*, 1 h, 5°C) ranged from ~0.55% (FA7.0-5) to ~14.88% (FA5.8-10; $P < 0.05$). Shear stress-strain Lissajous plots were used to express rheological fingerprints of phase stable EFG samples (0.1–500% strain). Gels exhibited plastic behavior with both shear thinning and strain stiffening texture profile. Tribology analysis (Stribeck curves) of FA-EFG samples showed that limiting friction was improved from ~0.4 (FA5.8-0) to ~0.3 (FA5.8-10) by adding optimized amount of Ca^{2+} ($P < 0.05$). The current findings demonstrate that FA emulsion gels may be utilized as a functional texturizer to modulate the creaminess of processed semi-solid foods.

Key Words: fractal assemblies, emulsion, rheology

2532 Use of an immunochromatographic method to investigate illegal addition of cheese whey to milk.

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Milk is one of the most important dietary foods around the world. However, it is often adulterated with the addition of extraneous substances. For example, cheese whey is frequently used as a milk adulterant. The Brazilian official method to detect cheese whey addition to the milk is the caseinomacropeptide (CMP) quantification by HPLC. Caseinomacropeptide is a polypeptide released from κ-casein hydrolysis by chymosin during cheese production, but in good milk quality it is supposed to be present only in low amounts. The objective of this work was to evaluate an immunochromatographic method to investigate the addition of cheese whey to the milk in comparison with the HPLC method. Samples of raw (6), retailed pasteurized (6), and recently processed UHT (6) milks were diluted with cheese whey (control, 1%, 2%, 3%, 4%, and 5%), and analyzed for composition (fat, protein, lactose, total solids, solids nonfat, casein, milk urea nitrogen) using FITR (Lactoscope, Delta Instruments). Detection of CMP was done by an immunochromatographic method (kit Somaticell, Bioeasy) with both the visual and spectrophotometric readings. The same samples were analyzed by HPLC, using a Shimadzu CLASS VP 6.1, and Zorbax GF250 column and UV-detection. Compositional data and CMP levels were analyzed by multivariate general linear model using Tukey test for post-hoc pair-wise comparison of the treatments at $P < 0.05$ level for significance. Performance metrics of the kit were obtained. Results from compositional analyses showed that even at the highest dilution level of milk at 5% of cheese whey addition, milk components were within legal requirements for fat (except for the commercial samples previously standardized to 3% fat), protein, and solids nonfat. Therefore, it is not possible to rely only on compositional data to detect illegal whey addition to milk at the tested levels. However, the kit performance for CMP at the detection limit was about 25 mg/L, which corresponded to about 2% of added cheese whey. At this limit, the accuracy was 100%, close to the legal limit of CMP level allowed for fluid milk in Brazil, which is up to 30 mg/L. Hence, the evaluated kit detected CMP at concentrations which correspond to illegal cheese whey addition to the milk, and could be used as a screening test for posterior confirmation with the official method.

Key Words: cheese whey detection, caseinomacropeptide method, kit for caseinomacropeptide

2533 Use of artificial neural network to detect addition of cow milk to buffalo milk.

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The mixture of milk from different species is allowed, however must be properly identified. Nonetheless, in Brazil, this practice often happens as a consequence of adulteration. The objective of this study was to develop models of artificial neural networks (ANN) using compositional data obtained from spectroscopic analysis (FTIR) to detect the addition of cow milk to buffalo milk. A total of 300 buffalo milk samples and 300 cow milk samples were collected, during the months of October 2021 to March 2022, from refrigerated tanks. Murrah and Mediterranean buffalos and Holstein-Gyr cows were the predominant breeds. Each 10 samples were mixed as pools, resulting in 30 samples of buffalo and 30 samples of cow milk. These were prepared in 9 levels of addition of cow milk to buffalo milk (1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 75%) and 2 levels without mixing (0% = pure buffalo milk, and 100% = pure bovine milk) with a total of 330 samples, which were analyzed by FITR (Delta Instruments) for composition (fat, protein, lactose, total solids, and SNF). Additionally, 1,481 compositional results of buffalo milk and 1,500 of cow milk were processed to obtain the neural network algorithms. These results were obtained from the laboratory database using an initial number of about 15,000 samples, which were randomly treated to reach a more balanced number of samples for the networks. Multilayer perceptron neural networks (MLP) with 1 and 2 hidden layers and radial base function neural networks (RBF) were tested using IBM SPSS Statistics software. Training, validation and testing were done with 60%, 20%, and 20% of the data, respectively. Results were analyzed using descriptive statistics and performance indexes. Each network type was repeated 10 times at all the treatment levels for post-hoc comparison using the Tukey test. The MLP resulted in accuracy of up to an average of 97.4% and 97.0% for 1 and 2 layers, respectively, while the RBF obtained 97.3% accuracy. Accuracy values increased with the amount of cow milk addition ($P < 0.05$), but were above 90% for the tested levels. The results showed good accuracy, sensitivity, specificity and precision for the neural networks, showing that they are potential tools to screen illegal addition of cow milk to buffalo milk in a dairy plant.

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Key Words: buffalo milk, neural network, milk quality

2534 A colloidal perspective of the changes occurring to milk protein concentrates produced by membrane filtration.

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Membrane filtration is a widespread technology used to produce milk protein concentrates and micellar casein concentrates. Diafiltration (DF) is applied to increase the flow during membrane filtration and the protein-to-solids ratio of the product. However, DF can be applied in different ways leading to differences in composition and changes in mineral equilibrium between the colloidal and soluble phases, which are in turn critical in terms of functionality. In this work, we report on the colloidal behavior of casein micelles depending on the DF extent and membrane selectivity. Samples were concentrated to 4× volume concentration factor using ultrafiltration (UF) and microfiltration (MF) with no (NONDF), low (LDF), and high (HDF) DF, using water as DF medium. Concentrates had comparable casein concentrations (between 9.4 and 10.1 wt/vol %), whereas the soluble phase composition differed. Total, soluble calcium and phosphate and the protein composition of the samples were analyzed using chromatographic techniques. Acid- and heat-induced changes were investigated using rheology. Total whey protein depletion (%) was 41 ± 6, 64 ± 6, and 88 ± 6 for MF-NONDF, MF-LDF, and MF-HDF, respectively. We found that DF caused the dissociation of casein micelles, and UF showed higher soluble caseins, calcium, and phosphate compared with MF, in both LDF and HDF samples ($P < 0.05$). The onset of acid-induced gelation was shifted to higher pH with increasing DF extent (in the order NONDF < LDF < HDF, $P < 0.05$), and MF retentates had a higher gelation pH compared with the corresponding UF retentates ($P < 0.05$). Heat treatment of the concentrates at 85°C for 1 min showed a higher viscosity for UF concentrates compared with MF concentrates ($P < 0.05$), indicating a higher amount of heat-induced aggregates in the presence of whey proteins. The results demonstrated that whey protein depletion affects the colloidal properties of casein micelles and impacts their processing functionality.

Key Words: casein micelle, soluble phase, membrane filtration

Dairy Foods 4: Microbiology

2535 Evaluation of a multi-step bioconversion process of salty whey for production of renewable products. C. R. Surana[1,2,3], E. Byrne[1], B. Barry[1,2], M. Callanan[2,3], E. W. J. van Niel[4], and O. McAuliffe*[1,3], [1]*Department of Food Biosciences, Teagasc Food Research Centre, Fermoy, Co. Cork, Ireland,* [2]*Department of Biological Sciences, Munster Technological University, Cork, Co. Cork, Ireland,* [3]*VistaMilk SFI Research Centre, Teagasc Agricultural Food Research Center, Fermoy, Co. Cork, Ireland,* [4]*Division of Applied Microbiology, Lund University, Lund, Sweden.*

Efficient and cost-effective transformation of underutilized dairy processing streams represents a roadblock to sustainable food production. Salty whey (SW), a by-product of the production of semi-hard and hard cheese such as Cheddar, represents a significant waste stream in many countries. The management of this waste constitutes a financial and technological challenge for the industry. However, this nutrient-rich waste stream represents an opportunity for microbial valorization for the production of renewable, bio-based products. In this study, we are conducting a "proof of concept" investigation of the valorization of SW using a multi-step bioconversion approach, where effluent generated from the first step is used as feedstock for the subsequent step. For the first step, exopolysaccharides (EPS) were identified as potential value-added end products. Initially, a bank of lactic acid bacterial strains were screened for EPS production on modified MRS. A total of 28 strains, mostly lactobacilli, produced EPS within the range of 0.5–1.0 g/L. When cultivated on 40% SW, the EPS producers generated less EPS, with yields in the range of 0.07–0.23 g/L. *Lactobacillus casei* DPC2047 and *Lactobacillus ozensis* DPC6850 produced the highest yield of EPS in 40% SW each. The EPS yield is being further evaluated under controlled fermentation conditions. For the second step, the effluent remaining following the growth of the EPS producers on 40% SW was used as feedstock for polyhydroxybutyrate (PHB)-producing species; PHB is a biologically produced moldable plastic generated naturally by numerous bacteria and archaea. Strains of *Bacillus megaterium*, *Cupriavidus necator*, *Alcaligenes latus*, *Paraburkholderia fungorum* and *Halomonas halophila* were capable of growth on 40% SW effluent generated from cultivation of the selected EPS producers, including *L. ozensis* DPC6850. The PHB yields from these fermentates are currently being evaluated. This cascading approach to the use of biomass could potentially yield multiple bio-based products from a single input substrate, improving the circularity and resource efficiency of dairy processing.

Key Words: multi-stage bioconversion, exopolysaccharide, polyhydroxybutyrate

2536 Evaluation of the antibacterial and antifungal activity of microorganisms isolated from artisanal cheeses against major cheese contaminants in a model cheese matrix. A. Commenges*[1,2], M-H. Lessard[1], F. Coucheney[2], D. Drider[2], and S. Labrie[1], [1]*Department of Food Sciences, Institute of Nutrition and Functional Foods (INAF), STELA Dairy Research Centre, Université Laval, Quebec City, QC, Canada,* [2]*UMR Transfrontalière BioEcoAgro No. 1158, Univ. Lille, INRAE, Univ. Liège, UPJV, YNCREA, Univ. Artois, Univ. Littoral Côte d'Opale, ICV–Institut Charles Viollette, Lille, France.*

Cheese ecosystems harbor great microbial diversity that contributes to the typicity of each cheese variety and is an opportunity to discover potential candidates with technological, probiotic, or antimicrobial activities. The isolation of new antimicrobial strains from cheese is becoming increasingly attractive, especially for the inhibition of spoilage or pathogenic microorganisms. In this project, 2 different artisanal cheeses (Bourle Roncquoise and Carré du Vinage) made from thermized milk, typical of northern France, were selected. Using systematic isolation, more than 800 bacteria, yeasts and molds were isolated from these 2 cheeses and screened for their antimicrobial activities by spot testing on double-layer agar. The antifungal activity was tested against the yeasts *Candida parapsilosis*, *Yarrowia lipolytica* and *Rhodotorula mucilaginosa* and the undesirable filamentous fungi *Penicillium commune*, *Cladosporium herbarum*, *Cladosporium cladosporioides* and *Mucor racemosus*. Antibacterial activity was tested against the pathogenic or spoilage bacteria *Listeria innocua*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Escherichia coli* and *Clostridium tyrobutyricum*. Among the 800 isolates tested, the 36 that demonstrated an inhibition capacity toward the different contaminants were identified by 16S rRNA (bacteria) or ITS (yeasts) sequencing. However, only the 2 yeasts *Metschnikowia pulcherrima* LMA-2038 and *Trichosporon asahii* LMA-810 that showed both antifungal (anti *Y. lipolytica*, *R. mucilaginosa*, *C. cladosporioides* and *P. commune*) and antibacterial (anti *L. innocua* and *C. tyrobutyricum*) activities were selected for further characterization. Their potential as biopreservatives was tested in model cheeses, incubated for 7 d at 14°C and 90% relative humidity. The addition of *Metschnikowia* or *Trichosporon* decreased the growth of both contaminating yeasts significantly ($P < 0.05$, Student's *t*-test), *Y. lipolytica* and *R. mucilaginosa* by 1.1 log UFC/mL and 1.5 log UFC/mL, respectively. The next steps will be to understand the mechanisms involved in their antagonist activities.

Key Words: cheese, antibacterial, antifungal

2537 Correlating genomic variation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* cultures with volatile and sensory evaluation of yogurt aroma, texture, and flavor. M. Siddiqi* and G. LaPointe, *University of Guelph, Guelph, Ontario, Canada.*

Volatile compounds and sensory attributes related to flavor development of yogurt are due to the association between *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Predictive models correlating yogurt creaminess with resistance to deformation have been generated by integrating sensory with rheological parameters. This study aimed to identify genomic markers for *S. thermophilus* and *L. bulgaricus* that differentiate the performance and flavor of yogurt cultures. Over a 5-week period, bench-scale fermentations (4 L) were carried out on the same batch of pasteurized whole milk adjusted to 6% (vol/vol) total milk fat that was used in the industrial production of yogurt. Each week, 2 out of 10 different starter cultures were inoculated into 4 L of pasteurized whole milk (6% vol/vol total milk fat) and incubated in 750 g plastic containers at 40°C. The 10 cultures were composed of mixtures of isolates obtained from 2 separate parent cultures. Each culture was designed to mimic the acidification ability of parent culture 2 or 3 by mixing isolates with slow or fast acidification rate. After 7 d of refrigerated storage, sensory evaluation was performed by a trained panel on a 10-point scale. At 7 d, volatile profiles were determined using gas chromatography-mass spectrometry (GC-MS). Shotgun Illumina sequences from mixtures provided frequencies of single nucleotide variants (SNV). Sparse partial least square discriminate analysis (sPLS-DA) identified sets of significant predictive variables for yogurt flavor and aroma. The SNV profiles of *S. thermophilus* showed pairwise correlations less than 0.5

($P > 0.05$) with volatile compounds but greater than 0.5 with sensory attributes ($P < 0.05$). The *L. bulgaricus* SNV had correlations greater than 0.5 ($P < 0.05$) with volatile compounds and sensory attributes. The *S. thermophilus* SNV from fast or slow-acidifying isolates were inversely correlated with acidity compared with acetoin and 2,3-butanedione. The *L. bulgaricus* SNVs from slow or fast isolates were inversely correlated with acetaldehyde and smoothness on spoon and in mouth. Genomic markers can thus be correlated with sensory and volatile data analyses. This approach may help culture companies to screen starter combinations for adapting flavor profiles.

Key Words: milk, fermentation, metagenomics

2538 The microbial diversity of cheesemaking facilities contribute to the typicity of Quebec's terroir cheeses.

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Cheesemakers carefully select the starter and ripening cultures for cheese production to ensure high quality and the development of optimal sensory qualities of cheeses. Some microbial species can nevertheless be present in cheeses, although they are not voluntarily introduced. This indigenous microbiota contributes to cheese typicity. This project aims to characterize the microbiota of milk and cheese plant environments to understand their contributions on the microbial composition of cheese ecosystems. For this purpose, artisanal and industrial cheese plants from the province of Quebec were recruited to monitor the production of 4 Cheddar and 4 washed-rind cheeses. Samplings were performed in fall 2021 and in summer 2022 in each facility. Raw and heat-treated milks, cheeses, surfaces, and air were collected. Bacterial and fungal enumerations were performed for all samples using traditional culture-dependent methods. In samples with low microbial counts, systematic isolations were performed. Using 16S rDNA or ITS sequencing, 1,528 strains were identified. The microbiota of the remaining samples were characterized using massive amplicon sequencing targeting 16S rDNA and metabarcoding analysis. Genera identified in heat treated milks were mostly lactic acid bacteria, *Microbacteriaceae* and *Micrococcaceae*, which were also detected in the corresponding washed-rind cheese samples. The surfaces of Cheddar cheese plants were less contaminated (10^0–10^3 cfu/cm^2) than those of washed-rind cheese plants (10^1–10^6 cfu/cm^2). The microbial population of air sampled in ripening chambers for washed-rind cheeses was mostly composed of psychrophilic and halophilic bacteria (*Brevibacterium*, *Corynebacterium*, *Microbacterium*, *Staphylococcus equorum*), and were the only ones containing fungi, mostly because they are part of the desired microbiota in these types of cheeses. These molecular and microbiological characterizations provided important insights that will help to measure the importance of the microbiota, and to better understand the sources of the indigenous microbiota in cheeses.

Key Words: environmental microbiota, metabarcoding, cheese

2539 Not all *Clostridium tyrobutyricum* strains are created equal—Some have limited ability to cause late blowing defect in cheese.

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Introduction: *Clostridium tyrobutyricum* is a spore-forming bacterium and is considered to be the main causative agent of late blowing defect of hard and semi-hard cheeses. However, the spoilage potential of *C. tyrobutyricum* is strain-dependent. Since previous experiments have been limited to laboratory media or experimental food models imitating cheese matrix, our objectives were to characterize 6 strains of *C. tyrobutyricum* (FSL E2-8993, FSL E2-8958, FSL E2-9031, FSL K5-5225 and FSL R10-3990) for their spoilage ability during cheese ripening. Methods: Washed-curd cheeses were made with pasteurized cow's milk, inoculated with 3–4 $\log_{10}$ cfu/mL of *C. tyrobutyricum.* After brining, the manufactured cheeses were cut in half and vacuum packaged. One of the cheese halves was aged at 10°C, and the other at 15°C. After 160 d of aging, the cheeses were evaluated for selected physical, chemical, and microbial parameters. Evaluation of gas production in cheese and in packaging was evaluated by a panel of 3 experts. A Student's *t*-test was performed to test whether cheeses that developed the defect differ in final pH and water activity. Results: Our results showed an increase of clostridial spore and vegetative cell counts in cheeses contaminated with FSL E2-8993, FSL E2-8958 and FSL E2-8979 strains after 160 d at 15°C, as well as a substantial gas production in cheese and package. Cheeses inoculated with FSL E2-9031, FSL K5-5225 and FSL R10-3990 and aged at 15°C showed no or low gas production with no effect on cheese body. At 10°C, none of the strains showed activity in cheese. Cheeses where no or low activity was observed showed reduction in clostridial spore and vegetative cell counts during aging. Moreover, the final pH in these cheeses with low activity (pH = 5.06) was significantly lower compared with cheeses with high activity (pH = 5.61; $P = 0.012$). Significance: Late blowing defect does not occur at ripening temperature of 10°C, while at 15°C, the defect development is dependent on the *C. tyrobutyricum* strain.

Key Words: cheese, late blowing, *Clostridium tyrobutyricum*

2540 Detection of bacterial pathogen residues in milk samples from mastitis-infected cattle with SERS-based biosensor.

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Dairy products associated with mastitis-infected cattle threaten consumer health and the dairy economy. Early-stage detection of pathogens will solve those snags and even save cattle life. Herein, we present a surface enhanced Raman spectroscopy (SERS)-based detection of *Escherichia coli* and *Staphylococcus aureus* in milk samples using Ag-modified pSi platform. The bacterial recognition was accomplished using selective bioreceptors against the respective pathogens. Further, the indirect approach was developed based on the separation of the bioreceptor (with a simple syringe filter) attached to the bacteria cell surface. The residuals of the unbound bioreceptors are evaluated by the SERS platform to quantify the concentration of bacterial cells. Based on the proposed assay, we successfully detected *E. coli* and *S. aureus* at concentrations ranging from 10^1 to 10^5 cfu/mL in a defatted milk sample with detection limits of 3 and 1 cfu/mL, respectively. Furthermore, the selectivity of the devised assay was confirmed with other bacterial strains, which confirmed sufficient selectivity toward the target pathogen and insignificant cross-reactivity with other common interfering pathogens. Finally, the developed assay's applicability was tested by utilizing the real milk samples spiked with the targeted pathogen from various dairy livestock, including bovine, goat, and sheep, as well as commercial pasteurized milk. The results were compared with the standard approach and revealed a recovery range of 78 to 115%. The developed biosensing

assay may also be utilized into the on-field detection of *E. coli* and *S. aureus* in fresh milk samples.

Key Words: pathogen detection in milk, mastitis, SERS biosensor

2541 Evaluation of the ability of biofilm formation of methicillin-resistant non-*aureus* staphylococci (MRNAS) isolated from milk.

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This work aimed to evaluate the phenotypic and genotypic ability to form biofilm by methicillin-resistant non-*aureus* staphylococci (MRNAS) isolated from bovine milk in Brazil. We performed PCR in 135 strains isolated from bovine milk to detected the presence of the *mecA* gene. Then, the species identification was performed by MALDI TOF, being, from 6 MRNAS, 5 *Staphylococcus epidermidis* (SE) and 1 *Staphylococcus chromogenes* (SC). The 6 strains were isolated from milk from healthy cows (n = 1), from cows with subclinical mastitis (n = 2), and from cows with clinical mastitis (n = 3), and evaluated for their ability to form biofilm in Congo red agar (CRA), as well as the presence of genes involved in biofilm formation (*icaA* and *icaD*), including MSCRAMS (*fnbA*, *ebps*, *cna*, *clfA*, *clfB*, *eno*, *fib*, *fnbB*). The results of the prevalence of biofilm formation genes found and the test in CRA can be seen in Table 1. The 6 MRNAS analyzed represent a danger to public health since 2 of the 6 strains analyzed come from the milk of cows with subclinical mastitis and 1 strain comes from healthy cow's milk and this milk goes to the tanks that will be commercialized. It is important to highlight that NAS could be a reservoir of resistance genes, and their capacity to form biofilm could be an additional concern in dairies. In addition, these MRNAS strains showed a wide variety of genes involved in biofilm formation, which demonstrates not only the resistance capacity but also the possible persistence in surfaces of these strains.

Key Words: mastitis, *mecA*, biofilm, *Staphylococcus epidermidis*, *Staphylococcus chromogenes*

Table 1 (Abstr. 2541). Prevalence of genes involved in biofilm formation and detection of the biofilm-producing phenotype by Congo red agar (CRA) test on methicillin-resistant non-*aureus* staphylococci isolated from milk of healthy cows and cows with mastitis[1]

Presence of genes and biofilm formation in CRA	SE 1	SE 2	SE 3	SE 4	SE 5	SC 1
icaA	+	—	—	+	+	+
ica*D*	—	—	—	—	+	+
fnbA	—	—	—	—	+	—
ebps	—	—	+	—	—	+
cna	+	—	—	+	—	+
clfA	+	—	+	—	—	+
clfB	—	—	—	—	+	—
eno	+	+	+	+	+	+
fib	—	—	—	+	+	—
fnbB	—	—	—	—	—	—
CRA test	—	—	—	—	—	—

[1] + = positive; — = negative. SE = *Staphylococcus epidermidis*; SC = *Staphylococcus chromogenes*.

2542 Toward spore-reduced milk powders: Microwave technology to continuously heat fouling-sensitive milk products.

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During processing of milk concentrates, the load of thermophilic spore formers and endospores may increase. Thus, not only the concentrate but also the thereof produced powder can contain high amounts of thermophilic spores, that finally germinate upon reconstitution. Their counts are reduced by high temperature treatments of dairy concentrates before the evaporation process (>100°C). However, such a treatment in indirect heating plants is limited due to extended fouling formation. Once fouling is initiated, it is an exponentially growing phenomenon, limiting the plant running time. It is hypothesized that through the lack of hot walls, continuous microwave (MW) heating of milk concentrates induces less fouling, retains the product properties, and diminishes the microbiological load of thermophilic spores. Model milk concentrates [27–36% dry matter (DM), reconstituted] were heated in a continuous MW heating plant in pilot scale ($f = 2450$ MHz, = 120 L/h). Temperatures ranging from 110 to 125°C were applied while holding times $t = 5$–270 s were maintained. The concentrates were analyzed by means of deposit formation, particle size, color, and whey protein denaturation. Moreover, thermophilic spores of the species *Geobacillus stearothermophilus* and *Anoxybacillus flavithermus* were added to the concentrates before heating to quantify their inactivation. The MW heating of model milk concentrates was feasible, while product properties were maintained. At turbulent flow conditions, particle formation was acceptable ($d_{90,3} = 5$ - 6 µm) while color changes (browning in CIE $L^*a^*b^*$ color space; MW 110°C, 27% DM: $b^* = -1.47 \pm 0.28^a$; indirectly heated 110°C: $b^* = -0.87 \pm 0.17^b$; $P = 0.007$) and whey protein denaturation (β-lactoglobulin; MW 110°C, 27% DM: denaturation = $55.31 \pm 0.58\%^a$; indirectly heated 110°C: denaturation = $87.34 \pm 4.31\%^b$; $P = 0.001$) were significantly reduced compared with indirect heating. Thermophilic spores were inactivated up to 3 $\log_{10}$ stages, depending on the heating temperature, holding time, and bacterial strain. The experimental results allow to set a process window for MW heating of dairy concentrates with minimal product changes and at the same time high spore inactivation. Thus, MW technology may present a new method to heat fouling sensitive milk products to produce spore-reduced powders.

Key Words: milk concentrate, fouling, thermophilic spores

2543 Enhancing physiological persistence of probiotic bacilli through adaptive geometrical structuring in acidic pH.

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The milk associated lactic acid bacteria (LAB), such as *Lactiplantibacillus plantarum* can positively modulate different aspects of human health. Nonetheless, they need to be physiologically functionalized to ensure their survivability during the passage through the upper gastrointestinal tract (GIT). Our microscopic observations show that cells of *L. plantarum* tend to complex geometrical rearrangements, for instance formation of V-shaped structures, during their growth in an acidic pH but not in control neutral pH environments. The real-time reverse transcriptase PCR analysis further indicates about activation of cell-cell communication mechanisms during V-shaped structuring as an adaptational transition from nearly neutral (control) to acidic pH environment. Furthermore, we find alterations in cell division machinery as well as an activation of primary metabolic pathways (glucose catabolism, fatty acid biosynthesis and methionine biosynthesis processes) in the cells structured within V-shapes. We thus propose that the V-shaped geometrical structuring represents adaptive developmental phase prior transition to protective–biofilm mode of life, which supports the survivability and persistence of LAB in acidic and other stress environments. We therefore believe that inducing physiological persistence of LAB, using acid adapting growth system, enables enhancing their survivability and probiotic functionality within various hostile conditions.

Key Words: functional probiotics, positive biofilms, probiotic bacilli

2544 Development of a spectroscopy-based device for the bacterial contamination in dairy product. L. Roy*[1], K. Bhattacharya[1], and S. Pal[2], [1]*University of Calcutta, Kolkata, West Bengal, India,* [2]*S. N. Bose National Centre for Basic Sciences, Kolkata, West Bengal, India.*

Detection of bacterial contamination in dairy products in daily use is a challenge worldwide. In this work, we have studied the spectroscopy of Methylene Blue (MB) dye in absence and presence of bacteria in aqueous solution to use the dye to probe bacterial contamination in dairy products. Here, we have considered pasteurized milk (sterilized milk) and *Escherichia coli* (*E. coli*) to be model dairy product and the contaminating bacteria respectively. The conversion of MB (blue color) to Leuco-MB (colorless), i.e., reduction of MB to Leuco-MB in presence of bacterial activity in milk reveals the presence of bacteria and this colorimetric change of MB dye has been utilized as the tool to detect presence of bacteria in milk. The absorbance peak for methylene blue (MB) at 662 nm decreases significantly in presence of E. Coli and the blue color becomes fade. In our study we have employed UV-vis spectrometry to count the bacteria present in the solution, which is found to be ~10^7 cfu/mL (inoculum size). We have further developed a prototype to detect the bacterial contamination for the detection of bacterial contamination in milk-selling outlet. In our experiment, we have observed a significant change in MB absorption in the milk contaminated with bacteria (*E. coli*). The indigenously developed sensor strips show colorless Leuco-MB compared with milk without the bacteria. Triplicate experimental result shows 5% standard deviation in the result (Table 1). The analysis of the strips has been measured in the developed device, called LOPA Device (Loading of Parasite Analyzer).

Table 1 (Abstr. 2544). Experimental results (LOD = limit of detection)

Experimental results	
LOD	2.473×10^6 cfu/mL
SD	5%

Key Words: bacterial contamination, quick detection of bacteria in dairy product, spectroscopy-based prototype

2545 Identification of *Bacillus cereus* and *Clostridium perfringens* isolated from a milk powder processing plant. G. Ünlü*[1,2], A. Sindi[1], and B. Nielsen[1], [1]*University of Idaho, Moscow, ID,* [2]*Washington State University, Pullman, WA.*

Bacillus cereus and *Clostridium perfringens* are abundant in dairy farms and capable of contaminating dairy products. In a collaborative project with a milk powder processing plant, we analyzed raw milk and finished product (dry powder) samples (3 samples each, each sample taken in duplicate). We determined process line profile of product before shutdown of the line for clean-up: 4 sampling locations between raw milk receiving to the spray drier were sampled (1 sample per location, each sample taken in duplicate) and analyzed. Liquid samples were 50–60 mL each and 25-mL aliquots were used for analysis. Powder samples were 50–60 g each and 25-g aliquots were used for analysis. We also determined process line profile of product contact surfaces: 9 equipment surfaces between raw milk receiving to the spray drier were swabbed and analyzed. Enumeration and confirmation of *B. cereus* and *C. perfringens* were accomplished according to FDA BAM. API 50 CH/CHB and API 20A were used for biochemical identification of *B. cereus* and *C. perfringens* isolates, respectively. We used MALDI-TOF (matrix-assisted laser desorption/ionization time of flight) for the proteotypic identification of the isolates. Multiplex PCR was used for toxin genotyping. We identified 1 *B. cereus* isolate and 2 *C. perfringens* isolates using microbiological, biochemical, and proteotypic identification methods. Three heat-labile enterotoxins known to cause *B. cereus* diarrheal illness are hemolysin BL (HBL), nonhemolytic enterotoxin (NHE), and cytotoxin K (CytK). The *B. cereus* isolate was determined to carry the *hblC*, *nheA*, and *cytK* genes. *C. perfringens* type A toxicoinfection is associated with type A isolate of *C. perfringens* with α toxin (cpa) and enterotoxins. Both *C. perfringens* isolates were confirmed to carry the *cpa* gene. Our results show that *B. cereus* and *C. perfringens* with enterotoxin production capabilities can be found in milk powder processing plants. It is important for the plants to validate the safety of skim milk powder and the controls for thermophilic spores; determine if source of spores is solely raw materials, or if there is an environmental source within the plant; determine potential for re-contamination of product after pasteurization; and determine if existing controls are sufficient to control hazard.

Key Words: *Bacillus cereus*, *Clostridium perfringens*, milk powder

2546 Withdrawn.

Production, Management, and the Environment 4: Greenhouse Gas Emissions

2547 Global warming potential star (GWP*) more closely represents modeled warming contributions from California dairy methane emissions. E. M. Pressman, C. J. McCabe*, S. Liu, and F. M. Mitloehner, *University of California, Davis, Davis, CA.*

Methane (CH_4) is by far the main greenhouse gas (GHG) of California dairy production and accounts for 45% of California's methane emissions. The 2 sources of CH_4 on dairy farms are in the form of enteric fermentation and manure management. The second most common GHG is carbon dioxide (CO_2), which accumulates in the atmosphere even if the rate of CO_2 emissions decline due to its long half-life of 120 years. In contrast, CH_4 has a half-life of 12 years and has been termed a short-lived climate pollutant (SLCP). Greenhouse gases have been compared using the accounting metric global warming potential 100 (GWP100), which integrates the warming of a GHG over 100 years. However, GWP100 does not accurately represent the warming contributed by CH_4 emissions in cases of increasing or declining emission levels. To overcome this misrepresentation of SLCP warming, global warming potential star (GWP*) has been developed. The objective of this research was to model the California dairy industry's warming contributions from 1950 to 2030 utilizing different scenarios under both GWP100 and GWP*. Lactating dairy cow enteric and manure management CH_4 sources were acquired from the California Air Resources Board. Scenarios analyzed included business-as-usual and reduction scenarios of a 40% reduction in manure CH_4 emissions and a 40% reduction in manure CH_4 along with an 11.7% reduction in enteric CH_4 emissions. Compared with GWP100, GWP* CO_2 warming equivalents (CO_2we) emissions were greater under increasing annual CH_4 emission periods but were lower under decreasing CH_4 emission rates and more closely matched modeled warming under CH_4 emission reduction scenarios. With assumptions in anticipated warming based on cumulative CO_2we emissions from 1990 to 2030, the business-as-usual scenario was assumed to add 1.16 mK of warming along with 1.10 mK and 1.09 mK for the 40% manure reduction scenario and the manure and enteric fermentation scenario, respectively. To meet temperature and policy emission reduction goals, GWP* may provide a more accurate representation for evaluating SLCP emissions impact on atmospheric warming.

Key Words: methane, global warming potential star (GWP*), sustainable agriculture

2548 Air filtering as alternative approach to combat emissions from cattle facilities. A. Kuipers*, P. Galama, R. Maasdam, S. Spoelstra, and P. G. Koerkamp, *Wageningen University & Research, Wageningen, the Netherlands.*

Various strategies can be applied to reduce ammonia and methane emissions. One strategy is to adapt the animal to the environment, like adding methane blockers to the feed or selecting for low methane animals, and the other is to adapt the environment to the animal. We study the simultaneously capturing of methane and ammonia from air, a practice belonging to the 2nd strategy. In some intensive European livestock areas, air scrubbers are common in pig houses to capture ammonia from the air. This technique has not been adopted in cattle housings, because these facilities have an open structure. Moreover, capturing of methane from cattle houses is complicated due to low concentration in barns (in 60 farms spread over Europe ranging from 5 to 80 ppm at 2 m height), while about 30% of methane comes from manure and 70% from the mouth of cows. We examined several options to remove ammonia and methane from dairy barns by use of a survey based on scientific literature, contacts with experimenting farmers and firms and experimentation on capturing more concentrated methane from air (with aid of hoods mounted over the lying place of cows and recirculating of air). We found no feasible technology to capture ammonia and methane simultaneously, because of the low concentrations and chemical and physical inertness of methane. Thus, 2 separate technologies need to be applied for capturing ammonia and methane, respectively. When stored manure is a significant source of ammonia and—temporarily—methane, the air can be guided to an air scrubber to remove ammonia by acid. As next step, a biobed depending on consortia of methanotrophs can be incorporated to deal with higher levels of methane. However, no technologies based on activity of methanotrophs or physical-chemical methods involving adsorption and oxidation of methane have been identified until now to treat the actual low levels of methane in barns. Concentrations to around 500 ppm seem to be a way out. Technologies like catching methane near the head of the animal, e.g., by a hood, and designing new ventilation systems are to be further explored to achieve the targeted high reductions in methane from cattle herds.

Key Words: climate, emissions, housing

2549 Assessment of greenhouse gas footprints on small and mid-sized U.S. dairy farms. L. A. Olthof*[1], K. R. Briggs[2], and B. J. Bradford[1], [1]*Michigan State University, East Lansing, MI,* [2]*Dairy Management Incorporated, Rosemont, IL.*

Greenhouse gas (GHG) emissions from the dairy industry have received increasing scrutiny as climate change concerns grow. Our objective was to estimate GHG footprints for 4 dairy farms (150 to 850 [BB1] cows; 89 to 353 ha) throughout the US using the Farm Environmental Stewardship (ES) and USDA Comet life cycle analysis models. Herds averaged 10,782 ± 2,037 kg/yr fat- and protein-corrected milk (FPCM; 4.0% fat, 3.3% protein) and 24 ± 2.8 kg/d dry matter intake during lactation. Data from 2021 were gathered through farm management software, producer interviews, and on-farm evaluations. Soil organic matter data for ≥7 yr were available on 3 farms. Emissions intensity was quantified as net CO_2 equivalents (CO_2e) emitted per unit of FPCM sold. Model output was compared with assess alignment across tools. Comet estimated greater GHG emissions compared with Farm ES on all farms. Mean GHG emissions for all farms were 0.94 ± 0.19 kg CO_2e/kg FPCM and 3.84 ± 4.62 kg CO_2e/kg FPCM in Farm ES and Comet, respectively. The Northeast dairy had the greatest FPCM/cow and the lowest emissions intensity at 0.52 kg CO_2e/kg FPCM in Comet, whereas in Farm ES, the Northwest dairy had the lowest at 0.33 kg CO_2e/kg FPCM. In both models, methane accounted for the largest share of GHG emissions on all farms. Comet estimated that manure accounted for 69 ± 29% of farms' total GHG emissions, whereas Farm ES attributed only 26 ± 11% to manure. Two farms that utilized manure separation had an average footprint 0.25 kg CO_2e/kg FPCM lesser than farms that did not. Average annual soil carbon sequestration rates ranged from 0 to 1.42 t carbon/acre. Farms which produced more FPCM/cow had lesser emissions per unit of milk, and manure management and cropping strategies also impacted emissions estimates. In conclusion, productivity, manure management, and cropping systems are important determinants of the GHG emissions intensity of milk produced on a given farm. Additionally, Comet is not a recommended tool for dairy farm GHG emissions estimates due to highly variable estimates, especially from manure, and misalignment with published dairy farm benchmarks.

Key Words: emissions, life cycle analysis, sustainability

2550 Investigating rumination and eating times to predict enteric methane (CH_4) emissions in dairy cows. A. Castaneda*[1,2], N. Indugu[1], K. Narayan[1], S. Rassler[1], J. Bender[1], T. Webb[1], B. Vecchiarelli[1], L. Baker[1], and D. Pitta[1], *[1]University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, [2]McGill University, Department of Animal Science, Ste-Anne-de-Bellevue, Quebec, Canada.*

Methane (CH_4) emissions pose energetic losses to the host and environmental costs. To develop a mitigation strategy, it is critical to quantify the animal's CH_4 emissions, although obtaining reliable data is expensive and challenging. A sound approach to quantifying CH_4 emissions is using behavioral traits as predictors. Studies have sought rumination and eating times as predictors of CH_4 emissions; however, the information available on these associations is inconclusive. Thus, this study investigated rumination and eating times as predictors of CH_4 emissions in dairy cows. We used the Dynamic Time Warping algorithm to select cows differing in rumination and eating times. The algorithm was developed using hourly data collected from dairy cows. Using principal components, we calculated the distances between cows differing in rumination and eating times and depicted them in a plot. The criteria used to select cows and further grouping into low rumination (LR) and high rumination (HR) clusters was cows in early lactation (30 to 100 DIM). Moreover, the cows with the lowest and highest rumination times were selected. From 55 cows, 20 were selected to represent the clusters LR and HR. These cows were enrolled in a 5-week trial: a 2-week training phase, a 2-week adaptation phase, and 1 week for sampling. Data for DMI, production responses, rumination and eating times, and CH_4 emissions were collected during this period. The CH_4 emissions were measured using the GreenFeed system (C-Lock Inc.). The correlation analysis revealed that only rumination time could predict CH_4 emissions. The daily average CH_4 mass flow (QCH_4) was higher ($P < 0.05$) in the LR group (430 ± 6.27) than in the HR group (404 ± 6.04). The CH_4 intensity (QCH_4 per DMI and QCH_4 per milk yield) was lower ($P < 0.05$) in the HR group (17.1 ± 064 and 10.3 ± 0.30) than in the LR group (23.6 ± 0.64 and 13.9 ± 0.30). The rumination and eating times were higher in the HR group (RT, 21 $\text{min}\cdot\text{h}^{-1}$; ET, 14 $\text{min}\cdot\text{h}^{-1}$) than in the LR group (RT, 17 $\text{min}\cdot\text{h}^{-1}$; ET, 12 $\text{min}\cdot\text{h}^{-1}$). In summary, rumination time can be used as a predictor to quantify CH_4 emissions in dairy cows.

Key Words: rumination, eating, methane emissions.

2551 Methane emissions from liquid dairy manure in Canada. A. VanderZaag*[1], H. Baldé[1], and C. Wagner-Riddle[2], *[1]Agriculture and Agri-Food Canada–Ottawa, Ottawa, Ontario, Canada, [2]University of Guelph, Guelph, Ontario, Canada.*

Methane (CH_4) emissions from liquid manure are an important contributor to the carbon footprint of milk. The dairy sector is interested in reducing emissions, and recently a target of net-zero by 2050 was announced by the Dairy Farmers of Canada. Although manure emissions are smaller than enteric emissions, manure storages are point sources with opportunities for mitigation. Quantifying the impact of mitigation strategies requires accurate knowledge of the baseline emissions under business-as-usual conditions. Methanogenesis is highly temperature dependent and manure storages in Canada experience a wide range of temperatures depending on the region, the season, and the quantity of manure in storage at a specific time (filling and emptying patterns). In this study we measured methane emissions in 2 contrasting regions to observe the impact of climate on baseline emissions. One farm was located in central Alberta (53°N, one of the coldest dairy production regions), and the other was located near Ottawa, Ontario (45°N, one of the warmest regions). Emissions were measured at both locations using the inverse dispersion technique. Upwind and downwind concentrations were measured using open-path CH_4 lasers (Boreal Laser, Edmonton, AB) while wind speed, direction, and turbulence parameters were measured with CSAT3 sonic anemometers. Emission rates were determined using the WindTrax dispersion model using 15-min input data. Emissions were measured during the warm season over a period of 6 years. The results show that emissions from the manure storage in central Alberta were less than half as much as the Ontario location. At the Alberta farm the manure temperature was rarely above 15°C in summer, while in Ontario the manure temperature exceeded 20°C consistently in the summer. The impact of interannual weather variability was also evident as peak emissions were 3× higher during the 2 warmest summers (35 $\text{g}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ in 2015 and 2021) compared with the other years. The results from this study will help improve the accuracy of baseline emission estimates for manure storages in response to weather and regional climate.

Key Words: methane, manure storage, emission reduction

2552 Comparing oxygen, carbon dioxide, and methane exchanges of dairy cows measured using GreenFeed versus respiration chambers. A. R. Bayat*, T. Stefanski, P. Mäntysaari, and P. Huhtanen, *Animal Nutrition, Natural Resources Institute Finland (Luke), Jokioinen, Finland.*

In an earlier study, methane (CH_4) emissions measured by GreenFeed indicated a good relationship with values predicted by equations derived from chamber data. If O_2 and CO_2 measured by GreenFeed have a good relationship with chamber data, it could pave the road for a novel approach (residual O_2 or CO_2) to measure feed efficiency using GreenFeed. The aim of this study was to evaluate the precision and accuracy of GreenFeed in measuring CO_2, O_2 and CH_4 exchanges measured by respiration chambers. Thirty-two lactating Nordic Red cows were used to compare CO_2, O_2 and CH_4 exchanges measured using GreenFeed units (n = 2) versus respiration chambers (n = 4) in a complete block design (8 blocks). The experimental period for every block lasted for 5 weeks; the first 2 weeks the cows were measured in GreenFeed, 3rd week in the chambers and 4th and 5th weeks again in the GreenFeed to partially balance the number of measurements for both techniques. The cows were fed a grass silage-based diets with 55:45 forage to concentrate ratio. For GF records, 2.5 × SD of all measurements for every cow over 4 weeks was used to detect outliers. Altogether 217 out of 3,188 datapoints were removed as the outliers. Overall average gas exchanges for every cow were used for statistical analysis. Average CO_2, O_2 and CH_4 exchanges measured using GF (12,890 ± 1,262, 8,882 ± 891 and 467 ± 61.4, g/d) were very similar to those measured using respiratory chambers (13,165 ± 1,445, 8,870 ± 968 and 453 ± 55 g/d), respectively. The Lin's concordance correlation coefficients between 2 techniques for CO_2, O_2 and CH_4 were 0.79, 0.82 and 0.68, respectively. Regression analysis of GF against chamber data provided the following equations indicating the strongest and weakest relationship for O_2 and CH_4, respectively: O_2 (g/d) = 618 + 0.929 × O_2 ($R^2 = 0.73$, RMSE = 511); CO_2 (g/d) = 1128 + 0.934 × CO_2 ($R^2 = 0.67$, RMSE = 850); CH_4 (g/d) = 158.5 + 0.630 × CH_4 ($R^2 = 0.49$, RMSE = 40.0).

Key Words: gas exchanges, GreenFeed, respiration chamber

2553 Effects of long-term supplementation with *Asparagopsis taxiformis* on enteric methane emission and lactational performance of dairy cattle.

D. E. Wasson*, S. F. Cueva, L. F. Martins, N. Stepanchencko, K. Welter, and A. N. Hristov, *The Pennsylvania State University, University Park, PA.*

This study investigated the effects of long-term supplementation of *Asparagopsis taxiformis* (AT), a known enteric methane (CH_4) mitigating feed additive, on performance and CH_4 emission in lactating dairy cattle. Following a 2-wk covariate period, 24 Holstein cows averaging 118 d in milk and 44 kg/d milk yield (MY) were assigned to one of 2 treatments in a 15-wk randomized complete block design experiment (2 wk for adaptation and 13 wk for data collection). Treatments were control (CON; basal diet) or basal diet supplemented with 0.25% AT [feed dry matter (DM) basis]. Inclusion of AT was increased to 0.5% in wk 10. Average bromoform (compound responsible for the antimethanogenic effect of AT) concentration in AT during the experiment was 3.8 ± 1.3 mg/g DM. Enteric CH_4 emission was measured over 3 d, bi-weekly using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS with fixed effects of treatment, week, and treatment × week in the model. Block and block × treatment were random effects. The average reduction in CH_4 emission (g/d) by AT over the entire experiment was 32% ($P \leq 0.001$). The mitigation effect of AT, however, diminished over time, being on average, 49% in wks 1–5, 14% in wks 7–9 and 33% in wks 11–13, following the increase in AT inclusion rate in wk 10. Overall, there was a trend for decreased ($P = 0.06$) CH_4 yield (g CH_4/kg DMI) in cows fed AT by 17%, with a 37% reduction ($P \leq 0.01$) during wks 1–3. Following the increase in AT inclusion in wk 10, CH_4 yield was again decreased ($P \leq 0.04$) 24% by AT compared with CON in wks 11–13. Compared with CON, AT decreased ($P = 0.002$) dry matter intake (SEM in parentheses) from 28.0 to 23.8 (0.96) kg/d. There was a trend for AT to decrease ($P = 0.10$) MY from 42.1 to 37.7 (1.54) kg/d. No other effects on milk components or production were observed. Automated body condition scores were lower ($P = 0.01$) for cows fed AT compared with CON (2.92 and 3.11, respectively). Interpretation of the data suggest that the mitigation effect of AT on enteric CH_4 emission may be transient, possibly as a result of rumen adaptation.

Key Words: seaweed, methane, production

2554 Dietary inclusion of *Fucus* species for methane mitigation in dairy cattle?

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In vitro experimentation has shown that 2 *Fucus* spp. have the ability to reduce methane formation associated with rumen fermentation. In contrast with the tropical red algae, *Asparagopsis,* the anti-methanogenic activity of *Fucus* species cannot be associated to halomethanes. The aim of this experiment was to investigate, whether the anti-methanogenic properties of the *Fucus* spp. can also be found when fed to dairy cows in vivo. Four multi-cannulated (rumen, duodenum and ileum) Danish Holstein dairy cows were used in a double (one complete and one incomplete) 3 × 3 Latin square design experiment, with 3 periods of 3 weeks duration, where cows were fed 3 different total mixed rations (TMR): a control TMR without seaweed (CON) and 2 rations where the control TMR had been diluted on dry matter basis with 4% of either *Fucus serratus* (SER) or *Fucus vesiculosus* (VES). The effects of adding seaweed to the rations were determined for methane emission, feed intake, milk production and milk composition. The first 14 d of each period was assigned to adaptation to the diet, followed by 3 d of digesta sampling and subsequently 4 d of gas exchange measurements in respiration chambers. Data were analyzed using the SAS MIXED procedure and differences between means were evaluated using Tukey multiple comparison test. Dry matter intake (DMI), milk production and energy corrected milk (ECM) were not affected by *Fucus* spp. supplementation. Data showed an increase in methane emission with SER compared with CON ($P = 0.002$) and also with VES compared with CON ($P = 0.018$). Methane yield (L/kg DMI) and methane intensity (L/kg ECM) was higher in SER compared with CON ($P < 0.05$). Milk protein production (g/d) was decreased with SER compared with CON ($P = 0.011$). In conclusion, supplementing with *Fucus* spp. did not reduce methane emission, but DMI and milk production were unaffected. Further analysis will be realized to determine impact of dietary addition of *Fucus* spp. on digestibility of the overall diets, metabolic profile of the cows and patterns of mineral excretion to evaluate the potential of *Fucus* spp. as dairy cattle feed additive.

Key Words: seaweed, methane emission, dairy cattle

2555 Effects of a forage additive on modeled greenhouse gas emissions from silage.

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Greenhouse gas emissions from silage comprise volatile organic compounds and CO_2 produced during ensiling. A model describing emissions from ensiling was recently reported, based on measures of volatile corrected dry matter loss (D_V), lactic acid (L), acetic acid (A), ethanol (E), and ammonia (N). Two experiments were conducted to test effects of a silage additive (Silo-King; Agri-King, Inc.) on these measures and modeled outcomes of global warming potential (GWP) as CO_2 equivalent (CO_2e) emissions. Experiment 1 utilized alfalfa haylage and experiment 2 utilized corn silage. In both experiments, freshly chopped forage was treated either with inert carrier as control (C) or the silage additive (S) at a rate of 0.25 $g \cdot kg^{-1}$ to provide 1.65×10^{10} cfu lactic acid bacteria per kg silage. Silos constructed of PVC pipe (n = 10 per treatment for both experiments; 3.0-L capacity) were packed to dry matter densities of 217 and 241 $g \cdot m^{-3}$ for experiment 1 and 2, respectively. Haylage was fermented for 50 d and corn silage for 30 d. After ensiling, final mass of silos was recorded, and contents were analyzed for measures stated above. Data from both experiments were analyzed by one-way AOV for effect of treatment with significance at $P < 0.05$. In haylage, measures of D_V, acetic acid, and ethanol were lower for S than C, whereas lactic acid and ammonia were greater for S. In corn silage, D_V was lower for S than C, but other fermentation measures were not affected. Data were used to model GWP as follows, with terms expressed in $mg \cdot kg^{-1}$ DM: $GWP_{20} = -3626.1 - 0.04343A + 0.8011E - 0.03173L + 1.46573D_V$; $GWP_{100} = -8526.1 - 0.22403A - 0.11963E - 0.03173L + 1.46573D_V$. Modeled CO_2e emissions as GWP_{20} and GWP_{100} were lower for S than C in both haylage and corn silage. Estimated CO_2e emissions in C haylage as GWP_{20} and GWP_{100} were 2.2 ± 0.6 and 0.5 ± 0.6% DM respectively, whereas S haylage was modeled as a net carbon sink with respective CO_2e emissions of −3.1 ± 1.0 and −4.3 ± 1.0% DM. In corn silage, respective values for C were 4.6 ± 0.7 and 1.8 ± 1.1% DM, compared with 1.6 ± 1.0 and −1.0 ± 1.1% DM for S. Reduction of modeled emissions with silage treatment supports that microorganisms in the additive promote CO_2 recycling in silage.

Key Words: silage, greenhouse gases

Table 1 (Abstr. 2556). Effects of barley and 4 varieties of oats on predicted in vivo methane and total gas production, and in vitro digestibility[1]

Item	Barley	Sonja	Niklas	Perttu	Fatima	SEM	P-value
CH_4, mL/g DM	43.5^a	42.2^a	39.9^b	39.6^b	38.0^b	0.55	<0.01
Total gas, mL/g DM	253^a	247^{ab}	236^{bc}	239^{bc}	230^c	3.6	<0.01
TDMD, %	89.0^a	87.0^b	86.5^{bc}	86.0^{cd}	85.2^d	0.22	<0.01

[a–d]Within a row, means without a common superscript differ ($P < 0.05$) adjusted by Tukey's test.

[1] CH_4 and total gas predicted in vivo according to Ramin and Huhtanen (2012); TDMD = true DM digestibility; SEM = standard error of mean.

2556 Evaluation of different oat varieties and cold-pressed rapeseed cake on predicted in vivo methane production. P. Fant*[1], P. Huhtanen[2], J. C. C. Chagas[1], S. Krizsan[1], and M. Ramin[1], [1]*Animal Nutrition and Management, Swedish University of Agricultural Sciences, Umeå, Sweden,* [2]*Animal Nutrition, Production Systems Natural Resources Institute Finland (Luke), Jokioinen, Finland.*

The objective was to evaluate 4 different varieties of oats differing in fat content and the replacement of rapeseed meal (RSM) with cold-pressed rapeseed cake (RSC) on methane (CH_4) production and digestibility in an in vitro gas production experiment. We applied a 3 × 5 factorial design replicated in 4 runs. Rumen fluid from 2 dairy cows was used as inoculum. The diets were incubated in buffered rumen fluid and consisted of 55% grass silage, 29–33% grain, and 7–16% of different levels of RSM to RSC. The grains were barley (control) and oats: Sonja, Niklas, Perttu, and Fatima (3.16, 4.12, 6.13, and 6.93% ether extract/kg DM, respectively). The RSM was replaced by RSC at isonitrogenous levels of 0, 50, and 100%. The gas data obtained from the in vitro system was used to predict in vivo total gas and CH_4 production according to Ramin and Huhtanen (2012). In vitro true DM digestibility (TDMD) was determined as described by Rodrigues et al. (2017). The data were subjected to PROC MIXED in SAS and the RSM replacement was tested using linear and quadratic contrasts. There were no interactions between grain and replacement levels of RSM ($P = 0.18$). The main effects of grain are presented in Table 1. Replacing RSM with RSC decreased linearly ($P < 0.01$) the predicted in vivo CH_4 production from 41.7 to 39.9 mL/g DM as well as the in vitro TDMD from 87.0 to 86.3%. In conclusion, both selecting a specific oat variety and replacing RSM with RSC show potential to reduce enteric CH_4 production, although diet digestibility might be adversely affected.

Key Words: rapeseed cake, oats, methane production

2557 Effects of dietary glycerol monolaurate supplementation on milk production and methane emissions in Holstein dairy cows: A pilot study. R. L. Culbertson*[1], P. Uzun[1,2], N. Seneviratne[1], A. B. P. Fontoura[1], A. N. Davis[3], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Isparta University of Applied Sciences, Isparta, Türkiye,* [3]*SUNY Cortland, Cortland, NY.*

Glycerol monolaurate (GML) has antimicrobial and anti-inflammatory properties in non-ruminants. Following incubation in rumen fluid, GML reduced methane production; however, the effects of dietary GML supplementation on milk production and methane emissions had not been evaluated in dairy cattle. In a randomized block design, 42 mid-lactation Holstein cows (3.10 ± 1.08 lactations; 40.76 ± 1.41 kg milk/d) were acclimated to a tiestall barn for 3 wk and assigned to 1 of 3 treatments (n = 14/group): unsupplemented (CON), low-dose GML (50 g/d; LD), or high-dose GML (150 g/d; HD) for a 21-d experimental period (EP). Cows were fed a base diet composed of corn silage, grass haylage, and concentrates. Cows were milked 3×/d. During the final 5 d of acclimation and EP, milk was sampled at 9 milkings/wk. Methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2) gas measurements were collected 3×/d over 4 d (12 samples/cow) using the GreenFeed system (C-Lock, Inc., Rapid City, SD). The statistical model included fixed effects of treatment, day, and treatment × day as well as random effect of cow. Planned contrasts to compare CON vs. LD and CON vs. HD were employed. HD cows made ~2 kg more milk on d 1, 11, and 21, relative to CON (treatment × day, $P < 0.001$). Milk fat contents (4.22 vs. 4.01%) and yields (1.76 vs. 1.62 kg/d) were greater in HD, relative to CON ($P = 0.05$). Milk protein contents were lower in LD (3.48%) and HD (3.42%), relative to CON (3.49%; treatment and CON vs. HD, $P < 0.05$). HD cows tended to produce more fat-corrected milk, relative to CON (46.6 vs. 44.8 kg/d; $P = 0.13$). The HD cows tended to have improved efficiency (i.e., fat-corrected and energy-corrected milk per unit of dry matter intake), relative to CON ($P < 0.15$). Milk protein yields were also lower for HD cows (CON vs. HD, $P = 0.05$). Energy-corrected milk yields, and milk lactose contents and yields were not modified by treatment. The CH_4, CO_2, and H_2 production and CH_4 intensities were not modified. Although GML modified milk production, short-term GML feeding did not modify enteric CH_4 production in dairy cattle.

Key Words: glycerol monolaurate, methane, milk

2558 Enteric methane emission and lactational performance of cows fed rapeseed cake and oats on a grass silage-based diet. S. E. Räisänen[1], Þ. H. Sigurðardóttir*[1], A. Halmemies-Beauchet-Filleau[1], O. Pitkänen[1], A. Vanhatalo[1], A. Sairanen[2], and T. Kokkonen[1], [1]*Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland,* [2]*Natural Resources Institute Finland (Luke), Maaninka, Finland.*

Effects of fat from oats and rapeseed cake on methane (CH_4) emissions and lactational performance was examined in 12 Nordic Red dairy cows, of which 4 primiparous, averaging (±SD) 37.8 ± 7.5 kg/d milk yield (MY) and 48 ± 23.9 d in milk. The experiment was a switch-back design, divided into three 4-wk periods. Cows were randomly assigned into 2 treatment groups, which received the experimental treatments in 2 different sequences. The treatments were 1) rapeseed cake and oats (RSC+O), and 2) rapeseed meal and barley (RSM+B). The diets consisted of partial mixed ration (PMR), which included grass and either oats or barley, and pellets, which included either rapeseed cake and oats or rapeseed meal and barley. The RSC+O and RSM+B diets contained on DM-basis: 18.0 vs. 17.2% CP, 41.1 vs. 35.1% NDF and 4.26 and 2.75% crude fat, respectively. Daily PMR intake was measured with the RIC system and MY, and treatment pellet intake was measured, and milk samples taken, in a Lely milking robot. Enteric CH_4, and H_2 production was measured with a GreenFeed unit attached to the milking robot. Statistical analysis was performed using PROC MIXED of SAS with treatment, period, block, and sequence as fixed effects and cow within a sequence as a random effect. Dry matter intake was lower ($P = 0.01$) for RSC+O vs. RSM+B (24.0 and 25.5 kg/d, respectively), and MY tended to be greater ($P = 0.10$) for RSC+O compared with RSM+B (34.7 and 33.6 kg/d, respectively), effectively increasing ($P = 0.001$) feed efficiency for RSC+O by 0.13 kg MY/kg DMI. No differences in energy-corrected milk yield (ECM) and milk components were observed. Methane emission as g/d, CH_4 intensity as g/kg MY and as g/kg ECM were 7.5, 9.8 and 8.5% lower ($P = 0.01$) for RSC+O vs. RSM+B, respectively. Hydrogen production for RSC+O vs RSM+B was lower ($P = 0.002$; 0.81 and 1.10 g/d). Grass silage-based diet including rapeseed cake with oats increased feed efficiency while decreasing CH_4 emission intensity by 9–10%. This provides a readily applicable and practical way of mitigating enteric CH_4 emission from dairy operations while increasing the production efficiency of the cows.

Key Words: enteric methane, dairy cow, dietary fat

Ruminant Nutrition Symposium: Advances in Fatty Acid Nutrition

2559 Best practices in fatty acid analysis. K. J. Harvatine*[1], T. C. Jenkins[2], and S. P. Alves[3], *[1]Penn State University, University Park, PA, [2]Clemson University, Clemson, SC, [3]University of Lisbon, Lisbon, Portugal.*

Fatty acid (FA) analysis has become routine in many dairy nutrition experiments but is challenging due to the diversity in sample types, FA of interest, and goals of projects. The presentation will overview the most commonly used procedures and highlight key issues and best practices. Methyl-ester derivatives are commonly prepared, but procedures vary in approach and chemistry. Direct methylation is more common in feeds, digesta, fecal, and tissue samples as it ensures more complete lipid extraction while extraction followed by methylation is more common in milk as it reduces loss of shorter chain FA and artifacts. Multiple internal standards from different classes (FAME, FFA, TG) are recommended and allow calculation of FA concentration and methylation efficiency. The stringency of methylation protocols can be increased by increasing reagent concentrations, reaction time, or temperature, but care must be taken to minimize oxidation and isomerization. Gas chromatography with flame ionization detection is the most common approach and is highly repeatable and linear, but column selection, column wear, and temperature programs influence results. Fatty acid recovery must be determined and optimization of integration setting should not be overlooked. Multiple inject may be required to separate or quantify some peaks. Advances in columns and mass spectrometry provide additional opportunities for identification of FA commonly found in samples from ruminants. Ruminant samples can contain hundreds of peaks and attempting to identify every peak can become extremely time-consuming and overwhelming. Considering the hypothesis and goals of the experiment are very important. Lastly, results must be converted to FA for reporting. One major issue is that some samples and derivation procedures create artifacts that can be up to half of the total peak area. Artifacts can be removed using solid phase extraction and is necessary if artifacts co-elute with FA. In conclusion, FA analysis is complex but robust procedures are available. Selection and execution of the right procedures for each experiment are important for robust hypothesis testing and increasing repeatability across laboratories.

Key Words: CLA, biohydrogenation, lipids

2560 Seventy years of research on ruminal biohydrogenation. A critical review. P. G. Toral*, G. Hervás, and P. Frutos, *Instituto de Ganadería de Montaña (IGM), CSIC-University of León, Grulleros, León, Spain.*

Our knowledge about ruminal biohydrogenation (BH) has improved enormously since this metabolic process was empirically confirmed in 1951. Until the 1990s, advances in BH research were modest, due in part to analytical limitations in the identification of fatty acid isomers. Poor understanding of the implications of BH for animal performance and nutritional quality of ruminant-derived products did not contribute either. For years, BH was mostly perceived as a process to be avoided for increasing the post-ruminal flow of unsaturated fatty acids. Two milestones changed this perception and aroused great interest in analyzing BH intermediates: in 1987, in vitro anticarcinogenic properties of conjugated linoleic acids (CLA) were described; and in 2000, the inhibition of milk fat synthesis by *trans*-10 *cis*-12 CLA was confirmed. Articles on BH grew linearly between both years, tripling the number of publications in the literature. In the 2000s, research on this topic grew exponentially, reaching a plateau in the 2010s. Numerous BH metabolites have been described both in small and large ruminants, and the major deviation from the common pathway (i.e., the *trans*-10 shift) is relatively known. However, less well-characterized alterations exist. In addition, expectations placed on this area of research have not always been materialized. Indeed, the exhaustive description of new BH intermediates (e.g., using isotopic tracers) has not been coupled with research on their actual biological effects. Furthermore, BH seems no longer perceived as a metabolic niche inhabited by few bacterial species with highly specific metabolic capability, but we have failed to elucidate which microbial groups are actually involved in the process and the basis for alterations in BH pathways (i.e., changes in microbial populations or their activity). Answering these questions would have relevant practical implications in animal science. In this review we summarize the state-of-the-art on ruminal BH, including a critical review of the achievements and failures accumulated over these decades, as well as the future approaches from the perspective of dairy research.

Key Words: fatty acid, lipid, rumen

2561 Odd- and branched-chain fatty acid metabolism: Food abundance and human physiology. J. T. Brenna*, *University of Texas at Austin, Austin, TX.*

Dairy products are the major sources of odd- and branched-chain fatty acids [(O)BCFA] in the diets of persons in North America. In the US cow's milkfat contains about 2% BCFA. The mean human dietary BCFA intakes in the US exceed 500 mg/d compared with less than 100 mg/d for the well-studied omega-3 long-chain polyunsaturated fatty acids. We established that BCFA are major components of the first solid meal of the prenatal (fetal) humans via oral intake of amniotic fluid-borne vernix caseosa particles, where BCFA averages 30%. Our objective is to review the body of recent evidence indicating that BCFA are key underconsumed nutrients for human gut health. Approximately 90% of branched fat swallowed as a component of vernix caseosa disappears in the human fetal GI tract, presumably absorbed by the fetal enterocytes. BCFA appearing in the meconium, the material accumulating the rectum throughout gestation, are dominated by longer chain (C16–26) BCFA with no detectable BCFA below C16. The BCFA are present in human milk at levels lower than in cow's milk. When human fetal or adult-like enterocytes are treated with BCFA as free fatty acids, they are taken up and rapidly incorporated into membrane phospholipids to 30–60% depending on structure. When administered in place of linoleic acid rich oils, they reduce the incidence of necrotizing enterocolitis in a neonatal rat model of the disease. In so doing, they increase the inflammatory cytokine IL-10 and shift the nascent microbiota toward organisms that contain high levels of BCFA in their membranes. The BCFA in California sea lion vernix develop mid-way through gestation, where they are found in vernix, stomach contents, amniotic fluid, plasma and meconium of fetal sea lions, all paralleling humans. Among ruminant milks, milks of yaks (*Bos grunniens* or *Poephagus grunniens*) are richest in BCFA, especially in the "half-lactating" yak where BCFA concentrations average 5.3%. Yak manure is particularly rich in BCFA, averaging almost 15% BCFA. Peoples of the Qinghai-Tibetan plateau rely on yak milk and dairy products as staples, consuming at the extremes 3,500–5,000 mg of BCFA per day.

Key Words: branched-chain fatty acids, yak, human nutrition

2562 Oxylipids: Mediators of the inflammatory process from initiation to resolution. G. A. Contreras*, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Oxylipids (oxylipins) are lipid mediators of inflammation derived from the oxygenation of ω-3, -6, and -9 PUFA. Oxylipids' biosynthesis starts with PUFA release from phospholipid membranes or lipid droplets into the cytoplasm by lipolytic enzymes (phospholipases and lipases). Next, enzymatic and non-enzymatic oxidation yield oxylipids. Generally, arachidonic (AA) and linoleic (LA) acids-derived oxylipids are crucial for inflammation's initiation stages. As the inflammatory process progresses, these ω-6 metabolites are "degraded" to epoxides, ketones, and other intermediate lipids by enzymes activated during different stages of inflammation resolution. Many of these oxylipids trigger the resolution of inflammation. As an example, LA's first oxidation products are HPODEs that are rapidly reduced to HODEs by glutathione peroxidases, followed by a dehydrogenation to oxoODEs (ketones) by hydroxy fatty acid dehydrogenase. In contrast to AA and LA-derived oxylipids, oxidation products of ω-3 PUFA, including ALA, EPA, and DHA, are considered pro-resolving. Given the number of chemical reactions involved in oxylipids biology, their profile is dynamic and varies by tissue. For example, during periods of intense lipolysis, such as parturition, the oxylipid content of adipose tissues is more dependent on the activity of hormone-sensitive lipase than on phospholipases. Consequently, the oxylipid profile of fat depots reflects on the PUFA profile of their triglyceride rather than their phospholipids. In contrast, in sites with an active bacterial infection, such as the mammary gland during mastitis, the oxylipid profile highly depends on immune cells' membrane phospholipid fatty acid content. Notably, not all oxylipids synthesized in tissues appear in circulation, and therefore a careful interpretation of plasma lipid mediator content is warranted. Nutritional and pharmacological interventions can modulate oxilipid profile in tissues and circulation. Although increasing the production of pro-resolving oxylipins may improve inflammatory disease recovery, it may impair physiological processes that rely on rapid and robust inflammatory responses.

Key Words: oxylipids, lipolysis, inflammation initiation resolution

Ruminant Nutrition 6: Gut Physiology, Fermentation, and Digestion

2563 A dynamic mechanistic model of microbial fermentation and methane production in the cow rumen. R. Muñoz-Tamayo*[1], S. Ahvenjärvi[2], A. R. Bayat[2], and I. Tapio[3], [1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants, Palaiseau, France,* [2]*Animal Nutrition, Production Systems, Natural Resources Institute Finland (Luke), Jokioinen, Finland,* [3]*Genomics and Breeding, Production Systems, Natural Resources Institute Finland (Luke), Jokioinen, Finland.*

Dynamic mathematical models have been developed to enhance understanding of rumen function. These models include Molly (Baldwin et al., 1987), the Dijkstra model (Dijkstra et al., 1992), Karoline (Danfær et al., 2006) and their extensions. Model evaluations showed that there is still room to enhance their accuracy for predicting volatile fatty acids concentration and methane production. Previously, Muñoz-Tamayo et al. (2016) developed an alternative model of rumen fermentation under in vitro conditions aimed at better representing the rumen microbiota and hydrogen dynamics. In the present work, we extended the previous model to account for in vivo conditions. Our objective was to evaluate the capability of our model to represent the dynamics of rumen fermentation. For that, we performed an experiment with 4 Nordic-Red dairy cows equipped with rumen fistulas to provide dynamic data to support model construction. The experiment determined animal daily patterns of feed intake, rumen fermentation and enteric methane production. Feed intake and methane emissions were measured in respiration chambers during 2 d after an adaptation period. Rumen liquid was collected every 3 h for determination of volatile fatty acids concentration. In the model, the rumen microbiota is represented by 3 functional groups namely sugar-, amino acids- and hydrogen-utilizers. The feed is expressed in 3 pools as neutral detergent fiber, non-structural carbohydrates and proteins. The model consists of 18 compartments described by ordinary differential equations. The model parameters were estimated using the maximum likelihood method implemented in the Matlab toolbox IDEAS (Muñoz-Tamayo et al., 2009). All data of the 4 cows were used for the model calibration. The model performance was satisfactory as evaluated by the coefficient of variation of the root mean squared error (RMSE/observed mean), which was 9% for acetate, 11% for butyrate, 13% for propionate and 21% for methane. Our model has the potential to be used as virtual platform to simulate the dynamics of rumen fermentation and methane production. Acknowledgments. The authors acknowledge funding from the MASTER project, an Innovation Action funded by the European Union's Horizon 2020 research and innovation program under grant agreement no. 818368.

Key Words: methane, modeling, rumen fermentation

2564 Determining net apparent appearance and disappearance of volatile fatty acid from time-series data. S. Sujani, C. Gleason, B. dos Reis, and R. White*, *Virginia Tech, Blacksburg, VA.*

Ruminal volatile fatty acid (VFA) concentrations and molar proportions are not representative of VFA supplied to the animal due to conflating effects of fluid dynamics and VFA interconversion. Although gold-standard methods such as isotope dilution, washed rumen techniques, and others are available to explore VFA fluxes, these methods often require developing artificial physiological states which fail to represent normal rumen conditions. Our objective was to explore whether apparent net appearance (NAA) and disappearance (NAD) rates for VFA could be derived from time-series VFA dynamics generated through meal-feeding. Six ruminally cannulated wethers were fed diets containing timothy hay or beet pulp (TH, BP) and soybean meal or heated soybean meal (SBM, HSBM) in a partially replicated Latin Square with a 2 × 2 factorial arrangement of treatments. Periods were 24 d (21-d adaptation, 3-d sampling). Baseline rumen samples were collected at 0800 h on sampling days, a bolus of polyethylene glycol (PEG) was given, and animals were fed. Samples were the collected every 30 min until 1200 h, and hourly until 2400 h. Samples were analyzed for VFA and PEG concentrations. Based on dilution kinetics, PEG data were used to estimate fluid passage and volume to calculate VFA mass and fluid-mediated exit. The NAA and NAD for VFA were estimated by deriving a 1-pool model for each VFA on a mass basis, with NAA estimated as an exponential decay function and NAP following mass action kinetics. Fluxes were analyzed using linear mixed effect models with fixed effects for fiber, protein, and their interaction, and random effects for animal and period. Fiber and protein source interacted to affect acetate NAA (*P* = 0.04) and NAD (*P* = 0.008), with TH^{+}HSBM and BP+SBM yielding higher fluxes than BP+HSBM and TH^{+} BM; and affected propionate NAD (*P* = 0.04), with TH^{+}HSBM resulting in greater NAD compared with other diets. Further work is needed to contextualize the relevance of these measurements compared with traditional methods.

Key Words: volatile fatty acid, flux, measurement

2565 Intraruminal aqueous carbon dioxide is a strong predictor of volatile fatty acid concentrations. K. Amirault, B. dos Reis, and R. White*, *Virginia Tech, Blacksburg, VA.*

Ruminal volatile fatty acid (VFA) concentrations and molar proportions are widely researched but typically analyzed through gas chromatography, which requires considerable delays between sampling and analysis, precluding real-time exploration of dynamics. Biosensors detecting relevant fermentation outcomes may provide value as real-time indicators of VFA dynamics within the rumen for use in precision feeding applications. Our objective was to determine the efficacy of intraruminal aqueous CO_2 sensing as an indicator of VFA concentrations across fermentation timeseries and diet types. Four ruminally cannulated dry cows were fed diets containing grass hay (GH) alone or top-dressed with soybean mean (+SBM), corn grain (+CG), or both (+Both) in a partially replicated Latin square. No adaptation period was used to replicate short-term shifts in fermentation which might be driven by precision feeding. During sampling, immediately before feeding, a set of weighted probes measuring rumen temperature, CO_2 and conductivity were placed in the center of the rumen. Sampling lines were used to collect rumen fluid samples from close to the probes. The probes logged every 3 min for 12 h post-feeding. Fluid samples were taken hourly, and were analyzed for VFA. To explore sensed capacity to explain variability in VFA, the sensor CO_2 readings were matched to VFA measurements and linear regressions relating the sensed metrics to each VFA were derived. The concordance correlation coefficient (CCC) of those regressions was compared with those obtained from linear, mixed-effect models of each VFA using fixed effects of diet, time, and their interaction, and random effects of animal and period. The CCC of VFA models from sensor data ranged from 0.78 to 0.86 for the VFA evaluated, while the models based on diet and time ranged from 0.74 to 0.92. Only valerate was better predicted by diet and time than by sensor information. These preliminary data suggest intraruminal CO_2 sensing may be a promising strategy to explore fermentation patterns in real-time.

Key Words: rumen, sensing, volatile fatty acid

2566 Modeling enteric methane emissions in heat-stressed lactating dairy cows.

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Current models to predict enteric methane (CH_4) emissions in dairy cows do not take into account the effects of heat stress (HS), which may affect enteric CH_4 emissions. Our objective was to model absolute enteric CH_4 emission (g/d), yield (CH_4/DMI; g/kg), and intensity (CH_4/MY; g/kg) in lactating dairy cows using generalized additive mixed-effects models. A database composed of individual records (n = 1,675) of DMI and MY from 82 heat-stressed (daily temperature-humidity index peaks of 83) lactating dairy cows housed in environmental chambers up to 20 d from 6 studies was used. Baseline enteric CH_4 emissions before HS exposure and days of exposure to HS were used as predictors. Absolute CH_4 emission was estimated based on DMI, fatty acids, and dietary digestible neutral detergent fiber content of the diets. Absolute CH_4 emission and yield reduced with the progression of HS up to 9 d when it started to increase again up to 20 d. From d 1 to 5, there was a 27.4 g/d reduction in absolute CH_4 emission, from d 5 to 10 there was a further reduction of 3.02 in absolute CH_4 emission, and from d 10 to 20 there was an increase (compared with d 5–10 period) of 4.13 g/d in absolute CH_4 emission. From d 1 to 10, there was a 0.14 g CH_4/kg DMI reduction in CH_4 yield, and from d 10 to 20 there was an increase (compared with d 1–10 period) of 0.06 g CH_4/kg DMI. Methane intensity was also reduced initially up to d 5 during HS exposure but then started to increase again up to d 20. From d 1 to 5, there was a 0.15 g CH_4/kg MY reduction in CH_4 intensity, from d 5 to 8 there was an increase of 0.09 g CH_4/kg MY, from d 8 to 11 there was another increase of 0.02 g CH_4/kg MY, and from d 11 to 20 there was a third increase of 0.20 g CH_4/kg MY. The models developed in this study indicate that enteric CH_4 emissions are reduced during the onset of HS but at the expense of decreases in DMI and MY, which is not desirable. Validation of the predictive capacity of the models developed herein using data from in vivo studies where enteric CH_4 emissions are measured in heat-stressed lactating dairy cows is required before these models can be used.

Key Words: heat stress, enteric methane, models

2567 Assessment of hindgut microbiota and its functional shift in postpartum dairy cows with nutritional diarrhea.

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Postpartum dairy cows (PDC) can have nutritional diarrhea and as a result, some cows develop enteritis that may significantly affect cow's health and performance. Our study aimed to identify the physiological and microbiological changes when PDC developed nutritional diarrhea and to assess whether it affects their milk production. Twenty-four multiparous Holstein dairy cows were selected from a cohort and separated into 2 groups based on the difference in their fecal scores: low fecal score (LFS) group (1.33 ± 0.42, mean ± SD, n = 12) and high fecal score (HFS) group (3.00 ± 0.29, n = 12). Milk composition, rumen fluid, and fecal fermentation profiles were measured from samples collected at 9 d in milk (DIM) and subjected to *t*-test analysis. Milk yield was recorded daily from DIM 1 to 19 and analyzed by a mixed model with the DIM, group, and their interaction as fixed effects, and cow as a random effect. Milk yield during the postpartum period was affected ($P < 0.05$) by the interaction between cow's diarrhea and DIM. Milk β-hydroxybutyric acid and acetone concentrations as well as ruminal propionate concentration were higher ($P < 0.05$) in LFS cows than those in HFS. The apparent digestibility of crude protein in LFS cows trended to be lower ($P < 0.10$) than that in HFS cows. In addition, LFS cows had higher ($P < 0.05$) fecal ammonia nitrogen concentration, isobutyrate, and isovalerate molar proportions and lower ($P < 0.05$) acetate and total volatile fatty acid (TVFA) concentrations than HFS cows. The relative abundance of *Frisingicoccus* and *Lachnospiraceae_UCG-001* trended to be higher in the feces of HFS cows (adjusted $P < 0.10$) based on Wilcoxon rank-sum test, which also showed a positive correlation with fecal acetate ($r > 0.60$, $P < 0.01$), butyrate ($r > 0.55$, $P < 0.01$) and a negative correlation with fecal ammonia nitrogen ($r < -0.45$, $P < 0.05$). Our results suggest that the dysbiosis in hindgut microbiota could be associated with diarrhea in PDC, resulting in altered protein digestibility and milk β-hydroxybutyric acid and acetone concentrations, which warrant future research.

Key Words: postpartum cows, nutritional diarrhea, gastrointestinal fermentation

2568 Using the rumen microbiome, genomic PTA, and artificial intelligence to predict feed and milk production efficiency in dairy cows.

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Optimizing predictive models for feed and milk production efficiency is an important strategy to reduce the carbon footprint in dairy farms. We hypothesized that the 16S rRNA rumen microbiome composition and previously predicted genomic PTA values are useful proxies to assess feed and milk production efficiency. We aimed to predict these traits using 495 genotyped lactating dairy cows from the US and Canada through artificial intelligence. Cows' DMI, milk production (MP), milk fat yield (FatY), and residual feed intake (RFI) were measured daily from approximately 50 to 99 DIM. Rumen contents were collected using an esophageal tubing procedure. Microbiome characterization was performed through 16S rRNA gene sequencing, and data were processed in DADA2. Predictions including only the microbiome and the microbiome plus genomic PTA values were performed using classical machine learning (ML) algorithms, including partial least squares, elastic net, lasso, ridge, and principal component regression. Deep artificial neural networks (ANN) were also used for the predictions. A 5-fold cross-validation was used in all models. The best-tested model for these variables was classical ML ridge regression with variable selection through backward elimination. Using only the microbiome composition, the best prediction was for MP ($R^2 = 0.57$ and MAE = 3.93 kg/d), followed by DMI ($R^2 = 0.54$ and MAE = 1.72 kg/d), FatY ($R^2 = 0.49$ and MAE = 0.173 kg/d), and RFI ($R^2 = 0.43$ and MAE = 0.766

kg/d). Predictions were improved by adding the genomic PTA values. The best prediction using the microbiome and genomic PTA was for DMI ($R^2 = 0.69$ and MAE = 1.45 kg/d), followed by MP ($R^2 = 0.65$ and MAE = 3.62 kg/d), FatY ($R^2 = 0.63$ and MAE = 0.152 kg/d), and RFI ($R^2 = 0.57$ and MAE = 0.704 kg/d). Overall, the rumen microbiome composition and genomic PTA values account for a major portion of the total variation in the studied production traits, which warrants further investigations on their prediction ability.

Key Words: machine learning, neural networks, bacterial community

2569 A multi-omics approach to characterize the role of the rumen microbiome on feed efficiency in dairy cows. H. Monteiro*[1], C. Figueiredo[2], B. Mion[3], W. Coelho Jr.[1], R. Bisinotto[2], M. Nehme[4], J. Santos[4], F. Peñagaricano[5], E. Ribeiro[3], F. Schenkel[3], B. Weimer[1], L. Guan[6], A. Neves[7], T. Brown[1], F. Lima[1], *[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, [2]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, [3]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [4]Department of Animal Sciences, University of Florida, Gainesville, FL, [5]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, [6]Department of Agriculture, Food, and Nutrition, University of Alberta, Alberta, Canada, [7]Department of Animal Science, University of Copenhagen, Copenhagen, Denmark.*

The precise understanding of modulators of feed and milk production efficiency remains a major challenge to optimizing the sustainability of dairy farms. We hypothesized that a multi-omics approach could reveal microbes, related host genes, and potentially active metabolites modulating residual feed intake (RFI). We assessed rumen microbiome, metabolome, and microbial enzymatic pathway differences in 2 groups of cows with extreme RFI from a large study cohort. A cohort of 495 lactating cows from the US and Canada had DMI measured daily between 50 to 99 (SD ± 13) days in milk. During this period, body weight and yields of milk and milk components were recorded, and rumen contents were collected using an esophageal tubing procedure. A total of 24 cows from the extremes of RFI distribution (12 most and 12 least efficient) were selected for rumen content 16S and 18S rRNA, untargeted primary metabolites, and dual-RNA sequencing analyses. Differential abundance analyses were performed through ANCOM-BC, MAaslin2, Linda, and ALDEx2. Statistical differences were considered when $P \leq 0.05$. There were 218 different bacterial genera between most and least efficient cows, and a major protozoal genus (*Entodinium* spp.; ~85% of total protozoa relative abundance) had greater counts in most efficient cows. From 232 primary ruminal metabolites identified, ruminal ornithine was in greater abundance in the most efficient cows, and it was the only metabolite consistently different across several validation analyses between the 2 groups. Rumen lauric acid was in lower abundance in most efficient cows and a major metabolite negatively associated with these animals. Preliminary data from dual-RNA sequencing analysis showed that the bacterial species candidate division TM6 bacterium GW2011_GWF2_36_131 (TM6) had increased mRNA abundance in the most efficient cows. Correlations of 0.52 and 0.61 between TM6 and ornithine and lauric acid were estimated, respectively. Overall, ornithine, lauric acid, *Entodinium*, and TM6 were the main metabolites and microbes found to be linked to RFI. Our ongoing study will continue to dissect the association of transcriptional co-occurrence profiles with microbes and metabolites and further explore their causation effects on the trait.

Key Words: residual feed intake, transcriptomics, metabolomics

2570 Rumen bacterial cluster identification and its influence on rumen metabolites and growth performance of young goats. D. Wang, G. Tang, X. Lei, L. Wang, J. Yao, and Y. Cao*, *Northwest A&F University, Xianyang, Shaanxi, China.*

The enterotypes have been found to relate to host metabolism and health tightly. However, this concept has never been used in rumen, and little is known about the complex biological relationships between ruminants and their rumen bacterial clusters. In this study, we used young goats (n = 99) as a model, fed them the same diet, and analyzed their rumen microbiome and corresponding bacterial clusters. The relationships of the bacterial clusters with rumen fermentation and growth performance in goats were further investigated. Two bacterial clusters were identified in all goats: P-cluster (dominated by *Prevotella*, n = 38) and R-cluster (dominated by *Ruminococcus*, n = 61). Compared with P-cluster goats, R-cluster goats had greater growth rates, the concentrations of propionate, butyrate, and 18 free amino acids, and the proportion of unsaturated fatty acids, but lower acetate molar percentage, the acetate-to-propionate ratio, and several odd- and branched-chain and saturated fatty acids in rumen fluid ($P < 0.05$). Several members of *Firmicutes*, including *Ruminococcus*, *Oscillospiraceae NK4A214 group*, and *Christensenellaceae R-7 group* were significantly higher in R-cluster, whereas some *Prevotellaceae* members were significantly higher in P-cluster ($P < 0.05$). Co-occurrence networks showed that R-cluster enriched bacteria have negative correlations significantly with P-cluster enriched bacteria ($P < 0.05$). Moreover, we found the concentrations of propionate, butyrate, and free amino acids, and the proportions of unsaturated fatty acids were positively correlated with R-cluster enriched bacteria ($P < 0.05$). The acetate molar percentage, acetate-to-propionate ratio, and the proportions odd- and branched-chain and saturated fatty acids were positively correlated with P-cluster enriched bacteria ($P < 0.05$). Overall, our result indicated that the rumen bacterial clusters can influence rumen fermentation and growth performance of young goats, which may shed light on modulating the rumen microbiome in their early life to improve the growth performance of ruminants.

Key Words: rumen microbiota, bacterial cluster, growth performance

2571 Feed-efficient dairy cows show potentially more significant protozoa activity towards microorganisms in the rumen. W. Coelho Jr.*[1], H. Monteiro[1], C. Figueiredo[2], B. Mion[3], P. Peixoto[2], R. Bisinotto[2], M. Nehme[4], J. Santos[4], F. Penãgaricano[5], E. Ribeiro[3], F. Schenkel[3], B. Weimer[1], L. Guan[6], T. Brown[1], F. Lima[1], *[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, [2]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, [3]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [4]Department of Animal Sciences, University of Florida, Gainesville, FL, [5]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, [6]Department of Agriculture, Food, and Nutrition, University of Alberta, Alberta, Canada.*

Previous data from our group indicate that the rumen microbiome plays an essential role in modulating residual feed intake (RFI). We hypothesized that cows with greater feed efficiency exhibit improved ruminal nitrogen metabolism. Our objective was to evaluate dietary nitrogen derivatives in the rumen of a subset of lactating cows from a large study cohort varying in RFI. A total of 495 lactating cows from the US and Canada had DMI measured daily between 50 to 99 (±13) DIM concurrent with daily yields of milk and milk components and body weight. Rumen contents were collected using an esophageal tubing procedure. A total of 24 cows from the extremes of RFI (12 most vs. 12 least efficient) were selected for the analyses of protozoa community composition and ruminal untargeted primary metabolites, ammonia-N (NH_3-N), and serum urea-N concentration. Protozoa community composition was characterized through 18S rRNA sequencing. Ruminal untargeted primary metabolome analysis identified 232 metabolites through gas chromatography TOF-MS. The relationships between ruminal untargeted primary metabolites, protozoa genera, ruminal NH_3-N, and serum urea-N concentration were evaluated. Statistical analyses were declared different at $P \leq 0.05$. Counts of the genus *Entodinium* (~85% relative abundance) were greater in the most efficient than the least efficient cows. This protozoan was primarily linked to hypoxanthine in the rumen, which is a metabolite from bacterial degradation. Ruminal NH_3-N concentration was also greater in most compared with least efficient cows (6.38 vs. 3.76 mg/dL, SEM: 0.89). Some of the main metabolites associated with the concentration of ruminal NH_3-N were derivatives of the urea cycle, which is primarily performed by protozoa in the rumen. Despite the relationship between ruminal NH_3-N and serum urea-N concentration ($R^2 = 0.50$), differences in the former were not translated to serum urea-N concentration. These differences in the rumen of most efficient cows suggest a potential greater protozoa predation of bacteria and greater ruminal bacterial-N recycling in these animals.

Key Words: rumen microbiome, ruminant nutrition, urea cycle

2572 Comparisons of ruminal microbiome and metabolites of low- and high-producing dairy cows. A. D. Ravelo*[1], P. Ferm[1], Y. Guo[2], B. O. Omontese[2], C. Chen[2], N. R. Noyes[1], and L. S. Caixeta[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN,* [2]*Department of Food Science and Nutrition, University of Minnesota, Saint Paul, MN.*

Few studies have reported the associations between the ruminal microbiome and the milk production genotypes in dairy cows. Thus, this study aims to compare the rumen microbiome and the metabolites between the second lactation dairy cows in the 75th percentile (n = 12) of production according to genomic PTA for milk (GPTAM) and their counterparts in the 25th percentile (n = 12). Two groups of cows were matched by days in milk (DIM), sire, occurrence of disease, and days open in previous lactation. Ruminal and blood samples were collected around peak lactation (65 ± 14 DIM). Rumen fluid was collected using an oro-ruminal gastric tube. Shotgun metagenomics was used for rumen microbiome profiling. Metabolite profiling of the rumen fluid and plasma samples was conducted using high-resolution liquid chromatography-mass spectrometry (LC-MS). No differences in the richness ($P = 0.71$), diversity ($P = 0.40$), or Bray-Curtis dissimilarity index ($P = 0.42$) of the microbiome were observed between high and low GPTAM cows. Benjamini-Hochberg adjusted *P*-value of Welch's *t*-tests were used to determine that there were no differentially abundant OTU identified by ALDEx2. Similarly, the concentrations of volatile fatty acids (VFA) and free amino acids in the ruminal fluid and serum had no differences between 2 groups of cows based on Student's *t*-tests. Pearson correlations were used to consider associations between serum metabolites and milk production. Serum valerate ($P = 0.05$; r = 0.40) and isovalerate ($P = 0.05$; r = 0.40) concentrations had a moderate positive correlation with milk production. Moreover, serum acetate ($P = 0.10$; r = 0.34), butyrate ($P = 0.09$; r = 0.35), and propionate ($P = 0.33$; r = 0.11) concentrations had a weak positive correlation with milk production. There were no significant correlations of rumen VFA with milk production. Overall, no differences were noted in the microbial composition which corresponded with no differences in concentration of amino acids between the groups.

Key Words: genotype, metagenomics, metabolomics

2573 High bioavailability of rumen-protected choline alters the vaginal discharge microbiota in dairy cows. T. Marques*[1,2], H. Monteiro[1], D. Melo[1], W. Coelho Jr.[1], S. Salman[1], D. Dubey[3], F. Sun[4], K. Leao[2], and F. Lima[1], [1]*Department of Population Health and Reproduction, University of California, Davis, CA,* [2]*Federal Institute Goiano, Rio Verde, Goias, Brazil,* [3]*Kemin Europa NV, Herentals, Belgium,* [4]*Kemin Industries Inc., Des Moines, IA.*

Rumen-protected choline (RPC) benefits dairy cows' intake, metabolism, lactation, and health. Recently, potential benefits to immune function were also identified. Our objective was to characterize markers of immune function (lipopolysaccharide-binding protein, LBP), acute phase proteins (haptoglobin, HAP), and vaginal discharge microbiota (VDM) as a proxy for uterine immune competence. Holstein cows at 245 d of gestation were blocked by parity within each block randomly assigned to Control or RPC. Cows enrolled in the RPC group received 15 g/d of CholiGEMTM (Kemin Industries Inc.) from 21 d prepartum and 30 g/d from calving to 21 d postpartum. During the transition period, blood was sampled weekly for LBP and HAP analysis. Vaginal discharge samples were collected at the calving and 7 d postpartum to evaluate VDM through 16S rRNA gene sequencing as amplicon sequence variants and data analysis using 4 multivariable in DADA2. Data were analyzed using the GLIMMIX procedure of SAS. There were effects of the week in relation to calving in serum concentrations of haptoglobin in primiparous ($P < 0.01$) and multiparous ($P < 0.01$) cows. However, RPC had no effect on the HAP serum concentration in primiparous ($P = 0.37$) and multiparous ($P = 0.81$). For LBP, there were effects of the week in relation to calving in primiparous ($P = 0.04$) but not in multiparous ($P = 0.52$) cows and RPC had no effect in primiparous ($P = 0.81$) and multiparous ($P = 0.39$) cows. For the VDM, the Shannon index showed lower richness and diversity in the RPC at calving than the Control ($P = 0.04$). Phylum differences between groups were found in multiparous and primiparous by principal coordinate analysis and PERMANOVA ($P < 0.01$). Cows in the RPC group had an altered VDM at genera and phylum at 7 d postpartum with a less relative abundance of *Fusobacteriota* ($P = 0.03$) and *Fusobacterium* ($P = 0.02$), a pathogen group associated with metritis in dairy cows. High-bioavailability RPC seems to impact the reproductive tract microbiota suggesting further research on its impact on uterine health warrants further investigation.

Key Words: *Fusobacterium*, uterine health, immune system

2574 Associations between residual feed intake (RFI) and digestibility or hepatic mitochondrial respiration in Holstein cows. M. N. Marinho*, S. E. Wohlgemuth, M. C. Perdomo, and J. E. P. Santos, *University of Florida, Gainesville, FL.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Objectives were to evaluate associations between residual feed intake (RFI) with total-tract digestibility (TTD) and hepatic mitochondrial (Mt) respiration in cows. A total of 117 Holstein cows were fed complete diets for 140 d. Individual dry matter intake (DMI), milk yield, and body weight (BW) were measured daily, whereas milk sampled from consecutive milkings twice weekly was analyzed for fat, protein, and lactose. Body condition (BCS) was scored twice weekly. Body energy change (BEC) was calculated as: [2.88 + (1.036 × BCS)] × BW change. Intake of DM was regressed as a function of energy in milk, metabolic BW, BEC, parity, treatment, and the random effect week cows were enrolled. The error term in the model defined RFI. Cows were categorized into 25% most (n = 29) and least (n = 29) efficient according to RFI and their rumen fluid, feces, and urine were sampled at (mean ± SD) 122 ± 20 and 184 ± 20 d postpartum. Liver tissue was sampled from a subset

Table (Abstr. 2574). Residual feed intake and performance, digestibility, and mitochondrial respiration[1]

Item	High efficiency	Low efficiency	SE	*P*-value
DMI, kg/d	21.1	24.2	0.43	<0.01
ECM, kg/d	39.6	40.6	1.11	0.53
TTD, %				
OM	76.7	77.0	0.52	0.73
CP	71.8	72.7	0.95	0.53
NDF	44.3	45.1	0.87	0.60
Starch	98.6	98.7	0.16	0.69
Rumen fluid				
pH	6.19	6.32	0.04	0.04
NH_3N, mg/dL	9.61	7.92	0.50	0.02
Acetate, m*M*	71.0	70.5	1.18	0.77
Propionate, m*M*	26.4	25.9	0.74	0.66
Butyrate, m*M*	16.3	14.9	0.32	0.01
Mt ATP synthesis efficiency, %	98.0	97.9	0.24	0.83

[1] TTD = total-tract digestibility; Mt = mitochondrial.

of 15 most and 15 least efficient cows at 184 ± 21 d postpartum, and Mt respiration assayed. Indigestible NDF was used to estimate TTD. Data were analyzed using mixed-effects models in SAS including the fixed effects of efficiency (high vs. low), period of collection or day when Mt was assayed, DIM, and the random effect of cow nested within efficiency. Improving efficiency was not associated with TTD or hepatic Mt respiration coupled with ATP synthesis; however, more efficient cows had smaller ruminal pH and greater ruminal concentrations of NH_3N and butyrate than less efficient cows (Table 1). Mechanisms underlying improved feed efficiency were linked with differences in ruminal metabolism rather than TTD or hepatic mitochondrial respiration.

Key Words: digestibility, efficiency, residual feed intake

ADSA-INRAE International Partnership Symposium: Milk—From Production to Effect on Human Health | The Latest Results of INRAE in Rennes in the PEGASE and STLO Research Units

2600 The structure of dairy products at different length scales drives the mechanism of digestion and the nutrient bioavailability. D. Dupont*[1,2], [1]*INRAE, Rennes, France,* [2]*Institut Agro, Rennes, France.*

Recent findings have demonstrated that the food matrix structure is one of the key drivers to control the fate of food in the digestive tract and, consequently, the kinetics of nutrient release. As an example, using the pig as a model of human, we have demonstrated that, at identical composition, differences in dairy product macrostructure (milk vs acid or rennet gels) lead to differences in gastric emptying, protein hydrolysis in the gut and amino acid bioavailability. Compared with dairy gels, milk goes quickly through the stomach to reach the small intestine where protein will be rapidly and extensively degraded. This generates a fast and intense peak of plasma amino acids. When macrostructures are identical, differences at the microscopic scale can also have a strong impact on dairy product digestion. For instance, the structure of infant formula (IF) has been shown to dramatically influence the hydrolysis of milk protein. Infant formulas were designed at INRAE's dairy platform and their in vivo digestion by piglets investigated. First, IF1 was a control IF with vegetable oil and milk proteins at the interface of the lipid droplet. In IF2, lipid droplets were stabilized by milk phospholipids to recreate a structure close to that of the milk fat globule. Finally, IF3 was also stabilized by milk phospholipids and 60% of the vegetable oil was replaced by milk fat. Piglets were fed 28 d with one of the 3 IFs and then slaughtered. Concentration of milk proteins in the jejunum and ileum was higher for IF3 whereas IFNg secretion was increased suggesting an improved intestinal immune system maturation such as observed in sow suckling piglets. Finally, the nature of IF was shown to drastically affect the intestinal microbiota composition of piglets. The structure of dairy products can therefore be considered as a lever to control the kinetics of nutrients release during digestion and fulfil the nutritional needs of specific populations such as elderly people, athletes, obese etc.

Key Words: digestion, food structure, nutrient

2601 Hormonal and nutritional regulations of lactation persistency in dairy cows. M. Boutinaud*[1], C. Gaillard[1], L. Herve[1], F. Dessauge[1], L. Delaby[1], P. Lacasse[2], and V. Lollivier[1], [1]*INRAE, Institut Agro, PEGASE, Saint Gilles, France,* [2]*AAFC, Sherbrooke R&D Centre, Canada.*

In dairy cows, it is believed that the decrease in milk production after the peak of lactation is largely caused by mammary epithelial cell loss. However, the physiological regulations of lactation persistency in dairy ruminants are not entirely elucidated. Therefore, several experiments were conducted to assess the effects of hormonal and nutritional challenges on milk production and the mammary tissue. In a first experiment, the effect of the long-term inhibition of prolactin release was assessed at early lactation with cows receiving or not daily injections of quinagolide for 8 wk. The prolactin inhibition induced a faster decline in milk production (−14%) associated with a reduction in cell proliferation and an increase in apoptosis in the mammary tissue. Another experiment was conducted to observe the effects of sex steroids. Multiparous cows were either ovariectomized or sham-operated around 60 d in milk and followed for 52 weeks. Ovariectomy slowed the decline of milk yield (+10%) and thus, improved lactation persistency. This effect was accompanied with modifications of apoptosis/proliferation balance in the mammary tissue and a reduction of mammary epithelial exfoliation into milk. The effects of feeding level were also investigated in 2 experiments. In the first experiment, dairy cows were assigned to a basal diet or a severe feed restriction from 2 wk before calving to wk 11 postpartum. The negative effect of feed restriction on milk yield (−38%) was accompanied with a higher level of apoptosis in the mammary gland without effect on cell proliferation but affecting the total amount of DNA in the tissue. A second experiment, a moderate feed restriction of cows at early lactation for 4 weeks, decreased milk yield (−8%) without affecting the apoptosis/proliferation balance but increasing mammary epithelial cell exfoliation into milk. Altogether, these experiments confirmed that the regulation of lactation persistency in the dairy cows is related to changes in the number of mammary cells through either the regulation of apoptosis/proliferation balance but also through the rate of mammary cell exfoliation into milk.

Key Words: apoptosis, proliferation, cell exfoliation

2602 Breeding factors of dairy cows, milk lipolysis, and consequences on semi-hard cheese and fresh cream. C. Hurtaud*[1], L. Bernard[2], A. Thierry[3], G. Garric[3], M. Harel-Oger[3], and C. Cebo[4], [1]*PEGASE, INRAE, Institut Agro, Saint-Gilles, France,* [2]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champanelle, France,* [3]*STLO, INRAE, Institut Agro, Rennes, France,* [4]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France.*

Milk lipolysis is defined as the hydrolysis of triglycerides. Short-chain fatty acids released in milk are responsible for rancid flavor that is unacceptable for consumers. In addition, the presence of partial glycerides impairs milk functional properties. Milk lipolysis represents an important criterion to assess the quality of milk. Within the framework of the ANR-19-CE21–0010 LIPOMEC project, we aimed thus to establish breeding and feeding strategies that ensure stable milk quality, and to characterize the effects of milk susceptibility to spontaneous lipolysis (SL) induced during milk transformation. Feed restriction in dairy cows induced SL, with higher value in evening milk. We therefore studied the impact on SL i) of milking intervals: 10–14, 14–10, and 12–12, and ii) of milking frequency, on 2 groups of cows that produced milk that was susceptible or not to SL before the experiment was started, by applying once, twice or 3-times-a-day milking. Regarding milking intervals, more SL was observed in the evening milk for 10–14 interval and in the morning milk for 14–10. A small but not significant increase in evening milk was observed for the 12–12 interval. Regarding milking frequency, SL was significantly lower with once-a-day milking, and higher with 3-times-a-day milking, with a larger increase for milks susceptible to SL. Regarding transformation, we compared the changes in lipolysis during processing operations for milk susceptible or not to SL. The processing steps (milk transport, transfer by pumping, heat treatment, skimming and cold maturation before renneting) induced an overall increase, which was significantly greater for susceptible milks. The most impactful steps were skimming and cold maturation. However, the differences induced were not significantly sensorially perceived, according to the results of triangle tests performed in the 2 mo-matured cheeses and in

2 mo-stored fresh creams. Taken together, our results demonstrate that farming practices such as feeding level, milking interval or milking frequency significantly affect the level of SL in milk and the level of induced lipolysis during milk transformation.

Key Words: milk, lipolysis, dairy products

2603 Eco-design approaches for developing sustainable processes: New opportunities for the dairy sector. G. Gesan-Guiziou*, *UMR STLO, INRAE, Institut Agro Rennes-Angers, UMR STLO, INRAE, Institut Agro Rennes-Angers, Rennes, France.*

More than 70% of all agricultural goods currently produced in the European Union are transformed into manufactured food products, and this trajectory is set to intensify as food manufacturing processes increasingly integrate the multiple fractionation, assembly and formulation steps required to propose a vast array of food products with specific properties. This increasing rate of food processing in addition to the increasing complexity of food manufacturing forces the food industry to address its impact on the environment. This talk presents the main approaches for ecodesigning food manufacturing processes. The first approach, based on minimizing material flows (particularly energy and water) within the process, either via modifications to operating conditions or via a redistribution or reuse of the flows within the process, is widely implemented at the industrial level. The second approach, based on comparative environmental assessment of either different processes or different scenarios for a particular process that is then iteratively improved, is starting to be used. This approach typically employs life cycle assessment to single out the most environment-negative processes and unit operations to help guide process engineers toward an improved design. New approaches based on modeling, simulation-optimization ecodesign approaches are also emerging. Only these approaches integrate the notion of compromise between different technological, economic and environmental objectives (minimizing key process-related quantities, i.e., inputs (water, energy and chemicals), environmental impacts and the related costs, while maximizing productivity and product properties) by co-optimizing conflicting objectives, and tease out the optimal solutions. These approaches will be illustrated with examples taken from the dairy sector, and explicitly discussed with respect to the potential rewards and challenges of their respective application.

Key Words: ecodesign, processing, food manufacturing

2604 Variation factors of milk calcium content in dairy cows and cellular mechanisms of milk calcium secretion. A. Boudon*, M. Boutinaud, and C. Hurtaud, *PEGASE, INRAE, Institut Agro, Saint-Gilles, France.*

Milk calcium (Ca) content has long been considered to be determined by milk protein content, with a strong influence of genetics of cows. Milk mid-infrared spectrometry analyses allowed showing a high heritability of milk Ca contents and a genetic correlation between this parameter and protein contents. This review of INRAE results aimed to illustrate that environmental factors also induce variations in milk Ca content. A survey based on the collection of 200,000 individual milk samples in the major areas of milk production in France showed that calendar month and cow feeding strategy affected milk Ca content, which dropped in the spring during grazing turnout and was lower when cows were fed fresh and conserved grass rather than corn silage. Complementary experimental studies clearly showed that the stage of lactation, which is an identified cause of milk Ca content variation, only explained a limited part of this variability. Thanks to a meta-analysis, based on experiments comparing diets in cows with similar stage of lactation, we showed a specific effect of diets on milk Ca content. Total, soluble and colloidal calcium contents of milk and ratio between milk Ca and protein contents were lower when diets were based on pasture rather than on corn silage, with intermediate values with diets based on grass silage or hay. Lower milk Ca contents with grass-based diet were not explained by an increased dietary cation anion difference. For some other environmental factors influencing milk Ca content, such as digestible protein and net energy balance or day length, we could observe parallel variations of milk Ca and casein contents. Milk Ca variations may be related to variations of the expression of mammary genes involved in calcium secretion pathways. Even though we observed a huge increase in knowledge in the regulation of Ca secretion in milk these last 15 years, the underlying mechanism for environmental milk Ca variation remain to be elucidated.

Key Words: dairy cows, feeding system, milk composition

2605 Milk microbiota: Potential allies for mammary gland health. C. Goetz[1], L. Rault[1], M. Boutinaud[2], C. Citti[3], H. Falentin[1], J. Guinard-Flament[2], P. Germon[4], M. Mariadassou[5], D. Morgavi[6], X. Nouvel[3], Y. Le Loir[1], and S. Even*[1], *[1]STLO, INRAE, Institut Agro, Rennes, France, [2]PEGASE INRAE, Institut Agro, Saint Gilles, France, [3]IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France, [4]ISP, INRAE, Université de Tours, Nouzilly, France, [5]Université Paris-Saclay, INRAE, MaIAGE, Jouy-en-Josas, France, [6]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champanelle, France.*

Bovine mastitis has long been thought to be the result of a host-pathogen interaction; however, the microbiota is now considered as a key player of this infectious disease process. This talk presents an overview of our research on the role of milk and mammary gland microbiota on mammary health and the opportunities it offers for a more sustainable management of dairy cows' health. The role of milk and mammary gland microbiota on mammary health was first highlighted by exploring these relationships either before or after mastitis events, i.e., some time from the infectious episode. Teat cistern microbiota (collected in foremilk) was found to be related to the history of animals with regard to mastitis. Besides, using changing from twice- to once-daily milking (ODM) to trigger an udder perturbation in initially healthy quarters of cows, a temporal relationship was reported between initial teat cistern microbiota and the immune response and mastitis development following transition to ODM. To identify determinants of milk microbiota composition, cows with different scores of susceptibility to mastitis were used to explore the microbiota in various body sites throughout lactation. The microbiota from milk, mouth, nose and vagina were specific and evolved throughout lactation. Interestingly, an important intra-animal sharing of microbes between anatomic sites was reported, whereas inter-animal microbial sharing was limited, suggesting a host regulation of bovine microbiota, including a slight role of the score of susceptibility to mastitis on milk microbiota. Within mammary gland microbiota, lactic acid bacteria (LAB), used as probiotic, showed promise for mastitis prevention. In vitro, LAB were able to compete with pathogens for epithelium colonization and exhibited immunomodulatory properties. A promising LAB candidate was recently evaluated in vivo through the assessment of its microbiological, immune and physiological impact on the mammary gland, following post milking application on teat skin. The safety of such topical application was confirmed, opening up an avenue for the development of new prophylactic strategies.

Key Words: milk microbiota, mammary health, probiotics

2606 Interfacial self-organization in droplets of dairy protein mixes: From skin formation to powder functional properties. L. Lanotte*, *UMR STLO, INRAE, Institut Agro Rennes-Angers, Rennes, France.*

Milk powders are nowadays highly added-value goods, whose demand continuously increases, driven by world population growth and globalization. The specificity of dairy powder end-users, as in the case of infant formulas and supplements for athletes, requires a tight control of functional and nutritional properties. This crucial question is still far from being resolved since the most employed technique for powder production, i.e., the spray drying, does not favor a direct insight into the mechanisms governing the droplet sol-gel transition at the industrial scale. Over the past decade, we implemented a multiscale approach to investigate the drying dynamics in mixes of dairy proteins (i.e., whey proteins and casein micelles), which represent fundamental components of milk. Coupling complementary techniques (e.g., profile visualization, microscopy, mass measurements), the evaporation steps have been characterized first in droplets of single classes of proteins and then in mixtures. Our outcomes revealed that the intrinsic signature of whey protein and casein micelle molecular characteristics on the structure of the so-called skin, leading to the formation of smooth and hollow dry particles in the first case and wrinkled ones in the latter. Interestingly, the study of protein mixtures highlights a behavior typical of polydisperse colloidal systems, consisting of the gradual accumulation of the smaller macromolecules, i.e., whey proteins, at the surface (smaller-on-top). Under certain experimental conditions, this auto-stratification conferred to whey proteins a predominant impact on dry particle shape. Surprisingly, these results have been confirmed also by tests performed using a pilot monodisperse dryer despite the significantly different drying time scale. The next studies will focus on more complex dairy systems to further shed light on the milk drying process and possibly provide a predictive method for tuning powder functional properties starting from sample composition and environmental conditions.

Key Words: drying, proteins, skin formation

2607 Variations in milk lactose content and the mechanisms underlying in dairy cows. J. Guinard-Flament*[1], A. Hamon[1], N. Decoopman[1], M. Boutinaud[1], C. Gaillard[1], C. Hurtaud[1], M. Gelé[2], L. Mériaux[3], S. Dufour[4], H. Larroque[5], and S. Lemosquet[1], *[1]PEGASE, INRAE, Institut Agro, Saint-Gilles, France, [2]IDELE, Paris, France, [3]EILYPS, Pacé, France, [4]Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, QC, Canada, [5]GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France.*

Lactose has not often been the subject of specific research as it is rarely included in milk payment systems. However, milk lactose content has been proposed as a potential indicator of udder health and metabolic disorders in dairy cows. Since these data are easy and quick to collect in dairy farms at a large scale and at a moderate price (MIR analysis), it is of interest to better understand its variations in milk to use it as an indicator. Lactose is synthesized from blood glucose in the mammary epithelial cells. It is the main osmotic agent in milk and determines the amount of water transferred from the blood compartment into the mammary tissue lumen. Thus, its synthesis and secretion largely explain the volume of milk produced by the animals and, for this reason, its content in milk varies little compared with milk fat and protein contents. The lactose content in milk is the result of 3 main mechanisms: 1) the regulation of the metabolic activity of the mammary gland for the synthesis of milk constituents, mainly based on the quantity of glucose taken up by the udder and its partition between the intra-cellular metabolic pathways, 2) the regulation of the osmotic pressure of milk related to the relative concentration of the different osmotic agents in milk (lactose, Na^+, K+, Cl^-, proteins, citrate), and 3) the integrity of the mammary epithelium which, if altered, is accompanied by passive transfers of soluble molecules (lactose, minerals) between the alveolar lumen of the udder and the blood (and vice versa). The aim of this review will be to discuss the mechanisms underlying variations in milk lactose content regarding variations reported in commercial dairy farms in the west of France and in Canada. In particular, it will highlight the interactions between the factors of variation, whether these factors are intrinsic or extrinsic to the animals in relation to the rearing conditions (feeding, milking, and health).

Key Words: milk lactose, variations, dairy cow

2608 How could a breakthrough innovation in cheese technology be accepted by the consumer? M. Harel-Oger[1], C. Martin[2], S. Marette[3], J. Chamberland[4], and G. Garric*[1], *[1]INRAE, INRAE, Institut Agro Rennes-Angers, UMR1253 STLO, Rennes, France, [2]INRAE, Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne, Dijon, France, [3]Université Paris Saclay, Université Paris-Saclay, INRAE, AgroParisTech, Paris-Saclay Applied Economics, Palaiseau, France, [4]STELA, Department of Food Sciences, STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Laval University, Quebec, QC Canada, [5]INRAE, INRAE, Institut Agro Rennes-Angers, UMR1253 STLO, Rennes, France.*

Traditional cheese technology first consisted in draining a coagulated milk to generate a curd that was eventually salted and ripened. From'Innov was suggested as a new simplified cheesemaking concept based on decoupling cheese texture development and aroma production. The production of flavor molecules is conducted externally, in fermented dairy matrices that are added in the cheese milk prior coagulation. Subsequent works generated the knowledge to modulate functional properties of From'Innov cheese, and the concept was also duplicated in the dry form (Valo'From) for large-scale export. The From'Innov concept has many advantages for dairy processors: it is an agile process (1 line for all products of a plant), adaptable to demand (1 "ripened" cheese is made in 5 d), and is more efficient than a conventional process (−25% energy and fluids, and margins increased by 50% per kg of cheese produced). However, one question remains: are consumers ready for this breakthrough innovation? A consumer test (142 participants) was carried out to compare, under blind conditions, the hedonic appreciation (HA) and willingness to pay (WTP) of 2 soft bloomy-rind cheeses obtained by the From'Innov process (one mild and one with more intense aroma) with a commercial product obtained in a supermarket: l'Ortolan Milleret cheese dairy (Bourgogne-Franche-Comté, France). From'Innov cheeses had a lower score for HA and WTP ($P < 0.01$). It was even further reduced when it was explained to consumers that these 2 cheeses were obtained using an innovative process. On the other hand, when consumers knew that the new process was more energy-efficient and that new cheeses contained −25% salt and fat, WTP increased and became very close to that of commercial cheese ($P > 0.05$). Consumers are therefore sensitive to the benefits of From'Innov for health and efficiency. Future work is still required to understand how this process could find its place in a mature cheese market to increase the offer for cheese having a lower environmental impact and better nutritional quality.

Key Words: cheese technology, innovation, consumers

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2761 The biosecurity basket: Using association rule learning algorithms to target recommendations more likely to be implemented by dairy farmers. F. Farison*[1,2], V. Régia Lima Campêlo[1,2], M.-E. Paradis[4,5], S. Buczinski[3], G. Fecteau[2,3], J.-P. Roy[2,3], P. Valdes Donoso[2,3], S. Dufour[1,2], and J. C. Arango-Sabogal[1,2], *[1]Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada, [2]Chaire de recherche de biosécurité en production laitière, Université de Montréal, Saint-Hyacinthe, Québec, Canada, [3]Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada, [4]Association des médecins vétérinaires praticiens du Québec (AMVPQ), Saint-Hyacinthe, Québec, Canada, [5]DSAHR Inc., Saint-Hyacinthe, Québec, Canada.*

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Biosecurity measures prevent the introduction and spread of pathogens on farms. Information about their implementation is collected through risk assessment questionnaires (RAQ) like the one completed during the mandatory Dairy Farmers of Canada (DFC) proAction initiative. Our objective was to explore the usefulness of Association Rule Learning (ARL), a non-supervised machine learning algorithm, to predict the biosecurity practices (BP) that will be more likely to be implemented by producers based on their responses to the RAQ. ARL has been widely used in marketing for consumer segmentation based on buying patterns and has the potential to help veterinarians to recommend BP that are more susceptible to be adopted by dairy producers. In total, 3825 RAQ completed between 2018 and 2021 by Québec dairy producers through the DFC proAction initiative were analyzed. Initially, 29 million rules were generated by the algorithm. Each rule is composed of one BP (i.e., consequent) associated with a combination of other BP already implemented in the farm (i.e., antecedent). The 60 best rules were retained based on the frequency (support), strength (lift) and likelihood of the association (confidence) between antecedent and consequent. Of the 32 BP initially assessed, the algorithm predicted 14 practices (consequents) with a confidence ≥ 70%. For instance, producers who disinfect sick cows' pens after each case and avoid contact of young animals with older cattle will have 91% probability of housing calves in a way that minimizes disease. This combination of BP was observed in 883 farms (23%). Recommending this consequent to producers who have not implemented it yet and have the same profile of antecedents might improve its adoption. ARL is an interesting methodology to analyze information collected through RAQ and study the relationships between BP on dairy farms. Since ARL identifies the practices more likely to be implemented by a given producer, it allows veterinarians to provide targeted recommendations that might improve producers' uptake of prevention and control programs.

Key Words: biosecurity practice, machine learning, recommendation

2609 Evaluating the efficacy of 2 footbath concentrates to control digital dermatitis in freestall dairy cows using a noninferiority study. S. Ordaz*[1], E. Shepley[1], D. Doepfer[1], K. Anklam[1], G. Cramer[1], and L. Caixeta[1], *[1]University of Minnesota, Saint Paul, MN, [2]University of Wisconsin–Madison, Madison, WI.*

Digital dermatitis (DD) is the most prevalent infectious hoof disease in dairy cattle and DD can decrease performance and farm profitability. Footbathing is the most common tool to control and prevent DD in dairy farms. The objective of this study was to determine the effectiveness of a glutaraldehyde-based product, Healmax (HM), compared with copper sulfate (CS), in controlling new cases of DD lesions over a 16-week period. Five freestall herds, 4 in Minnesota and 1 in Wisconsin, were enrolled from November 2021 to May 2022. Treatment was applied at the pen level and pens were randomly allocated to either the HM (2.5%) or CS (5%) footbath treatment. Footbaths were run on 4 consecutive days with footbaths changed every 200–400 cows and between treatments. All lactating cows were scored for DD lesions in the milk parlor biweekly using the M-stage scoring system, with 2 baseline scores taken before the study start. Cows were considered for inclusion in the study if no DD lesions on either hind foot were recorded for both baseline scores (HM = 4309, CS = 4521). To establish non-inferiority and to calculate sample size, a 25% non-inferiority margin was used. Data were analyzed using a discrete time survival analysis, with treatment, weeks in treatment, lactation, and DIM as fixed effect and pen as a random effect. Overall, 18.5% of the legs developed DD lesions, with the risk of DD lesion development being 19.9% (18.9–20.9%) in HM and 17.1% (95%CI: 16.2–18.1%) in CS. The hazard ratio for HM compared with CS was 1.03 (0.87–1.22), indicating a possible range in efficacy for HM of 13% fewer to 22% more DD lesions than CS. Based on the upper CI of the hazard ratio and our non-inferiority margin of 25%, HM was considered non-inferior to CS Farm-specific differences were observed, suggesting that certain farm-level factors influence the performance of footbath products. In conclusion, HM can be considered an effective and non-inferior to CS footbaths when used at a 2.5% concentration for at least 4 d a week.

Key Words: discrete time analysis, lameness, hoof health

2610 Impact of a first occurrence of digital dermatitis in early lactation on culling and pregnancy in dairy cows. E. Shepley*[1], S. Ordaz[1], D. Döpfer[2], K. Anklam[2], L. Caixeta[1], and G. Cramer[1], *[1]University of Minnesota, Department of Veterinary Population Medicine, St. Paul, MN, [2]University of Wisconsin–Madison, Madison, WI.*

Digital dermatitis (DD) is one of the most prevalent hoof lesions in dairy cows and can decrease cow performance and longevity. The objective was to investigate the impact of a first occurrence of DD in the first 60 d in milk (DIM) on culling and pregnancy in the first 180 DIM in dairy cows. As part of another study, lactating cows from 5 dairy herds (~1300–3000 cows/herd) were scored for DD lesion presence and severity biweekly in the milk parlor by trained observers for a period of 16 weeks (November 2021 to May 2022). Before enrollment, DD was scored in 2 baseline observations, 2 weeks apart. Health and reproduction records were extracted from farm software 6 mo following the completion of the study. To be considered for analysis, cows had to have no DD lesions recorded in both hind feet during the baseline period and be <60 DIM at the time of the study start. Cows were categorized as developing DD in either or both hind legs in the first 60 DIM (DD60) or as not having DD in the first 60 DIM (NoDD). Data were analyzed using Cox proportional hazard models, with time to event (i.e., time to cull/censor and time to pregnancy/censor) as the outcome, DD lesion occurrence by 60 DIM and lactation as fixed effects and accounted for clustering by farm. Overall, 83.9% of cows were pregnant by 180 DIM, with a pregnancy rate of 85.1% (222/261) for DD60 cows and 83.7% (2375/2836) for NoDD cows. The hazard ratio (HR) for pregnancy by 180 DIM for DD60 compared with NoDD was 1.1 (95% CI: 0.93, 1.29), indicating a possible higher likelihood of pregnancy in DD60 cows. The overall cull rate by 180 DIM was 5.9% (186 cows). The cull rate was 4.2% (11/261) and 6.0% (175/2,899) for DD60 and NoDD cows, respectively. The HR for culling was lower (0.65, 95% CI: 0.43, 0.98) for DD60 cows compared with NoDD cows. These results are unexpected; however, additional research into effects of recurrent DD is warranted to determine if chronic DD poses a more consequential risk to cow survivability and performance.

Key Words: hoof health, lameness management, longevity

2611 Evaluating machine learning algorithms to use accelerometer data for identification of lameness in dairy cows. R. Neupane[1], S. Paudyal*[2], A. Aryal[3], and P. Pinedo[4], [1]*Christian-Albrechts-Universität zu Kiel, Kiel, Germany,* [2]*Department of Animal Science, Texas A&M University, College Station, TX,* [3]*Department of Construction Science, Texas A&M University, College Station, TX,* [4]*Department of Animal Science, Colorado State University, Fort Collins, CO.*

The objective was to evaluate different machine learning algorithms for their effectiveness to identify cows needing corrective hoof trimming and treatment of lameness disorders. A total of 310 multiparous Holstein dairy cows from a herd in Northern Colorado were affixed with a leg-based accelerometer (IceQube Icerobotics, Edinburgh, UK) to obtain the lying time (LY; min/d), daily steps (ST; n/d), and lying bouts (LB; n/d). Study cows were monitored for 4 mo and cows submitted for HT. Cows were differentiated as receiving corrective interventions (TRM) or as being diagnosed with a lameness disorder requiring therapeutic intervention (DIS) by a certified hoof trimmer. A median filter was applied to smoothen the data to reduce inherent variability. Three different models were defined to fit each algorithm which included: simple features (containing daily steps, daily lying time, and daily bouts derived from the accelerometer), slope feature (slope feature extracted from each variable in simple feature), and all features (3 simple features and 3 slope features). The most important features within the models were selected using recursive feature elimination. Random forest (RF), Naive bayes (NB), Logistic regression (LR), and Time series (TS) with rocket classifier were used as ML predictive approaches. The machine learning algorithms were evaluated using leave-one-out cross-validation and the best-performing ones were reported. The TS approach presented the best results (accuracy: 0.89 ± 0.02, precision: 0.81 ± 0.03, recall: 0.95 ± 0.05, F1 score: 0.88 ± 0.03, AUC-ROC: 0.91 ± 0.02, specificity: 0.86 ± 0.02) utilizing all features to identify cows needing hoof trimming. For cows affected by specific lameness disorders, the models had low sensitivity using TS (accuracy: 0.90 ± 0.01, precision: 1.00 ± 0.09, recall: 0.4 ± 0.08, F1 score: 0.57 ± 0.10, AUC-ROC: 0.70 ± 0.05, specificity: 1.00 ± 0.01). Overall, accelerometer data and the features extracted could provide opportunities to identify and classify the lameness conditions, but more research is needed to improve the on-farm utility of these models.

Key Words: lameness, hoof trimming, machine learning

2612 Remote comparative lameness assessment in dairy cattle: A crowdsourcing approach. K. Sheng*[1], B. Foris[1], M. A. G. von Keyserlingk[1], J. Gardenier[2], C. Clark[3], and D. M. Weary[1], [1]*Animal Welfare Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada,* [2]*Australian Centre for Field Robotics, Faculty of Engineering, the University of Sydney, Darlington, NSW, Australia,* [3]*Livestock Production and Welfare Group, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, the University of Sydney, Camden, NSW, Australia.*

Lameness assessment in cows requires trained assessors to assign a single absolute gait score on a 4- or 5-level scale, which is subjective and laborious; this means that lameness assessments are rarely performed, impeding early diagnosis and treatment. Comparison-based lameness assessments improve accuracy and repeatability, even when performed by untrained assessors. We developed and evaluated a remote comparative lameness assessment method. Non-experienced participants were recruited via an online platform and asked to watch pairs of videos, showing 2 cows walking simultaneously, to record which cow they judged to be more lame and by how much on a 0 to 3 lameness difference scale. We created 88 video pairs for comparative analysis, and 50 online workers were recruited to evaluate each pair. In addition, 5 experienced lameness assessors evaluated all video pairs. We found moderate to high interobserver reliability among the online workers (intraclass correlation coefficient, ICC = 0.46 to 0.77, calculated in R using the “irr” package), and high agreement among the experienced assessors (ICC = 0.87). The average responses of the online workers demonstrated high agreement (ICC = 0.89 to 0.91) with the average responses of the experienced assessors. Based on average responses from experienced assessors, we further divided the video pairs into more easily discernible pairs (degree of lameness difference ≥1; ICC among experienced assessors = 0.88; n = 48) and pairs which were more difficult to discern (degree of lameness difference <1; ICC among experienced assessors = 0.21; n = 40). The agreement between online workers and experienced assessors was exceptionally high for the easily discernible pairs (ICC = 0.95), but low for those that were less discernible (ICC = 0.27). The proposed method offers rapid and cost-effective lameness assessment and enables the collection of large-scale data that can be utilized to train computer vision algorithms for automatic lameness assessments. However, further work is required to determine approaches for treating pairs that are less discernible.

Key Words: cattle lameness, wisdom of the crowd, click worker

2613 Interrogating the diversity of vaginal, endometrial, and fecal microbiomes in healthy and metritis dairy cattle. T. Tasara[1], R. Whiston*[2], M. Stevens[1], J. Wabui[1], A. Chapwanya[2], U. Bleul[3], [1]*Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland,* [2]*Ross University School of Veterinary Medicine, Basseterre, St. Kitts and Nevis, West Indies,* [3]*Department of Farm Animals, Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.*

Our aim was to determine whether pre-calving fecal, vaginal and endometrial microbiomes were altered compared with postpartum in healthy and metritic cows. Holstein Friesian cows (n = 39) were examined and sampled over a period of 9 mo. Retrospectively, those with extrauterine inflammation and/or antibiotic treatment were excluded from the study (n = 14). From the remaining, 10 were selected which exhibited puerparal metritis on at least one examination day and 10 animals were assigned control group, which did not show metritis or clinical endometritis on any day of examination. Pre-calving fecal and vaginal, and endometrial swabs at different postpartum interval were collected. Microbiomes were determined based on bacterial 16S amplicon sequencing and compared between healthy (H; n = 10) animals and cows that developed metritis (M; n = 10) within 21 d postpartum (DPP). PERMANOVA tests were used to test for differences in microbial β diversity between the H (n = 10) and M (n = 10) groups. Compared with healthy cows the precalving fecal and vaginal microbiomes of metritis cows were more abundant in sequences from the phylum Fusobacteria and the bacterial genera *Escherichia-Shigella* and *Histophilus*. In addition to low microbial species diversity the postpartum endometrial microbiomes of metritis cows displayed decreasing *Proteobacteria* and increasing *Fusobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* abundances at progressive postpartum intervals (DPP) when compared with healthy animals. The greatest taxonomic compositional deviations in endometrial microbial communities between the M and H cows were detected between 7- and 10 DPP. There was high taxonomic similarity detected between postpartum endometrial microbiomes and the pre partum vaginal and fecal microbiomes suggesting that colonization through bacteria ascending from the rectum and vagina to the uterine cavity may play a role in establishing the postpartum endometrial microbiome. Thus, a deeper understanding of establishment and dynamics of postpartum endometrial microbial communities in cows will provide crucial basic knowledge to guide development of genital microbiome manipulation strategies for preventing uterine disease and improving fertility in dairy cows.

Key Words: bacteria, endometritis, microbiome

2614 Predictive models for metritis cure using farm collected data and hematological variables measured at diagnosis.

P. R. Menta*[1], E. B. Oliveira[2], J. G. Prim[3], K. N. Galvao[3,4], F. S. Lima[2], M. A. Ballou[1], N. R. Noyes[5], and V. S. Machado[1], [1]*Department of Veterinary Sciences, College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX,* [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA,* [3]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL,* [4]*D. H. Barron Reproductive and Perinatal Biology Research Program, Gainesville, FL,* [5]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.*

Our objective was to evaluate the accuracy of predictive models for metritis spontaneous cure (SC) and treatment failure (TF) using farm collected data only, and with the addition of hemogram variables and circulating concentration of metabolites, minerals, and biomarkers of inflammation measured at time of diagnosis. Data related to parity, calving related issues, body condition score, rectal temperature, and days in milk at metritis diagnosis was collected from a randomized clinical trial that included 412 metritic cows from 4 herds in TX, CA, and FL. Metritis was defined as the presence of red-brownish, watery, and fetid vaginal discharge, while cure was defined as the absence of metritis 14 d after initial diagnosis. Cows were randomly allocated to receive systemic ceftiofur therapy (to determine TF) or to remain untreated (to determine SC). At enrollment (day of metritis diagnosis), blood samples were collected and submitted to cell blood count (CBC) and measurement of 13 minerals and biomarkers of metabolism and inflammation (BM). Univariable analysis to evaluate the association of farm collected data and blood assessed variables with metritis cure were performed, and variables with $P \leq 0.20$ were offered to multivariable logistic regression models and retained if $P \leq 0.15$ or if they improved model accuracy. The area under the curve (AUC; 95% CI) for models predicting SC using farm data only and farm + BM, was 0.70 (0.62 – 0.78) and 0.76 (0.67 – 0.82), respectively. Cell blood count were not retained in the models. For models predicting TF, the AUC (95% CI) was 0.75 (0.68 – 0.82), 0.77 (0.69 – 0.83), 0.80 (0.73 – 0.87), and 0.80 (0.73 – 0.86) for models using farm data only, farm + CBC, farm + BM, and farm + CBC + BM, respectively. Predictive models of metritis cure had fair accuracy, with SC models being less accurate than TF models. Additionality, adding BM variables marginally improved the accuracy of models using farm collected data, while CBC data did not improve the accuracy of predictive models.

Key Words: metritis, cure, predictive models

2615 Association between vaginal discharge scores with rumination time, activity time, a health index score, and milk yield in lactating dairy cows.

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The objective was to characterize the association between the pattern of rumination time (RT), activity time (AT), a health index score (HIS), and milk yield (MY) with vaginal discharge scores (VDS) from 2 to 20 DIM in dairy cows. Data for RT, AT, and HIS were generated by an automated monitoring system with a neck-attached sensor and MY data with milking parlor sensors. Data were gathered from 529 lactating Holstein cows. At 4, 7, and 10 DIM, vaginal discharge was collected with a Metricheck device and scored (VDS0: no mucus; 1: clear mucus; 2: clear mucus+flecks of pus; 3: mucopurulent with < 50% pus; 4: mucopurulent with ≥ 50% pus; 5: watery, brown/reddish and fetid). Cows were grouped based on highest VDS at 4, 7, or 10 DIM resulting in VDS2 ($n = 26$), 3 ($n = 90$), 4 ($n = 319$), and 5 ($n = 56$). Cows with VDS0 and 1($n = 1$ each) were added to VDS2. Data for RT, AT, HIS, and MY were analyzed using ANOVA with repeated measurements with cow as random effect and the subject of repeated measurements. Binary outcomes were analyzed with logistic regression for which cows in VDS2 were included to the VDS3 group ($n = 116$). There was an interaction between group and day for RT ($P < 0.01$), HIS ($P < 0.01$), and MY ($P = 0.01$). Cows in VDS5 had smaller RT than cows in VDS2, 3, and 4 from 3 to 12 DIM. Cows in VDS5 had smaller HIS than cows in VDS2, 3, and 4 from 3 to 13 DIM. Cows in VDS5 had smaller MY than cows in VDS2, 3, and 4 from 3 to 20 DIM. At 8, 10, and 11 DIM, cows in VDS2 had greater MY than cows in VDS4. Finally, cows in VDS5 (461 ± 10 min/d) had less AT ($P = 0.04$) than cows in VDS2 (503 ± 12 min/d), 3 (489 ± 7 min/d), and 4 (488 ± 4 min/d) up to 20 DIM There was no association between P/AI at first service and VDS group ($P = 0.10$; VDS3: 30.5%; VDS4: 36.1%; VDS5: 26.1%) or the proportion of cows that were sold and died by 129 DIM and VDS group ($P = 0.33$; VDS3: 18%, VDS4: 9%, and VDS5: 14%). We conclude that behavioral and milk yield data might be used to differentiate cows with different VDS. Specifically, sensor data could be used to differentiate cows with VDS4 from cows with VDS5 which is observed in cows with metritis

Key Words: dairy cow, vaginal discharge scores, sensors

2616 Effects of systemic or intrauterine lipopolysaccharide challenge in cows at 5 or 40 days postpartum on clinical signs, uterine inflammation, feed intake, and milk yield.

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Our objective was to investigate the effects of intravenous (IV) or intrauterine (IU) lipopolysaccharide (LPS) infusion at 5 or 40 DIM on clinical signs, uterine inflammation, dry matter intake (DMI), and milk yield (MY). Holstein cows ($n = 48$) were blocked by DIM (5 or 40) and parity and randomly assigned to receive IV-LPS [0.0625 mg/kg BW (5 DIM) or 0.1 mg/kg BW (40 DIM) over 1h], IU-LPS [100 mg (5 DIM) or 300 mg (40 DIM) in 20 mL saline], or 20 mL saline IU (CON). The proportion of polymorphonuclear (PMN) cells was measured by endometrial cytology at d −1, 1, 4, and 7 relative to infusion day. Vital signs were assessed for 12 h and DMI and MY for 7 d. Data were analyzed in multivariable linear regression models with treatment, DIM, parity, time, and interactions, baseline values as a covariate, block as a random effect, and accounting for repeated measures. Peak rectal temperature was greater in IV-LPS than IU-LPS or CON (5 DIM: 39.8 vs. 39.3 and 38.9°C; 40 DIM: 40.3 vs. 38.9 and 38.9°C). Rumen contraction nadir was lesser in IV-LPS than IU-LPS or CON (0.3 vs. 1.4 and 1.7 per 3 min). Regardless of DIM, the relative increase from baseline of endometrial PMN was greater in IU-LPS (55%) or CON (35%) than IV-LPS (5%), and the decrease in DMI was greater at d 0 in IV-LPS (−24%) than in IU-LPS (−4%) or CON (−8%). Multiparous IV-LPS cows had a greater decrease in MY (−19%) at d 0.5 than IU-LPS (−9%) or CON (−5%). In primiparous cows, the decrease in MY was greater at d 1 in IV-LPS (−52%) than IU-LPS (−23%) or CON (−16%), greater at d 3, 4, and 7 in IV-LPS (−15 to −17%) than CON (+2 to +6%), and greater at d 5.5 in IV-LPS (−16%) or IU-LPS (−19%) than CON (+3%). Irrespective of DIM, IV-LPS induced changes in clinical signs, DMI, and MY, but IU-LPS only resulted in subtle changes in clinical signs and in MY in primiparous cows. Endometrial influx of PMN was greater in IU-LPS than IV-LPS, but not different than CON, which received saline IU. Further steps include assessing inflammatory markers in circulation, liver, and uterine tissues.

Key Words: endotoxin, inflammation, transition health

2617 Vaccination protocols recommended by veterinarians in Québec dairy herds. M. P. Morin*[1,2], J. P. Roy[3,4], M.-E. Paradis[5,6], G. Fecteau[3,4], and S. Dufour[7,2], [1]*Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada,* [2]*Research Chair in Biosecurity in Dairy Production, Université de Montréal, Saint-Hyacinthe, Québec, Canada,* [3]*Bovine Health Research Group, GRESABO, Saint-Hyacinthe, Québec, Canada,* [4]*Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada,* [5]*Association des médecins vétérinaires praticiens du Québec, AMVPQ, Saint-Hyacinthe, Québec, Canada,* [6]*DSAHR Inc., Saint-Hyacinthe, Québec, Canada,* [7]*Regroupement pour un lait de qualité optimale, Op+lait, Saint-Hyacinthe, Québec, Canada.*

In-depth knowledge of the vaccination practices used by dairy producers is necessary to identify opportunities to improve the prevention and control of infectious diseases in dairy herds. This study aimed to describe the current vaccination practices of Québec dairy producers. A cross-sectional study using the electronic data collected as part of a mandatory biosecurity program (proAction) and describing the farm's vaccination procedures was realized. A total of 3,825 standardized vaccination procedures performed between June 2018 and January 2021 were analyzed. Results indicate that at least one vaccination target was recommended in 90% of dairy herds. Eighty-eight percent of producers were recommended to vaccinate against respiratory disease and/or embryonic mortality, 22% for mastitis, and 18% for calf diarrhea. The basic vaccination protocol most frequently recommended by veterinarians (528 herds; 14%) included solely a live vaccine for the following pathogens: bovine viral diarrhea (BVD) viruses type 1 and 2, bovine respiratory syncytial virus, para-influenza virus 3, and infectious bovine rhinotracheitis. Actually, 2,802 (73%) of producers had a vaccination plan based on these latter vaccinal targets, to which various other targets were added. The most frequent complements were to add: inactivated leptospirosis vaccinal targets (241 herds; 6%), inactivated BVD1 or BVD2 targets to a subpopulation of the herd (most likely pregnant animals; 186 herds; 5%), an *Escherichia coli* mastitis target (142 herds; 4%), or inactivated vaccinal targets for BVD1, BVD2, bovine respiratory syncytial virus, para-influenza virus 3, infectious bovine rhinotracheitis, and leptospirosis to the basic live targets plan (122 herds; 3%). The next most frequent approach was to only use the inactivated BVD1, BVD2, bovine respiratory syncytial virus, para-influenza virus 3, infectious bovine rhinotracheitis targets with (118 herds; 3%) or without leptospirosis (91 herds; 2%). This study provides useful and interesting information on veterinary recommendations for vaccination in Québec dairy herds. Avenues for improvement to better prevent infectious diseases and thus reduce the use of treatments were identified.

Key Words: vaccination protocol, disease prevention, veterinarian

2618 Evolution of the within-herd prevalence status of bovine leukosis between 2017 and 2022 in Quebec dairy herds. K. G. Solano-Suárez*[1,2], J. P. Roy[2,3], J. C. Arango-Sabogal[1,2], E. Molgat[4], J. Durocher[4], and S. Dufour[1,2], [1]*Université de Montréal, Faculté de médecine vétérinaire, Département de pathologie et microbiologie, Saint-Hyacinthe, QC, Canada,* [2]*Research Chair in biosecurity of dairy production, Saint-Hyacinthe, QC, Canada,* [3]*Université de Montréal, Faculté de médecine vétérinaire, Département de sciences cliniques, Saint-Hyacinthe, QC, Canada,* [4]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

Our objective was to compare the change in the within-herd prevalence (WHP) status of the bovine leukosis virus (BLV) between 2017 and 2022 in Quebec dairy herds. Convenience sampling was done in 140 Quebec dairy herds for this descriptive longitudinal study, and bulk tank milk samples were collected on each farm in 2017 and in 2022. Samples were analyzed by Lactanet using ELISA tests. The WHP status for each year was categorized as low (LO: < 10%), medium (ME: 10–30%), or high (HI: > 30%) based on the ELISA result. The proportions and 95% confidence interval (CI) of herds changing their status between 2017 and 2022 were described and compared using the Chi-squared test (χ^2). The proportion of LO herds slightly increased from 19% in 2017 (n = 26) to 20% in 2022 (n = 28), and of ME herds increased from 14% in 2017 (n = 20) to 26% in 2022 (n = 36). Conversely, the proportion of HI herds decreased from 67% in 2017 (n = 94) to 54% in 2022 (n = 76; *P* < 0.001, χ^2 test). An improvement in the farm's WHP was observed in n = 27 herds (19%, CI: 13 - 27%); more specifically, 2% changed from HI to LO, 14% from HI to ME, and 4% from ME to LO. Conversely, n = 10 herds (7%, CI: 3 −13%) had a small increase in their status, either from LO to ME (4%; n = 6) or ME to HI (3%; n = 4). The proportion of herds with a decrease in the WHP status was significantly greater than the proportion of herds experimenting an increment (*P* < 0.001; χ2 test), thus, indicating a general correction of the BLV WHP. Finally, in n = 103 herds (74%) the BLV WHP status did not change. In conclusion, a moderate improvement of the BLV WHP status was observed in our sample of Quebec dairy herds between 2017 and 2022 (i.e., the BLV WHP status was moderately reduced in 5 years). Limitations of this study include using different ELISA kits in 2017 vs. 2022. Future work includes assessing the relationship between the bulk tank milk ELISA result and the WHP to monitor more precisely the change of BLV.

Key Words: leukosis, prevalence, Canada

2619 Risk factors for *Salmonella* Dublin on dairy farms in Ontario, Canada. K. V. Perry*[1], D. F. Kelton[1], S. Dufour[2], C. Miltenburg[3], S. G. Umana Sedo[1], and D. L. Renaud[1], [1]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada,* [2]*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, Quebec, Canada,* [3]*Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada.*

Salmonella Dublin (S. Dublin) is an emerging pathogen on dairy farms in Canada. In Ontario, S. Dublin has been increasingly isolated from laboratory diagnostic samples, but its herd-level prevalence is unknown. The objective of this cross-sectional study was to identify management

practices that are associated with herd positivity. A convenience sample of 100 Ontario dairy farms were visited once from April to August 2022 to collect blood samples from 20 heifers between 4 and 24 mo old, sample bulk tank milk and administer an in-person questionnaire on management and biosecurity practices. An additional bulk tank sample was collected before the visit by milk transporters. Bulk tank milk and serum underwent ELISA testing to determine S. Dublin positivity. Overall, 25% of farms tested positive, meaning at least one serum or bulk tank sample was interpreted as positive (≥35%PP). Of the 1,990 heifers sampled, 44 (2.2%) animals were positive for S. Dublin. At least one seropositive heifer was identified on 24% of farms. Of the bulk tank samples collected during both sampling periods, 4% of farms were positive for S. Dublin. A logistic regression model was built using STATA17 to explore associations with S. Dublin positivity. Introducing purchased animals within the last 2 years increased the likelihood that farms were positive for S. Dublin. Farms that had at least one animal leave and return to the premises for cattle shows, embryo collection, or loan within the last 2 years were also at higher risk for S. Dublin. Farms that removed manure from the surface of bedding in calving pens monthly or less often were at lower risk for S. Dublin than farms that removed manure more frequently. Farms that added bedding material to calving areas once or twice weekly were at lower risk for S. Dublin compared with farms that added bedding less than once weekly. In addition, farms that kept 3 cows or less per pen in calving areas were at lower risk for S. Dublin. Test positivity for S. Dublin among Ontario dairy farms sampled is high, and dairy producers should consider avoiding management practices that are associated with an increase in the risk of S. Dublin infection.

Key Words: infectious disease, biosecurity

Breeding and Genetics 2: Emerging Issues in Dairy Genetics

2620 Development of genetic evaluations to enhance disease resistance. C. Lynch*[1], R. Bongers[1], F. Schenkel[1], N. van Staaveren[1], F. Miglior[1,2], D. Kelton[3], and C. Baes[1,4], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Lactanet Canada, Guelph, ON, Canada, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [4]Institute of Genetics, Department of Clinical Research and Veterinary Public Health, University of Bern, Bern, Switzerland.*

Growing consumer awareness means that the dairy industry needs to continuously review and improve standards of animal health and welfare. Genetic selection is a tool which can contribute to this, however, limited research has been conducted on the genetics of resistance to enzootic bovine leukosis (LEUK) and calf-hood diseases in Canadian dairy cattle. Leukosis can lead to lymphocytosis and tumors, and is associated with decreased production, immunity and fertility. Major calf diseases include respiratory problems (RESP) and diarrhea (DIAR), which are both associated with increased mortality and rearing costs. This study aimed to understand the impact of LEUK and calf diseases on Canadian farms, by investigating incidence rates, estimating genetic parameters, and providing industry recommendations. For LEUK, 117,349 milk enzyme-linked immunosorbent assay test records on 96,779 Holstein cows from 950 Canadian herds taken between 2007 and 2021 were provided by Lactanet Canada (Guelph, ON). Each cow was classified as test-positive or test-negative for LEUK. For RESP and DIAR, producer recorded data comprised of 69,695 Holstein calf disease records, from 62,361 calves collected on 1,617 Canadian dairy herds between 2006 and 2021 were provided by Lactanet Canada. Single and multiple trait analysis using a linear animal model were carried out. Two approaches with respect to minimum required disease herd-year incidence (1% vs. 5%) were applied to determine how different thresholds impact selection potential. Roughly 77% of herds contained a positive case for LEUK; and within those herds, an average of 39% of cows tested positive. Heritabilities of 0.10 and 0.07 were estimated for LEUK resistance using a linear animal model applying BLUP and single-step GBLUP methodology, respectively. Heritability estimates for RESP and DIAR ranged from 0.03 to 0.08 across analyses, while estimated genetic correlations ranged from 0.53 to 0.62. Results from the current study are promising, however, industry outreach on the value of recording of calf disease information and testing for LEUK is required for effective genetic evaluation.

Key Words: genetic evaluation, health

2621 Early-lactation health event frequency of cows that carried beef or Holstein-sired calves. B. L. Basiel*, T. L. Felix, and C. D. Dechow, *Pennsylvania State University, University Park, PA.*

The objective was to determine if sire breed, beef or Holstein (HO), of the calf a cow carries influences the number of health events she experiences through 60 d postpartum. Health event records were extracted from herd management software from 10 primarily HO herds located in the Northeast and Midwest US. Lifetime records were available for 30,005 multiparous cows. Cows mated to dairy breeds other than HO were excluded from data. All complete lactations beyond parity 1, as well as current lactations of multiparous cows that were at least 60 d in milk, were included in analyses. Calvings by service sire breed included HO (40,132 calvings; 2,980 sires), Angus (AN; 619 calvings; 64 sires), Charolais (61 calvings; 5 sires), Limousin (221 calvings; 8 sires), Simmental (SM; 366 calvings; 26 sires), crossbred beef (115 calvings; 22 sires), and other beef breeds (80 calvings; 17 sires). Health events were classified in 5 categories: reproductive (RE), metabolic (MET), lameness (LA), mastitis (MAS), and other (OT) events. An event was considered distinct from a prior event of the same type if it occurred 14 d or more after the last event. Mixed models of each event category and of the sum of all events (ALL) in the first 60 d of lactation included service sire breed, herd, parity (2, 3, 4, or 5+), and number of health events experienced in the first 60 d of the previous lactation. Individual service sire and herd-year-season of calving were random effects. Means differences by sire breed were Tukey-adjusted. There were no differences in MET, MAS, OTH, or ALL events based on calf sire breed. Lameness events differed ($P = 0.02$) by sire breed; cows that previously carried HO calves had 0.21 ± 0.03 LA events while cows that carried SM-sired calves had 0.35 ± 0.06 LA events. Early lactation RE events, including retained placenta, metritis, vaginitis, and pyometra, occurred in greater frequency ($P < 0.01$) in cows that had HO calves (0.27 ± 0.03) than in cows with AN-sired calves (0.19 ± 0.03). Reduced reproductive health events occurred in cows that carried AN-sired calves and more lameness occurred in cows that had SM-sired calves compared with cows that had HO calves.

2622 Estimation of genomic parameters for automated milk feeding behavior and bovine respiratory disease in preweaning Holstein heifers. J. R. Graham*, M. E. Montes, V. B. Pedrosa, J. Doucette, J. P. Boerman, and L. F. Brito, *Purdue University, West Lafayette, IN.*

This study used data recorded by automatic milk feeders (AMF) for studying the genomic background of early in-life indicators of health and behavior traits. We analyzed feeding records to indirectly select for bovine respiratory disease (BRD) and to estimate genetic parameters for early-life indicators. Data from 8 Förster-Technik AMF for ~10,000 preweaning Holstein heifers for a period of 6 years included daily and per-visit milk consumption (DMC, MCPV), drinking duration (DDS, DDPV), and rewarded and total feeder visits (DNRV, TNV) over a 60-d pre-weaning period. Additional traits were derived from these variables, including consumption, duration raw, log variances (CV, DV, CLV, DLV), feeding interval (FI), drinking speed (DS), and pre-weaning stayability (ST). Two BRD traits were derived from the reported treatment, whereby the affected period was considered as 7 d pre and post treatment, including successive treatments within this window (BRD) to account for the prodromal period and decline, and a single value was included to represent the total number of BRD treatments (NTT). All traits were analyzed using single-step GBLUP mixed-model equations and random regression modeling (RRM) in the BLUPF90 suite of programs with ~9,000 calves genotyped with a 50K SNP chip. Using a repeatability model with all data included, we found that feeding traits had low heritability (h^2) estimates (e.g., DDS = 0.07). By truncating the first 32 d preweaning, h^2 for the same trait increased to 0.11. Applying a quadratic RRM to the full data set, h^2 increased to 0.17, showing RRM preferable in modeling longitudinal calf feeding traits due to step-wise calf weaning causing heteroscedasticity across time. A moderate negative genetic correlation (−0.39 and −0.45) between BRD and several traits (e.g., DMC and DS) and a correlation of −0.59 between DMC and NTT suggests AMF records have potential as indicators of BRD resilience in dairy calves. These preliminary results provide a better understanding of the genetic background of early in-life traits, which could be important for selecting animals for improved health.

Key Words: calf health, resilience

2623 DNA methylation of first exons negatively correlate with gene expression during *Staphylococcus chromogenes* subclinical mastitis.

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Staphylococcus chromogenes (SC), a common coagulase-negative staphylococci in dairy farms, causes subclinical mastitis. However, the host responses and regulatory mechanisms underlying SC subclinical mastitis (SCSM) are rarely reported. This study explored the DNA methylation status of first exons (MethFE) and their associations with gene expression (GE) during SCSM. Genomic DNA from milk somatic cells from 4 Holstein cows positive for SC and 4 healthy cows from the same Canadian dairy herd were subjected to whole-genome DNA methylation and RNA sequencing followed by bioinformatics and integration analyses. The MethFE was calculated for 16,209 genes by averaging the methylation levels of qualified CpGs, which was extremely low globally (~15%). Overall, a MethFE signature negatively correlated with GE at the whole-genome wide scale ($R = -0.235$, $P = 2.60 \times 10^{-192}$). Moreover, MethFE changes between SCSM and healthy cows also showed a significant negative correlation with GE changes ($R = -0.22$, $P = 9.89 \times 10^{-178}$). A total of 2956 genes were identified by Gaussian Mixture Module as having significant changes either in their MethFE and/or GE in SCSM ($P < 0.005$). A total of 836 genes having > 10% changes in MethFE and 2-fold changes or more in GE (referred to as DME-genes) were identified. About 78.35% of DME-genes had opposite changes in their MethFE and GE levels, including 394 DME-genes with higher MethFE and lower GE and 261 DME-genes with lower MethFE and higher GE in SCSM cows compared with control. The DME-genes were significantly enriched in 50 GO terms and 9 KEGG pathways related to cell migration and immune functions, including *Positive regulation of cell migration*, *Immune system process*, and *Cytokine-cytokine receptor interaction*, among others, suggesting their involvement in the regulation of the host defense against SC. In conclusion, the negative correlation of MethFE trend with global GE suggests the roles of DNA methylation and MethFE in the regulation of gene expression and consequently host responses during SCSM.

Key Words: milk somatic cells, integration analyses, immune functions

2624 Get test-day milk yields right: What have we learned?

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Milk recording is vital for herd management and genetic improvement in dairy cattle. Lactation yields are typically estimated from daily or test-day milk yields (DMY), with the latter calculated from partial daily milk yields using the correction methods developed primarily in the 1970s and 1980s. Recently, CDCB, USDA-AGIL, and the national DHIA initiated a joint effort to revamp the current methodology. Herein, we present a summary of the preliminary findings. An additive correction factor (ACF) model is comparable to a linear model that regresses TDMY on milking interval bins and relevant variables, but it assumes a fixed regression coefficient of 2.0 for single milkings. In contrast, linear regression models estimating these regression coefficients from the data provide more accurate DMY estimates than ACF models. Multiplicative correction factors (MCF) represent the ratios of daily to partial yields. Despite their different forms and statistical interpretations, various MCF models give similar DMY estimates. Overall, MCF models surpass ACF models in the accuracy of estimated DMY. The linearity assumption of the MCF current methods holds approximately true for cows milked 3 or more times daily, but not for those milked twice daily. In the latter cases, nonlinear models can enhance estimation accuracy. An exponential regression model has been proposed, yielding the most accurate DMY estimates. This model resembles an exponential growth function with a single milking yield as the initial state and the rate of change controlled by a linear function of milking interval time. However, a major concern with the current MCF methods is the loss of accuracy due to discretizing milking interval time into large bins. As an alternative, we demonstrate that it is feasible to derive MCF on a continuous milking time interval scale, which can improve the accuracy of estimated DMY.

Key Words: dairy cattle, exponential growth, milk yield

2625 Genetic analysis of lactation consistency using daily milk weights in US Holsteins.

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The ability of a dairy cow to perform similarly across time is an interesting trait to include in dairy cattle breeding programs aimed at improving dairy cow resilience. Consistency, defined as the quality of performing as expected each day of the lactation, could be highly associated with resilience, defined as animal's ability to maintain health and performance in the presence of environmental challenges, including pathogens, heat waves, and nutritional changes. A total of 21,326,257 total daily milk weights collected from 2018 to 2023 were provided for 96,697 first parity Holstein cows from 214 herds milked 3 times daily in conventional parlor systems throughout 30 states by Dairy Records Management Systems (Raleigh, NC). Consistency in milk production was measured from 0 to 305 d postpartum and computed as the log-transformed variance of daily deviations between observed and expected milk weights. Expected values were obtained using 3 nonparametric regression models: 1) LOESS regression with a 0.75 span; 2) polynomial quantile regression using the median (0.5), and 3) polynomial quantile regression using a 0.7 quantile. The statistical model included age at first calving as a fixed effect and herd-year-season and cow as random effects. Heritability estimates (standard errors) of consistency ranged between 0.154 (0.009) and 0.159 (0.009), demonstrating that consistency is a moderately heritable trait. Correlations among consistency traits were high (0.99), indicating that the model used to calculate consistency does not alter the ranking of PTAs. Genetic correlations between consistency traits and milk PTAs ranged from 0.38 to 0.39, while longevity traits included Productive Life (−0.21) and Livability (−0.26 to −0.25). Correlations between PTAs for log variance of daily milk yield and PTAs for early postpartum health traits ranged from −0.23 to −0.02, indicating that more consistent cows tended to have fewer health problems. Overall, our findings suggest that lactation consistency can be used to select animals that maintain expected milk production performance throughout the lactation.

Key Words: consistency, resilience, nonparametric modeling

2626 Milk fatty acid profiles of Holstein, ProCROSS, and Grazecross cows.

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Improving the milk fatty acid (FA) profile of milk from a human health perspective could open marketing opportunities for producers. The objective of this study was to compare FA profiles of Holstein (n = 49), ProCROSS (n = 170), and Grazecross (n = 98) cows at the University of Minnesota West Central Research and Outreach Center (WCROC). ProCROSS crossbreds are composed of the Holstein, Montbéliarde, and Viking Red breeds and Grazecross crossbreds are composed of the Jersey, Normande and Viking Red breeds. Total, de novo, mixed and preformed FA concentrations were provided by Minnesota DHIA (Buffalo, MN) from February 2021 to December 2022 test days. Eight test days per cow were required. For statistical analysis, fixed effects were breed group, parity (primiparous or multiparous), the interaction of breed group and parity, sire breed nested within breed group, herd (organic or conventional) and test date. Random effects were cow nested within breed group, DIM and error. Cows in the organic herd had lower ($P < 0.05$) de novo (0.90 g/100g) and higher ($P < 0.05$) preformed FA (1.51 g/100g) than cows in the conventional (0.96 and 1.38 g/100g, respectively) herd. The primiparous cows had higher ($P < 0.05$) total (3.93 g/100g) and preformed (1.53 g/100g) FAs compared with multiparous (3.70 and 1.36 g/100g) cows. The Grazecross cows had higher ($P < 0.05$) total FAs (3.93 g/100g) than ProCROSS (3.72 g/100g) cows, but were not different from Holstein cows (3.78 g/100g). For de novo FAs, Grazecross (0.98 g/100g) cows had higher ($P < 0.05$) concentrations than Holstein (0.91 g/100g) cows or ProCROSS (0.90 g/100g) cows. Grazecross cows (1.47 g/100g) had higher ($P < 0.05$) mixed FAs than ProCROSS (1.39 g/100g) cows and Holstein (1.43 g/100g) cows. Grazecross (1.48 g/100g) cows had higher ($P < 0.05$) preformed FAs than ProCROSS (1.42 g/100g) cows and Holstein (1.44 g/100g) cows. Dairy farmers may consider Grazecross cows to meet marketing interests for higher FA concentrations.

Key Words: milk fatty acids, crossbreeding, organic

2627 Effect of growth of organic calves and sire-breed on milk and component yield in later life.

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This study was designed to evaluate the effect of sire-breed on body weight (BW) and average daily gain (ADG) of organic calves and to evaluate the effect of calf BW and ADG on milk, fat, and protein yield in first lactation. BW and ADG were collected from 190 calves on 2 New York herds and 1 Minnesota herd. Sire-breeds of calves included Holstein (HO; n = 121), Jersey (JE; n = 22), Montbeliarde (MO; n = 16), Red Dairy Cattle (RDC; n = 16), and Other (n = 15). A total of 1185 BW measurements from birth to 6 weeks of age were available and 84 animals had 305-d first lactation records. BW at wk 1 (BW1), wk 6 (BW6) and ADG were evaluated with a model that included sire breed and herd. Mature equivalent milk yield (M305), fat yield (F305), protein yield (P305), and lactation average SCS were evaluated with mixed models that included herd, age at calving, and either BW1, BW6, or ADG as fixed effects with sire-breed as a random effect. Higher BW1 and BW6 were associated with higher M305 (regression coefficients (b) = 33.32 kg and 27.79 kg, respectively; $P < 0.01$) as was higher ADG (b = 1677.09 kg; $P < 0.05$). Regression on BW1 and BW6 was associated with increased F305 (1.08 kg and 0.78 kg, respectively; $P < 0.01$). Increased ADG, BW1, and BW6 were associated with P305 (b = 45.63 kg, b = 0.92 kg, and 0.76 kg, respectively; $P < 0.05$). Sire-breed was significantly associated with growth traits ($P < 0.01$). Average BW1 was 41.7 kg, average BW6 was 67.2 kg, and average ADG was 0.7 kg/d. MO calves were significantly heavier with higher BW1 (+5.2 kg), BW6 (+7.12 kg), and ADG (+0.06 kg/d) relative to average. Jersey calves had significantly lower BW1 (−6.93 kg), BW6 (−9.01 kg), and ADG (−0.06 kg/d) than all other breeds. In conclusion, MO sired calves were born larger and grew more rapidly than those of other breeds and higher within-breed BW and ADG were associated with higher producing first lactation heifers on organic farms.

Key Words: organic, calves, growth

2628 Identification of runs of homozygosity associated with male fertility in Brown Swiss cattle.

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Intensive selection for improved productivity has been accompanied by an increase in inbreeding rate and a reduction in genetic diversity. The increase in inbreeding tends to impact performance, specifically fitness-related traits, such as male fertility. Inbreeding can be monitored using pedigree information or genomic data. Alternatively, inbreeding can be quantified using runs of homozygosity (ROH), defined as contiguous lengths of homozygous genotypes observed in an individual's chromosome. The goal of this study was to evaluate the presence of ROH in Italian Brown Swiss cattle and assess its association with bull fertility. First, we evaluated the association between ROH and male fertility using 1,102 Italian Brown Swiss bulls with sire conception rate records and 567k SNPs spanning the entire genome. Second, we split the entire population into 100 high-fertility and 100 low-fertility bulls to investigate the potential enrichment of ROH segments in the low-fertility group. For each ROH segment, a one-tailed Fisher's exact test using a 2 × 2 table was performed. Finally, we mapped the significant ROH regions to the bovine genome to identify candidate genes that may affect male fertility in cattle. Notably, there was a negative association between bull fertility and the amount of homozygosity (regression coefficient $\beta = -0.008$, t-value = −4.3, P-value ≤0.01). Four different ROH regions located in chromosomes 6, 10, 11, and 24 were significantly overrepresented in low-fertility bulls (Fisher's exact test, P-value <0.01). Remarkably, these 4 genomic regions harbor many genes, such as *DPF3*, *7SK* and *WDR7*, that are related to sperm biology and male fertility. Overall, our findings suggest that inbreeding and increased homozygosity have a negative impact on male fertility in Brown Swiss cattle. The quantification of ROH can contribute to minimize the inbreeding rate and avoid its negative effect on fitness-related traits, such as male fertility.

Key Words: inbreeding depression, homozygosity, service sire fertility

2629 Identification and validation of functional candidate genes associated with pregnancy status in Holstein dairy cows.

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Challenges in selecting for improved fertility traits in dairy cattle exist, due to the polygenic nature of reproductive traits. Validation of key regulatory functional candidate genes associated with establishment of pregnancy in dairy cattle is needed to better understand cattle fertility and offer genetic markers for selection for reproductive traits. Our objective was to identify and validate functional candidate genes associated with early pregnancy status. We used 82 Holstein dairy cows which were enrolled in a pre-synchronized timed artificial insemination (AI) program at 35 ± 3 d in milk. Endometrial biopsies and uterine lumen swabs were collected from non-pregnant (NP; $n = 18$) and pregnant (P, based on embryo recovery or detection of IFN-tau in uterine fluid; $n = 18$) lactating dairy cattle, 15 d after AI (77 ± DIM). Differential gene expression (DEG) analysis using RNA-Sequencing was conducted and reads were annotated to the ARS-UCD1.2 assembly using CLC Genomics Workbench. An optimized RNA-Sequencing pipeline was used to identify functional variants within the DEG fixed in P or NP groups. Functional analysis including identification of biological processes and network analysis was conducted using STRING and the GALLO R package. We found 90 significantly DEG ($FDR < 0.05$, $FC \geq |2|$) between NP and P cows. Enriched QTL analysis showed that DEG were positioned within reproduction type QTLs. Functional analysis of the DEG revealed the most significant biological processes including response to biotic stimulus, immune effector process, and innate immune response. The 90 DEG associated with P and NP will be further validated by identifying overlapping functional variants (SNP and INDEL) and vaginal microbial profiles that may affect gene function, and therefore prioritize the key regulatory genes. The identified key regulatory genes and further validation analysis will provide insight into the genetics of reproductive function and may improve selection for pregnancy establishment in dairy cattle.

Key Words: RNA-sequencing, functional candidate genes, endometrium

2630 Genetic ancestry and admixture of *Bos taurus* and *Bos indicus* genotypes for African dairy production systems.

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African dairy production is based on local breeds with poor genetic potential for milk production. The introduction of exotic breeds increases milk production. However, exotic breeds have lower disease resistance and low heat stress tolerance, which reduces their milk production and exposes them to death. The study examined variations in genomic ancestor estimation for *Bos taurus* and *Bos indicus* dairy cattle breeds. 1645 animals were genotyped with the 777K Bovine Bead Chip. The principal components analysis (PCA) and linkage disequilibrium (LD) analysis were done using Plink 1.9 and R program 4.2.1. Admixture Software was used for admixture analysis. We used an additive model for PCA to identify the first 10 principal components for 25 dairy cattle breeds with 1645 individuals. The PCA analysis showed a high divergence between *Bos taurus* and *Bos indicus* dairy cattle. The admixture analysis result indicated that most individuals came from different ancestors. The LD visualization showed a very high LD value when the markers were very close to each other (<100 cM), but it decreased as the distance between markers increased. Generally, the genetic ancestry study showed a high divergence between *Bos taurus* and *Bos indicus* dairy cattle breeds. The African dairy breeds were clustered into 2 main groups. The project will generate improved and adaptive high-performing Holstein and Gyr animals to accelerate the genetic improvement of dairy animals in Africa, establish genomic tools to enhance the genetic modification of African dairy cattle phenotype, get regulatory approvals for using genetically improved animals in target countries, and commercial those lines for a stable breeding program to the sustainable improvement of African dairy production.

Key Words: admixture, *Bos taurus*, Africa

2631 Genetic trend partitioning in dairy sheep.

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The genetic progress can be understood as the cumulating positive Mendelian samplings from ancestors, selecting at each generation positive Mendelian samplings from current generation candidates to selection and passing on to descendants. This study decomposed the genetic gain for milk yield of 2 French dairy sheep breeds (Lacaune (LAC) and Basco-Béarnaise (BB)) in Mendelian samplings (MS) by categories of animals defined by sex and by selection pathways, and explored long-term genetic contributions. The decomposition of genetic trend into MS contributions by sex or categories showed that females play an important role in selection schemes. Across generations, females contribute to genetic progress as much as the males (BB) and even more (LAC) to the final genetic gain. We interpret that, even if males are more heavily selected, half their genetics come from their dam, whose positive MS are eventually selected and spread (often, through AI males) throughout the population. In addition, we computed long-term contributions from each individual to the following pseudo-generations (one pseudo-generation spanning 4 years). With this information, we studied the selection decisions (selected/not selected) for females, and the contributions to the following generations. The MS was more important than Parent Average to determine the selection of individuals and their long-term contributions. Long-term contributions were larger for AI males (with larger progeny sizes than females) and in BB than in LAC (with the latter being a larger population). The difference on the relative importance of the MS and Parent Average between breeds across generations could reflect different selection strategies in practice. The MS relies on within-family selection, while Parent Average is more related to across family variation.

Key Words: genetic gain, Mendelian sampling, long-term contributions

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2632 The relationship between heifer growth and cost of production. S. Gehrett, C. Yost, C. Becker*, and T. Beck, *Pennsylvania State University, University Park, PA.*

This study aims to determine how heifer production costs influence the dairy breakeven cost of production and how heifer growth differences impact the cost of raising a heifer from birth to first calving. Heifer growth (n = 544) and financial data from 20 organic (O) and 20 conventional (C) dairies in Pennsylvania was collected. Hip heights and body weights were recorded at 4 different time points, birth, weaning, pre-breeding, and freshening. Producers in the project completed a cash flow plan and a year-end financial analysis using FINAN. This included budgeting of crops and dairy cow, and heifer enterprises. Farm financial and heifer growth data have been averaged and summarized by profit group (high, medium, low) for 2021. The high-profit group heifers had the greatest average daily gain (ADG) for the first 2 measuring points. Conventional heifers in the high-profit group averaged 2.34 lbs/d, and O heifers averaged 1.85 lbs/d from birth to weaning. From weaning to pre-breeding, C heifers had a 2.15 lbs/d ADG and O heifers had a 1.89 lbs/d ADG. Compared with the low-profit C heifers with an ADG of 1.67 lbs/d and the O heifers with 1.75 lbs/d from birth to weaning. From weaning to pre-breeding, the low-profit C heifers had an ADG of 2.01 lbs/d, and the O heifers with a 1.65 lbs/d ADG. The dairy cow and heifer cost of production (COP) for C farms ranged from $14.77 to $25.92 per day. The COP to raise a C heifer from birth to first calving ranged from $1,489 to $3,789, with the average at $2,235. Dairy and heifer COP for O farms ranged from $22.69 to $37.81 per day. The heifer COP for O farms ranged from $1,082 to $4,384, averaging $2,149. Feed cost per day greatly contributed to the difference in heifer cost per day. With the data collected from this project, improved management strategies for heifer nutrition, housing, and health programs that can address the problems that contribute to a higher heifer COP will be shared.

Key Words: heifer, cost of production, growth

2633 Stakeholder engagement in developing the *Mooving Cows* learning tool. J. Van Os*[1], N. Cook[2], D. Ledesma[3], R. Cradock[4], O. Abraham[5], and M. Brauer[6], [1]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI,* [2]*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI,* [3]*Division of Extension, University of Wisconsin, Madison, WI,* [4]*University of Wisconsin Survey Center, Madison, WI,* [5]*Social and Administrative Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI,* [6]*Department of Psychology, University of Wisconsin, Madison, WI.*

Inappropriate cow handling negatively affects animal welfare and productivity and increases the risk of human and animal injury. Our objective was to engage stakeholders in developing an innovative learning tool for dairy farm staff to practice appropriate cow handling. *Mooving Cows* is a digital educational game played on touchscreen tablets. Players practice moving cows in simulated dairy farm environments. The learning objectives of the game are to understand how human actions affect cow behavior, stress, productivity, and worker safety. The content was informed by a multidisciplinary team with expertise in cow behavior, health, and welfare, human behavior, educational games, extension education, and linguistic and cultural relevancy. Using a prototype with a freestall pen scenario, we engaged stakeholders to inform the game development process and ensure relevance for our target audience of cow-handling staff on dairy farms. Impartial facilitators conducted 9 focus groups with 34 participants, grouped by role and language. A pilot group (n = 4) comprised university animal handling trainers. On campus, 3 groups (n = 10) were held with farm owners, bilingual consultants, and veterinarians (all in English). On 2 dairy farms, 5 groups were held with cow-handling staff (e.g., milkers; n = 20), separated by language (3 vs. 2 groups in Spanish vs. English) and supervisory roles. During 2 h, participants played the game prototype and then shared their impressions in discussions on the relevance of the handling scenario, challenges they encountered, and ideas to improve the game, with facilitators using a semi-structured interview guide. The discussion recordings were professionally transcribed then evaluated by the research team. Themes were classified into 3 categories: actionable feedback to improve the game, ideas for additional scenarios, and non-actionable reflections on real-life cow handling. The actionable feedback was incorporated into Version 1.0 of the full game. Further stakeholder engagement is planned to collect feedback to further improve the game and pilot-test its effectiveness for increasing knowledge of cow handling best practices.

Key Words: animal handling, stockmanship, simulation

2634 Hands-on learning to stimulate the next generation of dairy employees. J. A. Spencer*[1], J. Pineiro[1], R. Hagevoort[2], M. Berry[1], L. Jenschke[1], and B. Boyd[1], [1]*Texas A&M AgriLife Extension, Stephenville, TX,* [2]*New Mexico State University, Clovis, NM.*

The dairy industry continuously faces labor shortages, and youth (≤25) are disconnected from agriculture. As the dairy industry experiences technological advancements, there is a need to recruit younger employees with a greater understanding of technology directly from high school or secondary education. Texas A&M and NM State Extension, and TX dairy producers have formed 2 dairy youth workforce programs: Junior Dairy Consortium (JDC) and Dairy Honors Program (DHP). These programs aim to educate high school students (Jr./Sr.) about the dairy industry and careers through hands-on activities on a dairy. The objective of this study was to determine if JDC and DHP would increase students' knowledge and stimulate interest in pursuing a dairy career. Retrospective surveys were distributed after programs to assess change in dairy knowledge and career interest, and response rates were 84% (JDC) and 90% (DHP). The JDC 30-question survey assessed students perceived change in understanding of dairy and interest in dairy careers after the program. A *t*-test was used for JDC to analyze the independent variables of grade level (Jr./Sr.), race (white/other), gender (M/F), and residence (farm/other) using SAS 9.2 (SAS Inst. Inc. Cary, NC), significance was $P < 0.05$. Descriptive statistics are reported for the 11-question DHP survey. Since 2019, 268 students have participated. Results from JDC (n = 79) showed 79% of students increased their knowledge of dairy, and 74% were definitely or probably pursuing a dairy-related career. These results were not influenced by grade, race, or residence ($P > 0.05$); however, more female students would consider a career after higher education ($P < 0.01$). The DHP survey (n = 158) demonstrated the value of hands-on activities with an 85% excellent rating compared with 50% excellent classroom ratings. Also, DHP surveys showed that 88% would recommend it to others and 62% would definitely or probably consider a dairy-related career. These results indicate that JDC and DHP youth workforce programs engage students with hands-on activities, increase students' dairy industry knowledge, and may inspire future dairy employees.

Key Words: youth workforce development, dairy education, employee recruitment

2635 The impact of farm tours on public knowledge and perception of dairy farming. A. M. C. Smid*[1], H. W. Barkema[1], S. Roche[2], W. Ruiter[1], B. Traub[1], and B. A. Ventura[3], [1]*University of Calgary, Calgary, Alberta, Canada,* [2]*ACER Consulting, Guelph, Ontario, Canada,* [3]*University of Lincoln, Lincoln, Lincolnshire, United Kingdom.*

Events such as 'Breakfast on the Dairy Farm' (BOTDF) are frequently used to educate the public about dairy farming, with the assumption that education will improve public perception of dairy farming. However, education does not necessarily lead to improved public perception, as people's views are shaped by factors beyond information. Therefore, we investigated the effects of different communication approaches (i.e., one-way education vs conversational style) by farmers on public knowledge and perception of dairy farming in Canada. Dairy farmers (n = 30) were trained to deliver one of the 2 conversation styles during farm tours at BOTDF events (n = 3) in Alberta. People attending the events were invited to take part in before- and after-tour surveys to assess a potential shift in peoples' knowledge and perception of dairy farming. A total 308 people filled out both surveys; 160 people also filled out a third survey 2 weeks after their tour. Overall, visitors' performance scores on a knowledge 'quiz' about dairy farming increased by 30% after the farm tour, regardless of communication approach. A total 41% of participants had become more positive in their perceptions toward dairy cow quality of life immediately after the tour, whereas 8% became more negative, with no differences between communication methods. However, when comparing peoples' perceptions after the farm tour with 2 weeks after the event, 27% had become more negative in their perceptions about dairy cow quality of life. The most frequent concerns focused on cow-calf separation and the lack of outdoor access. As public education does not necessarily lead to improved public perception of dairy farming it may be important for the dairy industry to create non-judgmental spaces for open dialog to build better relationships between dairy farmers and the public. These conversations may help the dairy industry determine what factors are important for a socially sustainable dairy industry.

Key Words: questionnaire, social sustainability, public perception

2636 From farmer to farmer: Case-based learning as an evidence-based approach to improving dairy cattle welfare. J. Saraceni*[1], D. Kelton[2], D. Renaud[1,2], D. Haley[2], T. DeVries[2], K. Barrett[3], and S. Roche[1,2], [1]*ACER Consulting, ACER Consulting, Guelph, ON, Canada,* [2]*The University of Guelph, The University of Guelph, Guelph, ON, Canada,* [3]*The Ontario Association of Bovine Practitioners, The Ontario Association of Bovine Practitioners, Fergus, ON, Canada.*

Peer learning has been well documented as influential on behavioral change. The objective of this project was to combine farmer success stories with summaries of relevant scientific literature to provide farmer perspectives and evidence-based tools to support farmers in improving dairy cattle welfare across Ontario. A narrative literature review was done to synthesize the scientific literature on preventing, controlling, and treating injuries and lameness. Key evidence-based recommendations were prepared for a producer audience. Data from proAction were used to identify farmers that had significantly improved hock, knee, and neck injury prevalence and/or lameness in their herd. These farmers were invited to participate in an interview to discuss their approach to improvement. Interviews explored producer attitudes to the assessment, mindset, and approach to developing corrective actions, and the strategy, cost, and impacts associated with change. A total of 7 interviews were conducted, recorded, and turned into podcasts and/or written case studies as KTT resources for other farmers; the podcasts included key messages from the scientific literature. Producers were generally aware of their on-farm issues ahead of their assessment and were motivated to strive for continuous improvement on their own merit, with assessments having little influence on their actions to change. While some farmers noted significant changes (e.g., building a new barn), others mentioned smaller changes that had significant positive impacts on their farm (e.g., new stall mats). Positive improvements in health and production demonstrated a return on investment for most farmers. Participants noted that expensive investment was not always required and that smaller changes of lesser cost could improve health and welfare with strong farm management. Advisors and other farmers often played a key role in the action planning process. Improved animal health and welfare was motivating for producers, with many seeing an improvement in their animals as encouragement to look for new solutions and areas of betterment.

Key Words: lameness, behavioral change, knowledge translation and transfer

2637 Key performance indicators associated with housing and management characteristics of Canadian dairy herds. L. Solano*, D. Warner, S. Adam, and D. E. Santschi, *Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

Milk recording data can provide a dynamic picture of a herd's life cycle and status. Differences in management can be linked to herd performance measured by Dairy Herd Improvement data. Our objective was to investigate associations between management and housing practices common to high and low performing herds, as related to key indicators of longevity, transition success and herd management. A survey of 2,202 herds in Québec and New Brunswick, Canada was conducted from April 2020 to January 2021. Statistical analyses were performed using R (version 4.1). For preliminary univariable analyses, the top 10% were compared with the bottom 10% of herds for each key indicator using a Chi-squared frequency test. Study herds housed lactating cows in tiestalls (83%) and freestalls (17%); the latter with a milking parlor (10%) or an automatic milking system (7%). Preliminary results suggested that in terms of longevity (i.e., determined by the percentage of cows in 3+ lactations), the top 10% of herds (mean of 57%) offered milking cows access to pasture or an exercise pen (57/303; $P \leq 0.004$). Regarding transition success (i.e., percentage of cows with a positive Transition Cow Index; an indicator of the effectiveness of a herd's transition cow program), the top 10% of herds (mean of 11%) housed milking and transition cows in barns with a soft lying surface (133/337; $P < 0.001$), had stall alleys with grooved concrete or rubber (125/326; $P < 0.001$), and had a smooth manger surface (121/321; $P < 0.001$). Finally, in terms of herd management index (i.e., a 0–100 score for the optimal use of genetic potential based on standardized milk), the top 10% of herds (scored 90–100) housed milking cows in barns with a soft (mattress, deep-bedded) lying surface (120/319; $P \leq 0.003$); had stall alleys covered with rubber flooring (95/315; $P < 0.001$); provided 16–18 h of lighting with an intensity of >200 lx (120/213; $P < 0.001$); had a smooth manger surface (metal, ceramic, or plastic; 115/310; $P < 0.001$), and had hoof trimming performed by a professional ≥ 3 times per year (123/306; $P \leq 0.014$).The differences found between high and low performing herds provide a basis for tailoring recommendations rather than applying a blanket approach.

Key Words: benchmarking, cow comfort

2638 Bridging the research-practice gap in lameness: A multistakeholder view to inform knowledge uptake. L. Solano*[1], D. E. Santschi[1], A. M. Smid[2], H. Ganshorn[2], and D. Weary[3], [1]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*University of Calgary, Calgary, Alberta, Canada,* [3]*University of British Columbia, Vancouver, BC, Canada.*

Implementing changes to improve lameness in dairy herds is difficult, even when backed by scientific evidence. Dairy stakeholders view lameness as an important issue, but have differing views on how industry practices, programs, and policies might help. We conducted 1) a narrative review and 2) focus group sessions. The aim of this combined methodology was to identify gaps between the published literature and to identify perspectives of industry stakeholders that may inform the development of extension activities focused on lameness control. The objective of the narrative review was to summarize evidence on herd-level management practices associated with lameness. Four databases (Medline, CAB Abstracts, Scopus, and ProQuest Dissertations) were searched for peer-reviewed literature published in English since 2000. Search results were imported into Covidence systematic review software. Inclusion criteria for studies were: North American, primary source; analytic observational or experimental design; management practices applied at herd level. Our search yielded 1691 articles, from which 42 were retained. Practices associated with lower lameness were the use of deep and dry bedding, sand bedding, high-traction flooring, reduced time spent standing while waiting to be milked, time from lameness diagnosis to treatment and access to outdoor areas. The objective of the focus group sessions was to explore stakeholders' views of actions required to reduce lameness and barriers and motivators to doing so. Two homogeneous (producers) and 3 heterogeneous (hoof trimmers, veterinarians and advisors) focus groups, each with 6–11 participants (n = 52), moderated by 2 trained facilitators (LS; AS), were conducted in Alberta and Québec, Canada. Discussions were audio recorded, transcribed, and thematically analyzed. Preliminary thematic analysis revealed perceived barriers, motivators and potential solutions to adopting methods of lameness control. Participants perceived that a thorough understanding of causes of lameness was generally a challenge. Detection and treatment were viewed as hampered by the lack of a designated person for managing lameness: "No one owns lameness." Implementation of deep/sand bedding were perceived as costly and time consuming. Key actions identified by participants included: 1) supporting producers in the development design and use of better handling systems and equipment, allowing for improved cattle handling and in-house hoof trimming; and 2) tools and services to improve decision making regarding which treatment and risk mitigation strategies to apply. The combination of evidence from our literature review, and the results of our thematic analysis of transcripts from multi-stakeholder focus groups, provides a basis for tailoring extension strategies to reduce lameness on farms.

Key Words: qualitative methods, knowledge adoption, lameness

2639 Addressing on-farm antimicrobial drug use practices through a community of practice-based approach: A case study. B. Karle*[1], R. Busch[2], C. Meehan[2], and M. Smith[2], [1]*University of California Agriculture and Natural Resources, Orland, CA,* [2]*University of California, Davis, CA.*

Innovative approaches that seek to understand and acknowledge individuals' values, social trust, and personal experience are needed to effectively empower decision makers on farm. Communities of practice (CoPs) are structured networks of peers and represent a model for professional development whereby groups of individuals work toward shared goals. Learning within CoPs occurs within the context of social relationships with other members of the CoP who have similar, if not identical, issues and concerns. The objective of our project was to implement a community of practice-based approach to serve as a transferable model to help influence farm employee decision-making behavior change toward judicious use of antimicrobials on participating farms. We implemented 2 CoPs on a 1,900-cow California dairy: one focused on maternity and calf management and one focused on the hospital string. Members of the project team served as facilitators for a series of 6 meetings with employees responsible for each of the targeted areas on the farm. Each meeting focused on current challenges and successes in each of the management areas and employees were encouraged to take the lead to guide the informal discussion. The maternity and calf CoP had 9 participants and reported increased confidence in their ability to share ideas and improved communication between shifts by the end of the facilitated CoP meeting series. Challenges in implementing protocols and biosecurity practices were addressed and a new method for identifying high-risk calves by marking the calf's head with a colored paint stick was implemented after the second CoP meeting. The hospital CoP had 6 members and identified communication as a challenge, especially when implementing treatment protocols. Improved methods for marking sick cows with leg bands and identifying treatments were implemented as a result of the CoP. In the short term, no changes in antimicrobial use were reported, but CoPs, especially when implemented as short, direct meetings may be an effective method to improve employee well-being and engagement on dairies.

Key Words: community of practice, antimicrobial stewardship

2640 Development of a bovine continuing education program for early-career veterinarians to address clinical service shortage. A. Abuelo*[1] and S. Mann[2], [1]*Michigan State University, East Lansing, MI,* [2]*Cornell University, Ithaca, NY.*

There is currently a shortage of production animal veterinarians worldwide. Access to adequate continuing education (CE) increases the retention of healthcare workers in rural areas. However, little information is available about the preferences of bovine veterinarians in their first years after graduation. Our objective was to develop a readily available CE program tailored for recent veterinary graduates working in bovine medicine. For this, we conducted a needs assessment and subsequently designed a CE program based on these results. We conducted a national cross-sectional survey including open- and close-ended questions to identify early-career bovine veterinarian needs and their preferences to access CE conducted while practicing full-time. a multi-pronged approach was used for distribution, including professional listservs and attendees at meetings. Subsequently, we conducted a second online survey to triangulate their responses with the opinions of experienced veterinarians, where the experienced veterinarians ranked the relevance of the CE topics that resulted from the early-career veterinarian survey using a 3-point Likert scale. We received 132 valid responses from US bovine practitioners in their first 5 years after graduation, and 32 responses associated with bovine veterinarians with 10 or more years of experience. Our results showed that a combination of distance education and workshops to practice hands-on skills was the most preferred method for accessing CE among early-career veterinarians. Similarly, a commitment of 1–2 h/wk to distance education was the most preferred

option by recent graduates. From the list of 20 topics identified by early-career veterinarians, the experienced veterinarians ranked “calf/heifer management” and “immunology and vaccinology” as the most relevant ones for practice. With the information gathered from these surveys, we designed, implemented, and piloted a 180 h CE program on bovine health management with 40 participants. The program is delivered over 2 yr through asynchronous distance education and annual hands-on workshops. Participant feedback has been very positive. Overall, the survey results will serve to develop CE programs targeted to bovine veterinarians in their first years of practice. Future research is needed to evaluate this program’s success in retaining the bovine veterinary workforce within the US, particularly in rural underserved areas.

Key Words: dairy production medicine, professional development, veterinary practitioner shortages

Joint Growth and Development and Physiology and Endocrinology Symposium and Platform Session: From Fetus to Weaning— The Microbiome and Its Impact on Immune Development

2641 Gut-microbiome-organs system axes: The role of short-chain fatty acids and probiotics. S. P. Lerner*, *Chr. Hansen Inc., Milwaukee, WI.*

There is a large and continually increasing body of evidence that the gut microbiota plays an essential role in the physiologic health and mental well-being of humans, animals, and birds. Via actions that are dependent upon an initial production of short-chain fatty acids (acetate, butyrate, and propionate) in the intestinal tract of their hosts, the microorganisms of the microbiota indirectly, but significantly, impact the function of a multitude of organs systems, including, but not limited to, the digestive, pulmonary, hepatic, adipose, and central nervous systems. There is tremendous evidence from studies of germ-free and humanized mice that healthful benefits can be transferred to unhealthy individuals by transplanting the intestinal microbiota from a healthy donor. In production animal model systems, fecal transplantation from healthy donor sows and the use of microbiota-altering probiotic supplements have been shown to significantly ameliorate the actions of industry-plaguing respiratory and reproductive viruses and thereby reduce mortality and morbidity in pig flows. Finally, we've seen that the most successful individuals in a pen of broiler chickens had a demonstrably greater proportion of short-chain-fatty-acid-producing microorganisms in their microbiota compared with that found in the least successful individuals in the same pen. With the ever-present push to improve the efficiency of food production and adopt sustainable agricultural solutions, it is incumbent upon us to gain a better understanding of the remarkable role played by a balanced and robust microbiota in achieving those objectives.

Key Words: microbiota, short-chain fatty acids, probiotics

2642 Tyndallized *Lactobacillus helveticus* supplementation improves gut structure and function in dairy calves around weaning. M. F. Olmeda*[1], L. R. Cangiano[1,3], C. Villot[2], E. Chevaux[2], B. K. McNeil[1], T. J. DeVries[1], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Lallemand Animal Nutrition, Blagnac, France,* [3]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objective of this study was to evaluate the impact of tyndallized *Lactobacillus helveticus* supplementation on gut structure and function of Holstein bull calves exposed to an abrupt weaning model, consisting of a stepdown from milk replacer (MR) over 4d (d35–42). A total of 44 newborn bull calves were randomly assigned to 1 of 2 treatments: Control (CON, n = 22) fed a premix containing a carrier with no para-probiotic, and tyndallized *Lactobacillus helveticus* (TLH, n = 22) fed a premix providing 2×10^9 cfu/d/calf of TLH. Calves received 6 L/d of MR (150 g powder/L) split over 3 meals, which was increased to 9 L/d one week after arrival. Treatments were added to the MR at morning and evening feedings at a rate of 2.5 g of premix/feeding and fed until d42. Starter feed was offered ad libitum starting on d28. On d43, a subset of 19 calves were euthanized. Intestinal tissues were sampled at the proximal and distal jejunum, and the ileum to evaluate the surface area (SA). Blood samples were taken on d35, 42, 49, and 56 to evaluate serum amyloid A (SAA) during and after the weaning period. Gut permeability was measured on d34 and 40 by analyzing blood samples taken during 10 h of orally dosed lactulose, D-mannitol, and chromium-EDTA markers. Data were analyzed using a generalized linear mixed model in SAS with fixed effect of treatment, day, its interaction, and the random effect on group. There were no changes observed in the intestinal tissue SA with TLH supplementation ($P = 0.84$). A treatment by day interaction was observed in serum SAA concentrations ($P = 0.03$), wherein TLH had lower concentrations of SAA on d35, 42, and 49. No differences in gut permeability markers were detected between treatments, but the Lactulose:Mannitol ratio was lower and lactulose and chromium AUC were higher for the postweaning period compared with preweaning (1.02 vs. 1.26 µg/mL, $P < 0.01$; 60.36 vs. 68.40 µg/mL, $P = 0.05$; 2356 vs. 2925 mg/mL, $P < 0.01$ on d34 and 40, respectively). In conclusion, the abrupt weaning model increased intestinal permeability, and TLH administration may help regulate the inflammatory response following weaning.

Key Words: gut permeability, inflammation, weaning

2643 Immunomodulation strategies to control respiratory disease in preweaned calves. J. L. McGill*, *Iowa State University, Ames, IA.*

Bovine respiratory disease is the second leading cause of preweaned heifer mortality, and the leading cause of weaned heifer mortality. Young calves are highly susceptible to infection due to their immature immune system. Although maternal immunity protects calves in the early weeks after birth, this protection is not always effective against respiratory pathogens. As maternal immunity wanes, the calf's own adaptive immune system is naïve, leaving them highly susceptible to infection. The incidence of bovine respiratory disease has remained static for the last several decades, despite the widespread availability of therapeutics and vaccines, underlining the need for improved interventions strategies. The innate immune system is appealing as a target for novel therapeutic or preventative strategies. It is broadly specific and can therefore provide protection against multiple pathogens. The innate immune system acts quickly, responding within minutes to hours, rather than the days required by the adaptive immune system. In very young calves, the adaptive immune system may not be fully matured; however, the innate immune system is active and primed for protection. This talk will discuss our recent work on development of strategies to enhance innate immune function in young calves, including in-feed supplements such as phytogenic additives and postbiotics, nutrition approaches and the development of intranasal or systemic immunostimulant treatments to prime the innate immune system in calves during periods of high risk.

Key Words: bovine respiratory disease, innate immunity, preweaned calves

2644 IgG transport kinetics and histological features in the postnatal bovine intestine are maximized during very early life. R. Hiltz*, D. Vine, D. R. Barreda, and A. H. Laarman, *University of Alberta, Edmonton, Alberta, Canada.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Bovine calves are immunologically naïve at birth, requiring passive immunity transfer through colostral immunoglobulin (Ig) absorption. Knowledge regarding location and kinetics of Ig transport is limited. This study focused on the effect of time and colostrum feeding on IgG vesicle abundance and IgG transport kinetics in bovine small intestine within 24 h of life. Holstein-Angus calves (n = 36) were assigned to one of 5 harvest times in the postnatal period (1.5, 6, 12, 18, or 24 h) and were either fed colostrum (6C, 12C, 18C, and 24C) or not fed (1.5NC and 24NC). At harvest, a blood sample was taken for serum IgG analysis; segments of duodenal, proximal and distal jejunal, and ileal tissue were sampled and preserved for histology analysis, via periodic-acid-Schiff stain, or mounted in Ussing chambers containing Kreb's buffer. Mucosal buffer was spiked with 120 mg/mL IgG and 10 mg/mL biotinylated IgG (B-IgG); serosal appearance of B-IgG was measured for 4 h. Tissue was analyzed for PAS-positive vesicle density and size. Statistics were analyzed using a 2-way (Ussing flux, histology) or a one-way (serum IgG) ANOVA in R (version 4.2.2) with a Tukey post-hoc adjustment. Serum IgG peaked at 12C (1,314 mg/dL IgG, $P < 0.001$) and remained constant at 18C and 24C. B-IgG flux was highest for 6C calves in all tissue segments except proximal jejunum (duodenum 11.4 ± 9.85; prox. jejunum 0.755 ± 12.8; dist. jejunum 18.6 ± 8.52; ileum 13.2 ± 4.17mg/mL/hr/cm^2). Distal jejunum had the highest absorption of any tissue, and was highest in 6C calves (6C 18.6 ± 8.52; 12C 3.22 ± 6.19; 18C −0.43 ± 4.46; 24C 5.45 ± 5.05; 24NC 4.41 ± 4.94 mg/mL/hr/cm^2). Histologically, vesicle density was highest in 12C calves ($P < 0.001$; 6hr 9.59 ± 0.42, 12hr 13.58 ± 0.59, 18hr 4.3 ± 0.46, 24hr 7.8 ± 0.46, 24NC 1.2 ± 0.31 vesicles/villi); maximal vesicle density was in the jejunum ($P < 0.001$; duodenum 0.8 ± 0.19, prox. jejunum 9.17 ± 0.40, dist. jejunum 11.1 ± 0.48, ileum 8.02 ± 0.37 vesicles/villi). Vesicle size increased in fed calves ($P < 0.001$) and tended to increase with postnatal time ($P = 0.055$). The B-IgG flux indicated maximal IgG absorption occurred at 6 – 12hrs of life; increased vesicle number indicated IgG absorption was highest in the jejunum sections of the small intestine.

Key Words: colostrum, immunoglobulin G absorption, neonatal calf

2645 Early-life microbiome: Modulator of immunity and health. N. Malmuthuge*, *Agriculture Agri-Food Canada, Lethbridge, AB, Canada.*

The pioneer microbiota colonizing the neonatal gut primes the developing immune system and stimulates long-term immune memory. The immune memory stimulated by the gut microbiota during neonatal period (birth to weaning) can only be restored if interventions are done before weaning. In addition, the neonatal gut microbiota has recently been linked to immune responses generated following immunization, suggesting that early-life microbiota is vital for the active and passive immunity of the host. Therefore, microbial perturbations (dysbiosis) during early life lead to neonatal infections and long-term microbiome-linked pathologies. In calves, the diversity and density of gut microbiota are affected by various factors such as the calving method, colostrum and milk feeding, antibiotics, water feeding, and housing. Moreover, variations in the gut microbial communities have been linked to enteric infections in neonatal calves. Omics-based studies mining host-microbe interactions in the gut at the molecular level reported that beneficial microbiota such as lactobacillus and bifidobacteria are associated with the expression of genes and regulatory molecules (microRNA) involved in immune responses in calves. These studies together suggest that pioneer microbiota play a vital role in modulating immunity and health in calves. As a result, microbial interventions have become one of the highly sought-after areas in livestock research, including dairy calves. Prebiotics, probiotics, and fecal matter transplantation/fresh rumen liquid inoculant are popular interventions tested in neonatal calves. However, our understanding of the impact of such interventions on immune priming and immune memory is lacking in calves. Therefore, an in-depth understanding of the immuno-modulatory role of the early-life microbiome is essential to improve the health of calves by supporting the establishment of healthy/beneficial gut microbiota.

Key Words: microbiome, immunity, neonates

Joint CSAS (Canadian Society of Animal Science) and ADSA Production, Management, and the Environment Symposium: Mitigation Strategies to Achieve Dairy Net Zero

2646 Towards a net zero dairy future in Canada. F. Jackson*, *Dairy Farmers of Canada, Ottawa, ON, Canada.*

Dairy farmers are motivated to run their farms in the most efficient and sustainable way possible—to succeed as a business, and because it's the right thing to do. The DFC's recently launched net-zero goal aligns with the Canadian government's commitment to achieve net-zero GHG emissions across Canada by 2050 and is also aligned with many business and global commitments such as Pathways to Dairy Net Zero led by the Global Dairy Platform. The presentation will review DFC's most recent life cycle analysis, the strategy toward net zero and their recently launched best management guide.

Key Words: Dairy Net Zero, greenhouse-gas emissions, life cycle analysis

2647 Could nutrition have a meaningful impact on reducing the carbon footprint of milk? A. N. Hristov*, *The Pennsylvania State University, University Park, PA.*

Depending on the production system, the carbon footprint of milk (CFM) has been estimated at as low as 0.75 to as high as 1.21 and even over 5.0 kg of CO_2 equivalents/kg energy-corrected or fat- and protein-corrected milk, with the general trend being for lower CFM in intensive vs. extensive production systems. The allocation of the major greenhouse gases (GHG) to emission sources (i.e., enteric fermentation, manure management, feed production) also depends on the production system and varies among and within regions. Consequently, the role of nutrition in GHG and CFM mitigation would, to a large extent, depend on the production system. A recent comprehensive analysis of published data recommended several nutritional strategies that can reduce absolute or relative (per unit of product) enteric methane emissions in ruminants by 12 to 32%. It can be estimated that in extensive, pasture-based dairy production systems where $\geq 80\%$ the GHG can be from enteric fermentation, a 30% reduction in enteric methane emissions, with an effective feed additive such as the methane inhibitor 3-nitrooxypropanol, would result in 25% overall reduction in CFM; for comparison, in intensive dairy production systems (where the share of enteric methane emissions may be <50% of the total GHG), the effect of that same mitigation practice on CFM would be about 13%. Limited research has shown additivity of the mitigation effect of some nutritional strategies; if confirmed (these interactions need to be further studied), simultaneous implementation of 2 or more practices may increase the reduction in CFM from the above examples to 40 and 20%, respectively. Important questions that have not been adequately addressed are the persistence of the effect of nutritional GHG mitigation strategies over full lactation or multiple lactations and the effects of diet on manure composition and GHG emissions. In conclusion, nutritional approaches alone can have a significant mitigation impact on CFM, but that impact can be considerably greater if they are integrated, particularly in intensive dairy production systems, with manure- and animal management-related mitigation practices.

Key Words: carbon footprint, milk, greenhouse gas

2648 Accelerating the discovery, regulatory approval, and adoption of feed additives that reduce enteric methane emissions from livestock. J. W. McFadden*, *Cornell University, Ithaca, NY.*

Methane is a climate pollutant that contributes to global warming. In 2021, the Global Methane Pledge set a target to reduce global methane emissions 30% by 2030, relative to 2020 levels, to limit global warming to 1.5°C by 2030, while enhancing public health and agricultural productivity. Methane from livestock agriculture represents ~40% of global methane emissions. Although cattle are part of a natural carbon cycle, reducing enteric methane production from ruminants using feed additives has potential to lower global methane emissions, while enhancing the conversion of dietary energy to meat or milk production. To define efficacy and safety of methane-reducing feed additives, we must consider impacts on enteric and manure methane and nitrous oxide emissions, nutrient digestibility and partitioning, rumen fermentation and microbiome, N retention, health and fertility of the animal, and meat and milk composition. Long-duration feeding trials are needed to ensure animal safety and consistent efficacy over time. If methane reduction fades with time, we must consider co-supplementation or replacement strategies with alternatives to ensure persistence. Our understanding of mode of action, especially for plant-based additives, is poor but necessary to define. We must do better to define methane reduction within the framework of energetic utilization in different planes of nutrition, physiological states, and environments. Because no one additive is likely to be a global solution, we must ramp up studies that examine how additives reduce enteric methane emissions across different production systems. Attention needs to focus on alternative modes of delivery for such approaches. Methodological standards that validate enteric methane monitoring and efficacy are required. In parallel, we must scrutinize technology through proper life cycle assessment and evaluate scalability. Enhancing efficiency in the developing world cannot be ignored. To meet this call for action, we must encourage international engagement, infrastructure development, academic-industry partnerships, and consumer education efforts.

Key Words: feed additive, livestock, methane

2649 The future of breeding programs: Redefining sustainability. C. M. Richardson*[1], J. J. Crowley[2], and P. R. Amer[2], [1]*AbacusBio International Ltd., Edinburgh, UK,* [2]*AbacusBio Ltd., Dunedin, New Zealand.*

Reducing emissions is vital to improve sustainability and industry leaders have set emission goals to either reduce gross emissions, lower emissions intensity, or reach net-zero. However, additional traits should also be measured and compared in terms of their impact on the broader definition of sustainability. In addition to environmental impact, a sustainable breeding objective must consider profit, animal welfare, farmer well-being, and social responsibility. Traits to be considered include direct emissions (e.g., nitrogen and methane), production efficiency (e.g., feed efficiency, growth), closer to biology reproduction and fertility (e.g., oestrous strength and semen quality), health (e.g., calf and transition cow health) and welfare traits (e.g., polled). Many of these novel traits require labor intensive or expensive phenotyping, resulting in small data sets and low reliability estimated breeding values. Opportunities exist to overcome this limitation by utilizing international collaboration to combine international, developing inexpensive and easy-to-measure proxy traits, and expanding novel phenotype reference population using female driven reference population and young stock and males. Non-economic values can be estimated that quantify the impact a trait has on societal perspective (e.g., farmer preference) or environmental impact (methane emissions), and combined with economic weights to calculate aggregate weights for each trait. While validation techniques are still uncertain, the United Nations Sustainable Development Goals may be applied to determine the improvement in sustainability due to genetic selection. This approach allows for various perspectives of sustainability, such as in the developed vs developing world, to be considered. Number and quality of relevant phenotypes are currently the main limiting factor. As confidence continues to grow in the opportunity to improve sustainability through genetic selection, substantial new investment will be required both in phenotyping activities, but also into novel breeding structures and scheme designs that can maximize the value and impact of these phenotype.

Key Words: genetics, sustainability, environment

2650 Development of genomic evaluation for methane efficiency in Canadian Holsteins. H. Oliveira[1,2], S. Narayana[1], A. Fleming[1], H. Sweett*[1], S. Shadpour[3], F. Malchiodi[4], J. Jamrozik[1], G. Kistemaker[1], P. Sullivan[1], F. Schenkel[3], B. Van Doormaal[1], C. Baes[3,5], and F. Miglior[1,3], *[1]Lactanet Canada, Guelph, ON, Canada, [2]Purdue University, West Lafayette, IN, [3]University of Guelph, Guelph, ON, Canada, [4]Semex, Guelph, ON, Canada, [5]University of Bern, Bern, Switzerland.*

Rising methane emissions and their impact on the global temperature change has become an increasing concern. Although the dairy industry is not the sole source of increasing global methane emissions, it has the potential to mitigate this increase and contribute to climate cooling. In 2016, methane was the largest contributor to the milk carbon footprint at 48%, mainly due to enteric fermentation. Dairy cattle produce, on average, 150 kg of methane per lactation and these emissions can represent a loss of 4% to 7% gross energy intake for the animal. As such, using genetics to select for cows with reduced methane emissions is a strategy that can combat global warming and improve the efficiency of the dairy industry. In April 2023, Lactanet launched genomic evaluations for Methane Efficiency using mid-infrared (MIR) spectroscopy data. Research based out of the University of Guelph using a machine learning algorithm has shown great accuracy of predicting individual animal methane emissions for milk-recorded cows using milk MIR spectral data. Lactanet developed methane predictions using methane data collected from research herds in Canada through 2 research projects, the Efficient Dairy Genome Project and the Resilient Dairy Genome Project, and milk spectral data collected via our milk recording services. Predicted methane had a genetic correlation with collected methane of 0.85 and a heritability of 0.23 (0.01). Lactanet's genomic evaluation for Methane Efficiency was developed using a 4-trait single-step genomic evaluation for predicted methane for the Holstein breed, including milk, fat and protein yields as energy sinks. Methane Efficiency is defined as genetic Residual Methane Production in 120–185 DIM of the first lactation, independent of Milk, Fat and Protein via a linear regression approach. The average reliability of Methane Efficiency for genotyped young bulls and heifers is over 70%. Methane Efficiency is an important selection tool, allowing dairy producers to achieve an expected 20% to 30% reduction in methane emissions from their herd by 2050, without negatively affecting production.

Key Words: methane emissions, genomic evaluation, single-step evaluation

2651 How to mitigate methane and ammonia emissions at the farm level with innovative approaches. P. J. Galama* and A. Kuipers, *Wageningen Livestock Research, Wageningen, Gelderland, the Netherlands.*

Complying with intensive societal discussions, the Dutch Dairy chain has set goals for 2030 to increase the sustainability on the topics of climate, welfare, grazing, biodiversity, environment, new business model and land based farming. Also, a coalition of several Dairy organizations have set management goals about dilution of manure, grazing and protein in ration together with the Ministry of Agriculture to reduce the nitrogen losses, especially ammonia emission. The potential to reduce ammonia and greenhouse gas emissions with these management measures and investment in housing systems like floor types to separate feces and urine, daily removal of manure from the barn, different freewalk housing systems, Cow-toilet and air extraction systems will be shown. The challenge is to design a cow barn that improves animal welfare, manure quality and reduces emissions. These indicators were studied in case control studies with groups of 16 cows at research station Dairy Campus. The Cowtoilet is an automatic urinal that cows use voluntarily in a concentrate feeder. It collects 35% of the urine production and reduced the ammonia emission by around 35- 45%. A permeable plate on a slatted floor improves the walkability of the cows and collects all the urine underneath the floor. The ammonia emission can be reduced between 35 and 50% by acidification of the urine, flushing the plates with the urine or by spraying 20 L water per cow per day on the floor in combination with a urease inhibitor. A freewalk housing system with woodchips bedding material decreased the ammonia emission with 32% but did increase methane emission with 30%. A new development is a freewalk system with sand bedding that separates the urine by drains at the bottom of the bedding. The feces are picked up by a bedding cleaner behind the tractor. The data of 12.000 dairy farmers using the Annual Nutrient Cycle Assessment tool (ANCA) were analyzed and show the importance of fertilizing, feeding and housing systems on the emissions of ammonia and greenhouse gasses. It illustrates that low emissions of ammonia can go hand-in-hand with low emissions of greenhouse gasses.

Key Words: dairy, emissions, housing

2652 The key role of forage and manure management to achieve net-zero targets. J. Gamble*, *USDA-ARS-PSRU, St. Paul, MN.*

Dairy forage and manure management directly influence on-farm biogenic carbon (C) storage and emissions and are therefore key considerations for achieving net-zero greenhouse gas (GHG) emissions. However, in-field changes in biogenic C such as soil organic C (SOC) or flux-based net ecosystem C balance (NECB) are not commonly or consistently included in C footprint or life cycle GHG inventories of agricultural systems. This presentation will discuss the impacts of forage crop and manure management on changes in SOC and NECB in the Upper Midwest US; examine how critical C cycling feedbacks between manure and soil impact these outcomes; and highlight the importance of including these changes into broader assessments of GHG emissions and soil sustainability.

Key Words: net-zero, manure management, forage

2653 Embracing the challenge: Net zero and beyond. G. Dick*[1,2], *[1]Dicklands Farms, Chilliwack, BC, Canada, [2]Dicklands Biogas LP, Chilliwack, BC, Canada.*

Last year Dairy Farmers of Canada (DFC) unveiled a goal to reach net-zero greenhouse gas (GHG) emissions from farm-level dairy production by the year 2050. In this presentation we will discuss one farm's steps toward reaching this target. Topics discussed will included increasing efficiency through genetic progress and nutrition. An overview of the development, design, planning and construction of an integrated biogas and nutrient recovery facility. On farm opportunities for conversion to other energy sources from diesel. Introduction of a new barn design for the capture of methane from enteric fermentation. As well as on farm carbon capture methods and sequestration options. This presentation will show that to go beyond Net Zero there are many, many steps to be taken. However, with careful planning and an understanding of the end goal it can be achieved.

Key Words: net-zero, greenhouse gas emissions, barn design

Reproduction Platform Session: Epigenetic Impacts on the Next Generation of Dairy Cows

2654 Long-term transcriptomic and epigenetic effects of in vitro embryo production in dairy calves. M. B. Rabaglino*, *School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

In cattle, the in vitro production (IVP) of embryos can induce undesirable epigenetic modifications in the conceptus, altering the developmental programming of specific organs during the fetal stage and impacting the postnatal calf's physiology. Even when IVP calves are born apparently healthy and phenotypically similar to calves produced in vivo, they might have underlying aberrations in organs at the molecular level. Our studies aimed to determine epigenomic and transcriptomic deviations in crucial organs of healthy IVP male dairy calves compared with in vivo-produced calves, i.e., after multiple ovarian stimulations and embryo transfer (MOET). Tissue samples from the hypothalamus, pituitary, gonadal, and adrenal glands (HPGA axes), liver, and semitendinosus muscle were obtained from 3 mo old male calves born after IVP or MOET (n = 4 per group). In addition, blood samples were obtained at birth and 3 mo of age from the same animals to proxy methylation of the internal organs. The gDNA and RNA molecules were extracted from the samples and sequenced through whole-genome bisulfite sequencing and RNaseq, respectively. Data were analyzed by bioinformatic pipelines using the R software to identify differentially methylated cytosines and differentially expressed genes (FDR <0.05) in IVP versus MOET samples and to integrate both omics data. Results indicated an early activation of the HPG axis and an altered hepatic and muscular energy regulation in phenotypically normal IVP calves compared with MOET calves. Furthermore, we identified a set of biomarkers that allowed the prediction of the embryo origin based on the epigenomic profiles from blood or buffy coat samples, even when the latter were obtained at birth. In conclusion, the IVP process introduces long-term molecular modifications that persist in the endocrine and metabolic organs of the postnatal calf. Future studies can also help to evaluate the implications of these findings in practical assisted reproductive technologies based on IVP of cattle embryos.

Key Words: fetal programming, system biology multi-omics, reproductive technologies

2655 Associations of uterine luminal fluid composition with subsequent success of preimplantation conceptus development. G. Madureira*[1], B. Mion[1], J. F. W. Spricigo[1], E. Ticiani[1], M. R. Carvalho[1], J. V. Bishop[2], T. R. Hansen[2], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Biomedical Sciences, Colorado State University, Fort Collins, CO.*

Our objectives were to investigate potential differences in uterine luminal fluid (ULF) composition at late diestrus between lactating cows that successfully developed or not an elongating conceptus in the subsequent estrous cycle. Cows (n = 88) had their estrous cycle synchronized and received a transcervical flushing of the uterine horn ipsilateral to the CL with 30 mL of PBS on d 14 of the cycle (59 ± 3 DIM). After flushing, cows received an injection of $PGF_{2\alpha}$, followed 3 d later by GnRH and timed AI. On d 15 after AI, the uterine horn ipsilateral to the CL was flushed with 50 mL of PBS for diagnosis of pregnancy based on detection of IFN-τ in the ULF using an in-house immunoassay. Eleven cows diagnosed pregnant (PREG; IFN-τ positive) were paired with 11 cows that failed to become pregnant (NPREG; IFN-τ negative) for retrospective comparison of ULF composition of the first sample. Analyses of composition included the investigation of 168 primary metabolites, 501 complex lipids, and 69 oxylipins. Data were analyzed by ANOVA using MetaboAnalyst 5.0 and SAS OnDemand. Statistical models included the effect of pregnancy category and parity. No differences in primary metabolites (saccharides, free fatty acids, amino acids and derivatives) were identified. The total amount of oxylipins in ULF did not differ between PREG and NPREG cows (187 *vs* 185 ± 76 nM/L) but the concentration of 4 individual oxylipins (19,20-DiHDPE, 13S-HODE, 12,13-EpOME, 15,16-DiHODE) were 80–107% greater ($P \leq 0.04$) in PREG than in NPREG. As for complex lipids, the total concentration tended ($P = 0.07$) to be lower in PREG compared with NPREG (37.5 *vs* 57.1 ± 8.0 µg/mL), which was mostly explained by differences ($P = 0.05$) in concentration of sterols (5.5 *vs* 10.4 ± 1.7 µg/mL). For individual molecules, 34 were differently abundant in ULF between PREG and NPREG cows. Ten of those, mostly glycerophospholipids, were 75–123% more abundant in NPREG cows. The remaining 24 lipids, mostly triacylglycerols, were 74–100% more abundant in PREG cows. In conclusion, composition of lipids but not of primary metabolites in ULF at late diestrus were associated with subsequent success of preimplantation conceptus development in lactating dairy cows.

Key Words: fertility, pregnancy

2656 Nerve growth factor-β supplementation for in vitro fertilization and maturation media improve cleavage rates in bovine embryos. T. Marques*[1,2], M. Silva[1], I. Macedo[1], S. Martin-Pelaez[1], A. De La Fuente[3], S. Meyers[3], P. Dini[1], and F. Lima[1], [1]*Department of Population Health and Reproduction, University of California, Davis, CA,* [2]*Federal Institute Goiano, Rio Verde, Goias, Brazil,* [3]*Department of Anatomy, Physiology, and Cell Biology, University of California, Davis, CA.*

Nerve Growth Factor-β (NGF) is a seminal plasma protein that improves embryo development in rabbits, sheep, mice, pigs, and cattle when added to maturation media. Further improvements in sheep were shown when NGF was supplemented in the fertilization media. Our objective was to determine the effects of adding 0, 10, 100, and 1000 ng/mL of NGF to fertilization (IVF-10, IVF-100, and IVF-1000) or maturation medium (IVM-10, IVM-100, and IVM-1000) on blastocyst rate and quality. Dulbecco's phosphate-buffered saline at 150 m*M* was added to 0, 10, and 100. Abattoir-derived bovine ovaries were used for in vitro embryo production. Cumulus-oocyte complexes (COC) (n = 1,025) were selected and divided among the groups in each replication (n = 7), placed in maturation medium for 22h, and incubated at 38.8°C, 5.6% CO_2 in humidified atmospheric air (21% O_2). After the maturation, COCs were denuded partially, transferred to the fertilization medium, and co-incubated with frozen-thawed semen prepared with a final concentration of 2×10^6 spermatozoa/mL at the same conditions of maturation. After 18–21h, presumptive zygotes were transferred into the culture medium (BO-IVC, IVF Bioscience), overlayed with oil, and incubated for 7 d at 38.8°C in 6% O_2, 5.6% CO_2, and 88% N_2, according to the manufacturer's instructions. Fertilization day was considered d 0, and zygotes were kept in culture until d 9. The cleavage rate was assessed after 72h post-fertilization. The blastocyst rate was evaluated on d 7, and the hatching rate was determined on d 8 and 9. Data were analyzed using the GLIMMIX procedure of SAS. The cleavage rate increased ($P < 0.05$) in IVF-1000 (87.6%), followed by IVM-1000 (69.9%), IVF-100 (64.7%), IVM-100 (63.2%), IVF-10 (57.9%), IVM-10 (54.1%), and Control (49.0%). However, NGF revealed no effects on blastocyst rate on d 7 ($P = 0.65$) or hatching rate on Day 8 ($P = 0.77$) and Day 9 ($P = 0.41$). These results suggest that a dose-dependent effect of NGF is only present for cleavage rate, and no differences exist after that stage of development. In vitro fertilization or in vitro maturation supplementation of NGF for bovine embryos was not different in the current study for any stage of development.

Key Words: oocyte competence, blastocyst rate, hatch rate

2657 Genetics causes of bovine male reduced fertility: Environment and genetics meeting in the aryl hydrocarbon receptor gene. R. Raz[1,2], Z. Roth[2], A. Komsky-Elbaz[2], D. Kalo[2], and M. Gershoni*[1], [1]*Institute of Animal Sciences, Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel,* [2]*Department of Animal Sciences, Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University, Rehovot, Israel.*

Breeding programs for farm animals have led to significant progress in many livestock traits. However, such progress was not achieved for fertility-associated traits. This is likely due to the high heterogeneity of these traits, which involves multiple environmental and genetic variables. Typically, breeding indexes include female but not male fertility. Thus, identifying markers predicting male fertility is expected to facilitate improvement in overall herd fertility. Here, we aimed to discover genetic causes in bulls' sub-fertility. We reduced the subfertility etiological heterogeneity by producing and analyzing high-throughput phenotypic and genomic data. First, we identified bulls that presented significantly reduced conception rates; then, bulls (n = 20 Holstein) underwent genotype-based kinship analysis (nSNP = 50K) and clustered according to their familial affiliation. This analysis revealed that the cohort of subfertile bulls is mainly composed of 4 familial clusters. Thereafter, we performed an in-depth semen physiology analysis to identify the precise semen fertilization malfunction. Measurements included acrosome activity, sperm membrane integrity, mitochondrial membrane potential, sperm genomic stability, and the response of sperm to oxidative stress (representing environmental stressors). The analysis pointed to one family cluster (n = 4) in which all the bulls present a similar aberrant phenotype of significantly higher ROS production upon exposure to oxidative stress compared with the control ($P = 0.008$, n(control) = 51, MW 2-tail test). These 4 bulls are progenies of the same sire and were found to share a similar cellular response might suggest a similar genetic etiology. In support of this, a whole genome sequence analysis (WGS) of the subfertile and control bulls identified a polymorphism (Average MAF ~2.5%, n = 262; MAF ~4% in Holstein, n = 109) in the gene Aryl Hydrocarbon Receptor (AHR) which carried by all the affected bulls (n = 4) and was absent in control (n = 14). AHR is a transcription factor that enables the adaptation of cells to cellular metabolism and environmental changes. Similar to our findings, polymorphism in human AHR is associated with men's infertility and seminal oxidative stress. Therefore, it is suggested that, upon validation, the AHR allele could serve as a marker for bull fertility.

Key Words: fertility, genetics, Aryl Hydrocarbon Receptor allele

2658 Characterization of genomic predicted transmitting ability of females according to their reproductive efficiency as heifers and cows. I. Avalos-Rosario*, A. P. Silva, G. Madureira, B. Mion, M. R. Carvalho, and E. S. Ribeiro, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objective was to characterize the genomic predicted transmitting ability (GPTA) for production and reproduction traits in females categorized according to their reproductive efficiency as heifers and as first lactation cows. Pubertal heifers (n = 814) were categorized based on pregnancy success as 1) highly fertile (HF; pregnant at the first breeding); 2) moderately fertile (MF; pregnant at the second or third breeding); or 3) subfertile (SF; pregnant at fourth or later breeding). Heifers diagnosed as pregnant were followed up to 305 DIM in their first lactation. Reproductive performance of cows was classified with the same criteria used for heifers, and 4 groups of interest were formed: females with HF as heifer and HF as cow (HH group; n = 151); 2) females with HF as heifer and SF as cow (HS group; n = 84); 3) females with SF as heifer and HF as cow (SH group; n = 72); and 4) females with SF as heifer and SF as cow (SS group; n = 35). Genomic PTAs were obtained from a commercial panel. Data were analyzed by ANOVA using the GLIMMIX procedure in SAS. The LSMEANS statement was used for pairwise comparisons. Compared with HH females, HS females had lower GPTA for daughter pregnancy rate (DPR; 1.30 *vs* 1.70; $P = 0.03$), cow conception rate (CCR; 1.95 *vs* 2.43; $P = 0.02$), net merit (NM; 408 *vs* 444; $P = 0.04$), and productive life (PL; 4.13 *vs* 4.65; $P < 0.01$). No differences were found in GPTAs for heifer conception rate (HCR), milk, fat, and protein. Compared with SS females, SH females had lower GPTA for milk (627 *vs* 882; $P = 0.02$) and protein (24.7 *vs* 31.9; $P = 0.02$). No differences were observed in GPTAs for DPR, HCR, CCR, NM, PL, and fat. Comparing the extreme groups, HH *vs* SS respectively, differences were observed in DPR (1.70 *vs* 1.21; $P = 0.05$), milk (633 *vs* 883; $P = 0.01$), and protein (25.4 *vs* 31.9; $P = 0.02$). In conclusion, important differences in GPTAs were observed between groups of females with distinct fertility patterns. Reduction in reproductive efficiency during the heifer to first lactation cow period seemed to be associated with fertility traits. Nonetheless, improvements in reproductive efficiency during the heifer to first lactation cow period seemed to be associated with production traits.

Key Words: fertility, genetics

2659 Long-term consequences of postpartum inflammation on ovarian biology and preimplantation conceptus development in healthy cows. B. Mion*[1], G. Madureira[1], J. F. W. Spricigo[1], M. R. Carvalho[1], E. Ticiani[1], O. B. Pascottini[2], F. Peñagaricano[3], S. J. LeBlanc[2], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Our objectives were to evaluate the associations between postpartum concentrations of haptoglobin in serum and measurements of ovarian and early pregnancy biology in healthy cows. Postpartum cows were evaluated for incidence of clinical disease and serum haptoglobin. Cows with clinical disease were excluded and the remaining were classified as high (HHp) or low (LHp) haptoglobin based on the following cutoffs: 0.8 g/L on d 0, 3, 7, and 0.4 g/L on d 10 and 14 relative to calving. In study 1 (n = 182), we compared the interval from calving to resumption of ovarian cyclicity, the likelihood of estrus detection after Presynch (56 ± 3 DIM), and the characteristics of estrous behavior. In study 2 (n = 48), we compared the uterine luminal fluid (ULF) composition at late diestrus (59 ± 3 DIM). In study 3 (n = 97), we compared the sizes of preovulatory follicle (68 ± 7 DIM) and resulting corpus luteum (CL), concentrations of progesterone in plasma, and transcriptome of d 15 conceptuses. In study 4 (n = 105), we compared the expression of interferon-stimulated genes (ISGs) in peripheral blood leukocytes (PBL) on d 19 after AI. Data were analyzed using SAS OnDemand and MetaboAnalyst, with models accounting for the effects of group and parity. The HHp cows took longer ($P = 0.03$) to resume ovarian cyclicity (AHR = 0.7; 95% CI = 0.51–0.97) but no differences were observed in estrus detection and behavior, sizes of follicle and CL, or plasma progesterone. Composition of complex lipids and oxylipins in ULF were similar between groups, but HHp cows had reduced concentration of nonessential amino acids (fold changes: 0.46–0.53, $P \leq 0.05$) and glycerol (fold change: 0.51, $P = 0.04$). Differentially expressed genes (n = 185) indicated that VEGF and integrin signaling, GATA6 and NOTCH activities were inhibited in conceptuses of HHp cows. In multiparous cows only, ISGs responses to pregnancy in PBL were 1.4 to 2.8-fold lower in HHp than in LHp. The differences in ovarian cyclicity and early pregnancy biology reported above support the hypothesis that systemic inflammation is partly responsible for the long-term effects of postpartum clinical disease on reproductive biology of dairy cows.

Key Words: health, fertility

Ruminant Nutrition 7: Lipids

2660 Interaction of pretrial milk fat production and dietary fat supplementation on milk and milk fat yield in Holstein cows. Y. Adeniji*, R. Bomberger, and K. Harvatine, *Department of Animal Science, The Pennsylvania State University, University Park, PA.*

Fat supplements are fed to increase milk and milk fat yield, but the response may depend on cow specific factors. Our objective was to investigate the effect of pretrial milk fat production on response to a saturated fat supplement. The hypothesis was that fat supplementation would increase milk fat yield more in cows with higher pretrial milk fat yield. Twenty-seven multiparous and 20 primiparous lactating Holstein cows (DIM = 116 ± 10 and 189 ± 24, respectively) were separated into high (Prim = 1.73 ± 0.12 and Mult = 2.21 ± 0.48 kg/d) and low (Prim = 1.53 ± 0.26 and Mult = 1.52 ± 0.22 kg/d) groups based on pretrial milk fat yield and assigned to treatment in a crossover design with 28-d periods and a 7-d washout. Treatments were a low-fat (LF) diet and a high-fat (HF) diet that included a prilled free FA supplement at 2% of DM (36.9% C16:0, 38.5% C18:0, 8.86% C18:1). Data were analyzed with JMP Pro 16, and the model included random effects of cow, period, sequence, and the fixed effects of parity, fat yield category, treatment, and their interactions. Increasing dietary fat had no effect on milk yield but tended to increase dry matter intake by 0.72 kg/d ($P = 0.05$). The HF diet increased milk fat concentration similarly in both parities (3.40 vs 3.63%, $P < 0.001$). There was an interaction of treatment and pretrial production for milk fat percent with a 0.26% unit increase in low and only a tendency for a 0.20 unit increase in the high group ($P = 0.05$). The HF diet increased milk fat yield 95 g/d across all groups, but the increase was greater in primiparous than multiparous (130 vs 66 g/d) and was greater in the low group (132 vs 58 g/d). The HF diet increased yield of 16 C FA in the low group ($P < 0.01$) and reduced FA < 16 C in the high group ($P = 0.01$). The low group and primiparous cows had higher *trans-10* C18:1 in milk fat ($P < 0.01$) and was reduced by the HF diet. Milk protein concentration and yield decreased with the HF diet ($P < 0.05$) but milk urea nitrogen was not affected. In conclusion, fat supplementation increased fat yield of cows with lower pretrial fat yield more than cows with higher milk fat yield.

Key Words: dietary fat, milk fat yield, dairy cows

2661 Production responses to fatty acid supplementation are impacted by fatty acid profile rather than form of the supplement. A. M. Bales*[1], M. L. Miller[1], J. de Souza[2], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

We determined the effects of fatty acid (FA) profile vs the form of a FA supplement on production responses of post-peak dairy cows. Twenty multiparous Holstein cows (44.3 ± 3.00 kg/d of milk; 99 ± 23 DIM) were randomly assigned to treatment sequences in a replicated 4x4 Latin square design with 21-d periods. Treatments were a non-FA supplemented control diet (CON) and 3 diets incorporating FA supplements at 2.0% DM of total FA of 1) blend of FA supplements to achieve a ratio of 70% C16:0 + 20% C18:1 using FA prills and Ca-salts (FAB), 2) a Ca-salt containing 70% C16:0 + 20% C18:1 (SLT), and 3) a Ca-salt of palm FA distillate containing 55% C16:0 + 35% C18:1 (TRD). The 3 FA treatments replaced soyhulls in the CON diet. Diets contained similar (%DM) NDF (29.8%), forage NDF (18.4%), starch (28.6%), and CP (17.3%). The statistical model included the random effect of cow within square and the fixed effects of treatment and period. Pre-planned contrasts included CON vs the average of the 3 FA treatments (FAT), the form of the FA supplement (FAB vs SLT), and the FA profile of the Ca-salt (SLT vs TRD). Results in the text are presented in the following order: CON, FAB, SLT, and TRD. Overall, FAT increased 3.5% FCM (48.2, 49.0, 49.2, 49.5 kg/d; $P = 0.01$) and milk fat yield (1.72, 1.76, 1.79, 1.75 kg/d; $P = 0.01$) but decreased DMI (31.0, 30.9, 30.4, 29.4 kg/d; $P = 0.02$) and milk protein yield (1.54, 1.49, 1.48, 1.50 kg/d; $P < 0.001$). There was a tendency for SLT to increase milk fat yield ($P = 0.08$) compared with FAB, but there were no other differences in production responses for FAB vs SLT ($P > 0.33$). Compared with SLT, TRD decreased DMI ($P = 0.02$) and tended to decrease milk fat yield ($P = 0.07$), but increased milk yield ($P < 0.001$) and tended to increase milk protein yield ($P = 0.08$). In conclusion, overall FAT increased yields of milk and milk fat but decreased DMI. There were no differences between the 70% C16:0 + 20% C18:1 supplement fed as a blend or a Ca-salt. However, a traditional Ca-salt decreased DMI compared with the 70:20 Ca-salt, indicating that the FA profile of a FA supplement is more important than the form.

Key Words: calcium salt, oleic acid, palmitic acid

2662 The short-term effect of increasing doses of palmitic and stearic acid on milk fat production in Holstein cows. A. N. Staffin* and K. J. Harvatine, *Penn State University, University Park, PA.*

Fat supplements high in palmitic and stearic acid are commonly fed to dairy cows but there is limited data directly comparing these fatty acids (FA) at different dose levels. The objective was to compare the short-term effect of feeding palmitic and stearic acid at increasing doses on milk fat production. The hypothesis was that cows supplemented with palmitic acid would have a greater increase in milk fat yield at lower doses compared with stearic acid and that both FA would reach a plateau within the doses tested. Twelve second lactation Holstein cows (106 ± 31 DIM) were arranged in a replicated 3x3 Latin square design with a dose escalation design within period and a ≥ 10 d washout between periods. Treatments included a no-supplement control (CON), a FA supplement high in palmitic acid (PA; > 80% palmitic) and a FA supplement high in stearic acid (SA; 80% stearic and 10% oleic). The FA supplements were fed at increasing doses every 4 d targeting 0g, 150g, 300g, 500g, and 750g/d. Milk samples were taken on d 3 and 4 of each dose and were composited within dose level according to milk yield. Data were first analyzed by ANOVA with preplanned contrasts testing CON vs PA, CON vs SA, and PA vs SA at each dose level and second by random regression. Both analyses included the random effects of cow and period and the fixed effects of treatment, dose, and their interaction. PA progressively increased milk fat yield and concentration compared with CON and was higher than control at 500g of supplementation ($P = 0.03$, +132g/d for yield and $P = 0.003$, +0.37% for concentration) and greater. Stearic acid increased milk fat yield compared with CON only at 750g ($P = 0.03$, +138g/d). Neither PA nor SA modified milk yield compared with CON or each other and milk fat yield also did not differ between PA and SA. By regression analysis, there was a treatment by dose interaction for both milk fat yield ($P = 0.008$) and concentration ($P < 0.001$) where PA increased at a faster rate than SA or CON. In conclusion, PA increased milk fat yield over CON at lower doses compared with SA, but neither FA treatment reached a maximal response with the doses tested.

Key Words: lactation, nutrition, fatty acid

2663 Effects of increasing dietary palmitic acid inclusion on production by lactating Holstein cows. A. M. Dickerson*[1], L. Garcia[1], F. C. Cardoso[1], J. Albrecht[2], O. R. Drehmel[2], C. Soderholm[2], W. P. Hansen[2], J. R. Loften[2], M. F. Scott[2], and J. K. Drackley[1], *[1]University of Illinois, Urbana, IL, [2]Milk Specialties Global, Eden Prairie, MN.*

The optimal dietary inclusion rates of palmitic acid (C16:0) and stearic acid (C18:0) when oleic acid (C18:1) is held constant are unknown. Lactating Holstein cows (n = 20; 117 ± 21 DIM) were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced for carryover effects. Treatments were a basal diet with no added fat (CON), or the basal diet supplemented with 2% (DM) fatty acid supplements containing 9% C18:1 and 35% C16 (PA35), 50% C16 (PA50), 65% C16 (PA65), or 80% C16 (PA80), with the remainder being mostly C18:0. Each treatment period lasted 21 d with an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). Cows were milked 2 times daily at 0400 and 1600 h with milk weights recorded for each milking. Milk samples were collected at each milking on d 20 and 21 of each treatment period and composited by day. Data were analyzed using the MIXED procedure of SAS. Contrasts were CON vs fat supplementation and the linear, quadratic, and cubic effects of increasing C16:0. Significance was $P < 0.05$, trends were $P < 0.10$. As the C16 inclusion rate increased, PA65 produced the largest milk yield (37.9 ± 1.08 kg/d), although the quadratic effect did not reach significance ($P = 0.108$). The FCM increased linearly ($P = 0.004$) as palmitic acid increased. Fat yield increased linearly ($P < 0.01$); PA50 and PA65 had the greatest fat yield compared with CON (1.59 each vs 1.40 ± 0.74 kg/d; $P < 0.01$). Fat percentage increased linearly ($P < 0.01$), with PA65 having the largest fat percent compared with CON (4.34 vs 3.95 ± 0.14%; $P = < 0.01$). No differences were observed for DMI ($P = 0.75$). Increasing palmitic acid inclusion rates in fat supplements for Holstein cows improved milk yield, fat content, fat percentage, and FCM with no effects on DMI. The optimal short-term supplemental inclusion rate for C16:0 was between 50 and 65%, with 9% C18:1 and the remainder C18:0. Further research is needed to determine the long-term effects on DMI, production, body condition, health, and reproduction.

Key Words: fat supplementation, production, intake

2664 Effect of palmitic acid supplementation and a milk fat depressing diet on milk production, fatty profile, and polar metabolites. C. Matamoros*[1,2], F. Hao[2], A. D. Patterson[2], and K. J. Harvatine[1], *[1]Department of Animal Science, The Pennsylvania State University, University Park, PA, [2]Center for Molecular Toxicology and Carcinogenesis, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA.*

Milk fat yield can be decreased by diet-induced milk fat depression (MFD) and increased by dietary palmitic acid (PA) supplementation. Production responses to these diets are well characterized, but little is known of their effect on minor metabolites in milk. The objective of this study was to characterize the effect of MFD and PA supplementation on milk production, fatty acid (FA) profile, and polar metabolites. Twelve Holstein cows were used in a 3 × 3 Latin square design with 21-d experimental periods. Treatments were a control diet (17% CP, 32% NDF, and 27% starch), a high PA supplement at ≈2% DM of the control diet (88% PA), and a diet to induce MFD (17% CP, 26.5% NDF, 33.5% starch, and 2.2% soybean oil). Data were analyzed with a mixed model with the fixed effect of treatment and random effect of cow and period, and means were separated using a protected LSD. There was an effect of treatment on dry matter intake ($P < 0.001$), where MFD increased intake by 3.0 kg/d compared with control, and PA diet did not differ from control. There was no effect of treatment on milk yield ($P = 0.53$). Milk fat concentration and yield increased by 0.30 percentage units and 160 g/d with the PA supplement, and MFD decreased milk fat by 1.35 percentage units and 520 g/d ($P < 0.001$). The MFD diet decreased the concentration of milk FA <16 C and 16 C FA by 41 and 20% and increased the concentration of >16 C FA by 40% compared with control ($P < 0.001$). The PA supplement increased the concentration of milk 16 C FA by 40% and decreased <16 C FA by 16% compared with control ($P < 0.001$). The MFD diet altered the concentration of citric acid cycle (TCA) intermediary metabolites in milk, a significant source of NADPH for de novo lipogenesis; interestingly, citrate increased by 57% and succinate and α-ketoglutarate decreased by 27% and 66%, respectively, compared with control ($P < 0.001$). In contrast, the PA supplement had no effect on any TCA intermediary metabolites. The PA supplement and MFD resulted in the expected changes in milk fat yield and FA profile and modified milk polar metabolites that may reflect the changes in lipogenic pathways.

Key Words: lipids, metabolomics, citrate

2665 Effects of oleic and palmitic acids levels in a fat supplement on milk production in lactating dairy cows. S. L. Burtnett*[1], J. Albrecht[2], O. R. Drehmel[2], C. Soderholm[2], M. F. Scott[2], and K. J. Harvatine[1], *[1]The Pennsylvania State University, University Park, PA, [2]Milk Specialties Global, Eden Prairie, MN.*

Fatty acid (FA) supplements are fed to increase energy intake and positively impact milk and milk fat yield. Fat supplements commonly differ in oleic (OA) and palmitic (PA) acids concentration which may impact milk fat yield, digestibility, and nutrient partitioning. Our objective was to investigate the effects of increasing OA or PA in a prilled fatty acid supplement. We hypothesized increasing PA will increase milk fat yield and increasing OA will increase FA digestibility and milk yield. Eight multiparous and 8 primiparous Holstein cows (>70 DIM) were blocked by parity and randomly assigned to a sequence in a 4 × 4 Latin square design with 21 d periods. Treatments were 1) low fat control (CON), 2) high fat control (HF; 59% PA, 22% SA, and 10% OA), 3) high oleic supplement (HO; 60% PA, 18% SA, and 13% OA) and 4) high palmitic supplement (HP; 73% PA, 12% SA, and 9% OA). The FA supplements were fed at 2.0% of DM intake. Data were analyzed using the MIXED procedure of SAS. The model included the random effects of cow and period and the fixed effect of treatment, parity, and their interaction. Preplanned contrasts compared the low and high fat controls and effects of increasing oleic acid (HF vs HO) and palmitic acid (HF vs HP). The conventional fat increased milk fat yield regardless of parity compared with the low fat control (1.44 vs 1.52 kg/d, $P < 0.01$) and in multiparous cows also increased milk yield (41.1 vs 43.3 kg/d; $P = 0.03$) and protein yield (1.26 vs 1.32 kg/d; $P = 0.03$). Increasing OA in the supplement increased milk yield (40.1 vs 41.8 kg/d; $P = 0.02$) and milk fat yields (1.52 vs 1.58 kg/d; $P = 0.02$). Increasing PA did not change milk or milk fat yield compared with the conventional FA supplement. Fat supplementation increased 16 C and decreased de novo and preformed FA. Increasing OA had no effect on the sum of milk FA < 16, 16, and > 16 C FA, while increasing PA increased 16C FA in multiparous cows. Overall, fat supplementation improved milk fat yield and the observed benefit with increased OA may be due to changes in FA digestibility or nutrient partitioning.

Key Words: milk fat

2666 Oleic acid promotes lipid accumulation and improves mitochondrial function in bovine adipocytes. U. Abou-Rjeileh*, A.

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

In rodent models, oleic acid (OA) enhances mitochondrial biogenesis and function. In periparturient cows, OA limits lipolysis and improves adipose tissue (AT) insulin sensitivity. However, the mechanisms behind OA effect in cows are still unknown. The objective of this study was to determine the effect of OA on lipogenesis and mitochondrial function in bovine adipocytes. Pre-adipocytes were isolated from subcutaneous AT explants (n = 9, nonlactating, non-gestating dairy cows) and induced to differentiate. Mature adipocytes were cultured with standard differentiation media (CON) supplemented with palmitic acid (PA)or OA at 100, 200, and 300 μ*M*, or mixed PA:OA at 60:40, 50:50, and 40:60 ratios at 300 μ*M* for 7 d. All fatty acids (FA) were solubilized in albumin (10% BSA). Intracellular lipid droplets were quantified using Adipored assay (RFU/ng DNA). Expression of lipogenic and mitochondrial gene networks was evaluated using RT-qPCR. Protein was quantified by capillary electrophoresis. The statistical model included the random effect of cow and fixed effect of treatment. Compared with CON, the 300PA, 200OA, 300OA, 60:40, 50:50, and 40:60 treatments enhanced lipid accumulation ($P < 0.01$). 300OA and 40:60 stimulated lipid uptake and adipogenesis through increasing the expression of *PPARγ* compared with all other treatments ($P < 0.01$). The 300OA and 60:40 treatments tended to increase expression of *PPARα* compared with 40:60 ($P = 0.06$). Moreover, compared with 300PA, 300OA and 40:60 tended to increase expression of insulin-regulated glucose transporter *GLUT4* ($P = 0.06$). Compared with CON and 200PA, 300OA and 50:50 tended to increase lipid droplet associated protein PLIN5 content ($P = 0.10$). Within the mitochondria, 300PA tended to decrease the expression of FA transport protein system (*CAC*, *CPT1*, *CPT2*), complex I protein (*NDUFS1*), *SIRT1*, and *PGC1α* ($0.05 \leq P \leq 0.07$) compared with CON, 300OA and 40:60 treatments. This shows that OA, in combination with PA, restores mitochondrial biogenesis and improves oxidative phosphorylation. Our results provide mechanistic evidence for the use of OA in dairy cow diets during the periparturient period to enhance lipid accumulation.

Key Words: oleic acid, lipogenesis, PLIN5

2667 Increasing dietary inclusion of high oleic acid soybeans increases milk production of high-producing dairy cows. A. M. Bales* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of increasing dietary inclusion of roasted and ground high oleic acid soybeans (HOSB) on production responses of high-producing dairy cows. Conventional soybeans contain ~15% oleic acid and ~50% linoleic acid whereas HOSB contain ~75% oleic acid and ~7% linoleic acid. Twenty-four multiparous Holstein cows (50.7 ± 4.45 kg/d of milk; 122 ± 57 DIM) were randomly assigned to treatment sequences in a replicated 4x4 Latin square design with 21-d periods. Treatments were increasing doses of HOSB at 0, 8, 16, and 24% DM. HOSB replaced conventional soybean meal and hulls to maintain diet nutrient composition (%DM) of 28.0% NDF, 21.3% forage NDF, 27.3% starch, and 17.8% CP. Ether extract of each treatment was formulated to contain 3.25, 4.52, 5.80, and 7.08%DM, respectively. The statistical model included the random effects of period and cow within square and the fixed effect of treatment. Pre-planned contrasts included the linear (L), quadratic (Q), and cubic (C) effects of increasing HOSB. Results in the text are presented in the following order: 0%, 8%, 16%, and 24% HOSB. Increasing dietary inclusion of HOSB decreased DMI (31.2, 31.3, 30.8, 30.5 kg/d; L $P = 0.01$) and milk urea nitrogen (11.3, 10.5, 9.57, 8.46 mg/dL; L $P < 0.001$) and increased yields (kg/d; all $P < 0.001$) of milk (47.8, 51.2, 51.7, 52.5; L), 3.5% FCM (48.5, 50.9, 51.7, 52.5; L), ECM (49.1, 51.3, 51.9, 52.5; L), milk fat (1.67, 1.75, 1.77, 1.83; L), and milk protein (1.57, 1.63, 1.63, 1.61 kg/d; Q $P < 0.001$). Due to the increase in milk component yields and decrease in DMI, there was an increase in feed efficiency (ECM/DMI; 1.57, 1.65, 1.69, and 1.72, L $P < 0.001$). There was no effect of treatment on BW, BW change, BCS, or BCS change (all $P > 0.20$). In summary, increasing dietary inclusion of HOSB up to 24% DM increased production responses of high-producing dairy cows by increasing yields of milk, milk fat, and milk protein. Future research will examine differences between raw vs. roasted HOSB and interactions with other dietary nutrients and fatty acid supplements that may support improvements in yields of milk fat and protein.

Key Words: oleic acid, soybean, milk fat yield

2668 High oleic soybean oil maintains milk fat, increases fat digestibility, and fat deposition of lactating dairy cows. S. L. Hanno*, A. M. Hurst, K. A. Weaver, A. T. Richards, M. E. Montes, and J. P. Boerman, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Due to the bioactive properties of oleic acid, the objective of this study was to feed high oleic soybean oil (HOSO) to lactating cows and evaluate milk production, body composition, and digestibility variables. Thirty Holstein cows (n = 16 primiparous, n = 14 multiparous) at (87 ± 26 DIM at start of trial) were utilized in a crossover design with periods lasting 21 d. The treatments were a control (CON) diet with no added soybean oil and a HOSO diet with 1.5% diet DM of high oleic soybean oil. Dry matter and milk production data were collected the last 7 d of each period, and milk component information was collected the last 3 d of each period. Fecal samples were collected 6 times during the last 3 d of each period. Body weights were collected on the last 3 d of each period after the morning milking and ultrasound scans of the longissimus dorsi on the last d of each period. Data were analyzed in SAS; models included the fixed effects of treatment, parity, period, the interaction between treatment and parity, and the random effect of cow. Compared with CON, HOSO did not affect DMI, milk production, and milk component yields. However, a parity effect was observed with multiparous cows having increased DMI (5.9 kg/d), milk production (11.2 kg/d), and component yields (all $P < 0.01$). Similarly, multiparous cows had increased feed efficiency (ECM/DMI kg/kg: $P < 0.01$) with no treatment differences observed for feed efficiency ($P > 0.05$). Milk fat concentration tended to be greater for HOSO cows ($P = 0.07$). Body weight data tended to have an interaction between treatment and parity, with multiparous HOSO cows having increased BW (49 kg) compared with CON with no effect in primiparous cows ($P = 0.08$). Similar treatment by parity effects were observed for BCS ($P = 0.03$). Compared with CON, HOSO increased fat depth by 0.44 mm ($P = 0.03$) and fat digestibility by 12 percentage units ($P < 0.01$). The results of this study indicated no detrimental effects of HOSO on milk production parameters with an increase in milk fat concentration, fat digestibility, and fat deposition compared with a control diet.

Key Words: high oleic soybean oil, fat digestibility, parity effects

2669 The in vitro effect of different sources of DHA on the fatty acid profile and microbiota of ruminal fluid of lactating dairy cows. J. Ding, T. Zhan, D. Bu, and L. Ma*, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Docosahexaenoic acid (DHA, C22:6), a kind of n-3 polyunsaturated fatty acid (PUFA), has been widely used in the dairy industry. Fish oil (FO) and *Schizochytrium* sp. (SZ) are the main sources of DHA production, with high contents at 25.13% and 25.22%, respectively. However, those 2 DHA sources have varied fatty acids profiles. Whether this difference may induce microbiota changes in rumen of dairy cows is still unknown. Thus, this study was conducted to provide further insight into the changes of rumen lipid fermentation and bacterial community based on different DHA sources in vitro. Diets supplemented with 4 DHA sources at 1.25 g/kg DHA of DM, which were SZ, SZ oil (SZO), FO, and DHA standards (DHAS), were compared with a control diet (CON) without additional DHA. After 24 h anaerobic incubation by batch feeding technique in vitro, samples were collected to detect rumen fermentation parameters, fatty acids and microbiota profiles. Data were analyzed with one-way ANOVA and Tukey's method for multiple comparisons with 5 treatments as the factors (CON, SZ, SZO, FO, DHAS) using SAS software (version 9.4). The results showed that NH_3-N and pH of the rumen fluid were not affected by different sources DHA ($P > 0.05$), while microbial crude protein was significantly improved in SZ and SZO groups compared with CON group ($P < 0.01$). DHAS significantly reduced ruminal total volatile fatty acid concentrations and methane production compared with the other groups ($P < 0.01$). Branched-chain fatty acids (BCFA) and C22:6 contents significantly increased in SZ group ($P < 0.01$), while significantly decreased in DHAS group ($P < 0.01$). The difference of C22:6 and BCFA contents were insignificant between SZO and FO groups ($P > 0.05$). The microbiome analysis using 16S rRNA sequencing revealed that, at the phylum level, the abundance of *Firmicutes* decreased ($P < 0.01$), in all treatment groups compared with CON group, while that of *Bacteroidota* ($P < 0.05$), *Proteobacteria* ($P < 0.01$) and *Verrucomicrobiota* ($P < 0.01$) increased. Overall, compared with *Schizochytrium* oil and fish oil, *Schizochytrium* sp. can be more efficient to improve ruminal concentrations of C22:6 and BCFA as dietary supplementation in dairy cows.

Key Words: *Schizochytrium* sp., rumen fatty acids, microbiota

2670 Effects of abomasal infusion of lecithin from different sources on milk production and nutrient digestibility in lactating dairy cows.

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Phospholipid composition due to origin source may impact the outcome of dietary phospholipids to enhance fat digestion. Eight multiparous Holstein cows (99.4 ± 9.2 d in milk [DIM]; 48.9 ± 3.8 kg milk yield [MY]/d) were enrolled in a 3 × 3 incomplete Latin square design with 3 treatments provided as continuous abomasal infusates spanning 14-d experimental periods: water (CON), soybean lecithin (SOY; 28% phosphatidylcholine [PC]; 3% lyso-PC [LPC], 74.5 g of BergaPur; Berg+Schmidt); or sunflower lecithin (SUN; 23% PC, 14% LPC, 133.5 g of BergaThin SF Lyso; Berg+Schmidt). Cows were fed a corn silage-based diet (47% DM, 27% NDF, 26% starch, 5.1% EE). The statistical model included the fixed effects of treatment, period, square, treatment × period, and the random effect of cow. Tukey's test was performed for mean comparisons. Dry matter intakes were not affected by treatment. SUN cows had greater MY than SOY and CON (49.1, 46.4, and 45.4 kg/d, respectively; $P < 0.05$). SUN cows tended to produce more energy-corrected milk [ECM] than SOY and CON ($P = 0.10$). SUN cows had greater feed efficiency [MY/DMI] than SOY and CON ($P < 0.05$). Milk fat, protein, and lactose contents were not affected by treatment ($P > 0.05$). Although milk fat yield was not modified by treatment, yields of total solids and protein were greater in SUN than SOY and CON ($P < 0.05$). Treatment did not modify plasma glucose, fatty acid, or insulin concentrations ($P > 0.05$). SUN cows had greater total fatty acid [TFA] and 18C intake than SOY and CON ($P < 0.05$); however, C16 intake was not modified by treatment ($P > 0.05$). Treatment did not modify dry matter, TFA, 16C, or 18C digestibility ($P > 0.05$). De novo (<16C) milk fatty acid concentrations were greater in SOY and SUN, relative to CON ($P < 0.05$). Milk mixed (16C) fatty acid concentrations were greater in CON than SOY and SUN ($P < 0.05$). Milk preformed (>16C) fatty acids concentrations were higher in SUN than SOY and CON ($P < 0.05$). Abomasal infusion of lecithin from different sources did not modify fatty acid digestibility; however, yields of milk and milk protein were uniquely modified by dietary phospholipid source.

Key Words: milk fat, emulsifier, fatty acid

2671 Mammary gland responses to altering the dietary supply of de novo and preformed fatty acids: Effects on the yield of milk and milk components.

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We evaluated the effect of supplementing sodium acetate, palmitic acid, and whole cottonseed (WCS) on the yields of milk and milk components in high producing cows. Thirty-two multiparous Holstein cows (133 ± 57 DIM) were used in a 4x4 Latin Square split plot design to determine the interaction between acetate, palmitic acid, and long chain FA. Cows were blocked by milk yield and allocated to a main plot receiving a basal diet (n = 16) with no supplemental C16:0 (Low PA) or a basal diet (n = 16) with 1.5% DM C16:0-enriched supplement (85% C16:0; High PA). In each Latin Square, the following treatment diets were fed: 1) control (CON), 2) 12% DM WCS (CS), 3) 3% DM sodium acetate (AC), and 4) 12% DM WCS and 3% DM sodium acetate (CS+AC). The C16:0 supplement and sodium acetate replaced soyhulls and WCS replaced cottonseed hulls and meal. Diets were balanced, on DM basis, for 30.1% NDF, 23.0% forage NDF, 28.0% starch, and 16.9% CP. The statistical model included the random effect of cow nested in basal diet, fixed effect of basal diet, period, treatment, and their interactions. Treatment by basal diet interactions were observed for yields of milk fat and ECM where AC increased milk fat ($P = 0.04$) and tended to increase ECM yield ($P = 0.11$) compared with CON in Low PA but not in High PA. Compared with Low PA, High PA increased milk fat content (3.77 vs 4.14%; $P = 0.02$) but had no effect on DMI ($P = 0.55$) or yields of milk ($P = 0.59$) or milk fat ($P = 0.26$). Treatment results are presented in the following order: CON, CS, AC, and CS+AC. Compared with the other treatments, CS+AC increased yields (kg/d) of milk (47.8, 48.0, 47.1, 49.1; $P < 0.01$), 3.5% FCM (50.1, 49.8, 51.8, 53.1; $P < 0.01$), and ECM (50.3, 49.8, 51.7, 52.8; $P < 0.01$). Compared with CON and CS, AC and CS+AC increased milk fat yield (1.82, 1.80, 1.94, 1.97; $P < 0.01$) and content (3.80, 3.84, 4.07, 4.13%; $P < 0.01$). Treatments had no effect on milk protein content (3.24, 3.22, 3.23, 3.21%; $P = 0.45$) or yield (1.53, 1.53, 1.52, 1.56; $P = 0.18$). Our results emphasize the importance of balancing de novo and preformed FA supply for improvements in yields of milk and milk fat.

Key Words: fatty acid, mammary gland, milk fat

Ruminant Nutrition 8: General

2672 Effect of tef *(Eragrotis)* hay inclusion in dairy cows rations on production and performances. P. Wagali*[1], G. Ngomuo[1], J. Kilama[1], C. Sabastian[1], Y. Saranga[2], S. Ben-Zeev[2], Y. A. Ben-Meir[3], N. Argov-Argaman[1], and S. J. Mabjeesh[1], [1]*Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food, and Environment. The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*The Robert H. Smith Institute of Plant Sciences & Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel,* [3]*Institute of Animal Science, Agricultural Research Organizations, Rishon LeTsiyon, Israel.*

Objective was to assess the effect of replacing wheat hay by tef hay in rations on feed intake, digestibility, and lactational performance of dairy cows. Thirty-four multiparous (≥3rd parity) Israeli Holstein Friesian dairy cows averaging (±SD) 182 ± 8 DIM, 45 ± 4.8 kg/d of milk yield (MY), and BW 647.1 ± 51 kg at the beginning of the study were used in a 6 wk feeding trial. Cows were randomly divided into 2 balanced groups based on parity, DIM, and MY. Cows were housed at research barn capable of recording individual intake and subjected to the experimental rations in which the first 14 d were considered as covariate. Cows were subjected to 2 low roughage dietary treatments (~30% roughages); a control ration and tef based ration. Production performances, DMI, and nutrients digestibility were calculated. Milk samples were analyzed for compositional, and FA profile. Blood samples were used to measure metabolite concentrations. Ruminal metabolites and pH were determined on rumen liquid samples. Data collected were analyzed using JMP Pro. The statistical model included fixed effects of dietary treatments, time, and random effect of cow nested in treatment. Production data and feed intake were analyzed as repeated measures using covariance structure. Dietary treatments did not affect DMI (26 kg/d), however, CP digestibility was higher ($P = 0.04$) in tef ration. Tef inclusion increased ($P < 0.05$) MY by 1.5 kg/d. PUFA and Omega 3 FAs profiles in milk were greater ($P < 0.02$) in tef than the control rations. The NEFA and β-Hydroxybutyrate concentrations in circulation were higher in control treatment, while BUN was higher in tef compared with control ration. Acetic to propionic acid ratio in rumen liquid was higher ($P = 0.014$) in control compared with tef ration (2.90 vs. 2.43). However, NH_3-N concentration was higher in tef than control diet ($P = 0.04$; 18.5 vs. 15.8 mg/dL). Conclusively, the inclusion of tef hay in the rations of high-producing dairy cows significantly improve the performance and production of the animals.

Key Words: tef hay, milk yield, dairy cows

2673 Effects of particle size of ground corn on feeding behavior, lactation performance, and metabolic status of fresh Jersey cows. M. N. T. Shipandeni[1,2], G. Esposito[1,3], L. Bailoni[4], and E. Raffrenato*[1,4], [1]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,* [2]*Department of Animal Science, University of Namibia, Windhoek, Namibia,* [3]*Department of Veterinary Medicine, University of Parma, Parma, Italy,* [4]*Department of Comparative Biomedicine and Nutrition, University of Padova, Padova, Italy.*

The objective of this study was to evaluate the effects of starch fermentability of diets fed during the early postpartum (PP) period on feeding behavior, dry matter intake (DMI), lactation performance and energy status of fresh dairy cows. A total of 116 Jersey cows were used in a randomized block design and 58 cows were randomly allocated to each treatment, blocked by parity and balanced for calving date, milk yield, BW and BCS. Treatment diets were fed as a oat-silage based total mixed ration (TMR) from calving to 30 d PP and formulated to contain 28% of starch, with varying particle size of ground corn (3 or 6-mm screen sieve; 26% DM of the TMR), as the primary starch source. Milk yield (MY), DMI and body weight were measured daily; milk compositions, body condition score (BCS) and blood metabolites were measured weekly. Data were analyzed in SAS with a factorial arrangement of treatments with covariate adjustment for the pre-partum period. Feeding coarsely ground corn (CC) increased DMI (17.13 vs. 16.08 kg/d) and MY (21.70 vs. 20.41 kg/d) during the early PP period compared with finely ground corn (FC). Diet did not affect DM eating (581.13 vs. 583.57 min/d) and rumination time (308.58 vs. 315.35 min/d, FC vs. CC) across the whole period. The reduction in DMI in cows fed FC than the CC supports the hepatic oxidation theory of control of feed intake. Diets had no effects on yields of milk protein, ECM and MUN (0.74 and 0.76 kg/d, 26.91 and 27.67 kg/d, 9.61 and 10.27 mg/dL, respectively in FC and CC diet). Milk lactose was increased in CC than FC diet (4.70 vs. 4.61%) whereas fat percentage tended to be greater (5.57 vs. 5.27%) in FC than CC diet, but milk fat yield was not different (1.12 vs. 1.13 kg). Feed efficiencies were also not affected by treatments. Loss in BW and BCS was greater in cows fed FC (39.92 vs 32.24 kg and 0.23 vs. 0.14 units). Feeding CC diets decreased the concentration of plasma NEFA (0.71 vs. 0.56 mmol/L), suggesting that cows were in a better metabolic status, reducing mobilization of body reserves. Blood glucose levels were not affected by the diets. The increased DMI in cows fed CC diets could possibly be attributed to reduced production of propionate in the rumen, shifting of starch digestion to postruminally and by the decreased concentration of plasma NEFA.

Key Words: hepatic oxidation theory, transition cows

2674 Determining factors that affect negative production responses when feeding corn dried distillers' grains in lactating dairy cows. K. L. Clark*, K. Park, and C. Lee, *Department of Animal Sciences, The Ohio State University, Wooster, OH.*

We determined if PUFA or S were responsible for the negative effects of corn dried distillers' grains (DG) on production and if increasing dietary cation-anion difference (DCAD) could alleviate the negative effects of DG. Thirty lactating cows in a randomized complete block design were randomly assigned to the following treatments: a diet containing SBM and Energy booster (CON; 0.2% S, 4% FA,170 mEq/kg DM of DCAD on DM basis), CON with additional dietary S by adding sodium bisulfate (0.4% S; SBM+S), CON with corn oil replacing Energy booster (4% FA; SBM+CO), CON replacing SBM, Energy booster, and partly soyhulls with DG at 30% dietary DM (0.4% S, 4% FA, 55 mEq/kg DM of DCAD on DM basis; DG), and the DG diet with increased DCAD with sodium bicarbonate and potassium carbonate (300 mEq/kg DM; DG+DCAD). All diets contained 16% CP, 24% starch, and 31% NDF on a DM basis. All cows were fed CON for 10 d as a covariate followed by the experimental period for 35 d. Production data were analyzed using Proc Mixed of SAS (random, block; fixed, treatment, week, and interaction). While milk yield remained unchanged, DG tended to have greater DMI compared with CON (29.3 vs. 28.1 kg/d; $P = 0.06$). Milk fat concentration tended to decrease for DG (3.83 vs. 3.26%, $P = 0.09$) compared with CON, but milk fat yield did not differ. The treatment SBM+S did not affect DMI, milk yield, and milk composition when compared with CON. The treatment SBM+CO decreased ($P \leq 0.01$)

both milk fat concentration (3.83 vs. 2.10%) and yield (1.56 vs. 1.00 kg/d) compared with CON. The treatment DG+DCAD versus DG did not affect DMI, milk yield, and milk composition. However, numerical increases in milk fat concentration ($P = 0.17$) and energy corrected milk ($P = 0.17$) were observed for DG+DCAD compared with DG. In conclusion, high S supply did not appear to be a factor of DG causing negative production, but PUFA could be the major factor of DG causing negative production. A negative milk fat response to DG can be potentially alleviated by increasing DCAD. We did not see production differences between CON and DG, and it was against our hypothesis.

Key Words: dietary cation-anion difference, sulfur, polyunsaturated fatty acids

2675 Describing the distribution type of dry matter intake to predict the quantity for cow pens based on pen characteristics.

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Precision feeding meets individual animal requirements to increase efficiency but is not achieved when a group is fed as if all cows are the same. Cows are fed by pen, but diets are formulated to a single cow. If the DMIs were equal there would be no error, but pens are grouped on pregnancy and week of lactation. Our objective is to describe the distribution of DMI by week, then for pens of fresh, mid and late lactation cows and predict the total DMI with the best fitting distribution type. This will be compared with the NASEM 2021 equation of DMI with an assumption of uniform intake for a pen as in practiced in the industry. Our hypothesis is the distribution shape will have lower model error compared with the NASEM. Using a database of 16,500 weekly individual cow observations with the variables of DMI, milk yield and solids, and bodyweight and BCS 700 pens (fresh, mid and late, small and large dairy) were created by randomly sorting individual cow observations to each. The DMI distribution of each pen was fitted to the best shape and its parameters were used to generate random variates for that shape that were summed to predict the total pen DMI. The second model estimated an individual cow DMI for each replicate using the NASEM equation with the input values set at the mean of the pen, and then multiplied by the number of cows for total pen DMI. The β distribution type was the most common fit, at 85% of fresh, 60 – 80% of mid, and 30 – 60% of late pen replicates. The percentage error of total pen DMI for the distribution model was significantly lower than the NASEM, with error less than 1% for each pen type compared with a low of 10% for NASEM. For the distribution shape model more than 95% of the mean square predicted error was due to random variation, with 18 – 68% for the NASEM model, showing a better fit for the distribution model. Machine learning models were trained to predict the distribution shape and parameters for pens with unknown individual DMI values using these described pens. The training data set was labeled with the distribution type and its parameters as output, and descriptive statistics of milk yield and the weeks of lactation of the pen as the inputs. Random forest and neural net models were tested with k-fold cross validation for the lowest root mean square error. Describing the distribution and using it to predict a total pen DMI provides accurate and precise estimates of feed quantity for a group.

Key Words: machine learning, distribution, dry matter intake

2676 Aragonite as a rumen buffer and calcium source for lactating dairy cows.

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The objective was to evaluate performance, rumen fermentation, and blood pH and ionized calcium (iCa) in cows supplemented with aragonite as a source of $CaCO_3$. A replicated 3 × 3 Latin Square design experiment was conducted with 9 primi- and 9 multiparous Holstein cows averaging (±SD): 121 ± 98 d-in-milk (DIM) and 43 ± 8 kg/d of milk yield (MY) at the beginning of the study. Cows were grouped into 6 squares based on parity, DIM, and MY and were fed the same basal diet, except for the inclusion (as % of dry matter intake; DMI) of the following minerals: CON: 0.80% limestone and 0.55% NaCl; BICARB: 0.80% limestone and 0.80% $NaHCO_3$; ARAG: 0.80% aragonite (Rumen Cal +, Ag Source, LLC) and 0.55% NaCl. Data were analyzed using PROC MIXED of SAS with the fixed effects of treatment, time, period, and treatment × time in the model. Square and cow(square) were considered random effects. Treatments did not affect MY (38.0 kg/d), energy-corrected MY (34.5 kg/d), or yields of milk components. Compared with CON, DMI was decreased ($P = 0.02$) by 0.7 kg/d in ARAG and tended to be decreased by 0.4 kg/d in BICARB. Feed efficiency (FE) was increased ($P < 0.001$) by BICARB and ARAG (1.66 and 1.64, respectively vs. 1.59 kg/kg for CON; SEM = 0.091). Milk fat concentration was higher ($P < 0.01$) for BICARB and ARAG, compared with CON (3.58 vs. 3.32%; SEM = 0.152), whereas milk true protein concentration was decreased (by 2%; $P = 0.05$) by ARAG, compared with BICARB and CON. Rumen pH, VFA concentration, and acetate to propionate ratio were not affected by treatment. Rumen NH_3 concentration was higher ($P = 0.01$) for ARAG than BICARB, and butyrate concentration was higher ($P = 0.01$) for ARAG than CON cows. Blood pH was increased ($P = 0.04$) at 6 h after feeding by ARAG and BICARB, compared with CON (7.46 and 7.45 vs. 7.42 for CON; SEM = 0.009). Blood iCa concentration tended to be higher ($P = 0.07$) for ARAG than BICARB, but both were not different from CON. Rumen buffering capacity of ARAG appears to be similar to that of BICARB, which was supported by increased milk fat concentration. Improved FE was observed for both ARAG and BICARB supplementation.

Key Words: rumen buffer, calcium, milk production

2677 Effects of branched-chain volatile fatty acids at different levels of rumen degradable protein on milk production and nutrients digestibility in lactating cows.

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Branched-chain volatile fatty acids (BCVFA) have been known as growth factors for rumen microbes. The objective of the study was to determine the effects of BCVFA and its interaction with RDP on total-tract digestibility and performance of dairy cows. Eight ruminally cannulated cows (parity = 2 and DIM = 191 ± 41); were used in a replicated 4 × 4 Latin square design. Cows were randomly allocated to 4 treatments in a 2 × 2 factorial arrangement: 9 or 11% RDP with or without BCVFA (0.09% isobutyrate and 0.06% 2-methylbutyrate in dietary DM). The dietary RDP was mainly adjusted with soybean meal and AminoPlus, and RUP was constant (6% of dietary DM). Total collection of feces and urine were conducted on the last week each 4-wk period. Data were analyzed using the MIXED model with RDP, BCVFA, RDP × BCVFA, and period as fixed effects, and square and cow(square) as random factors. Milk yield and DMI were not influenced by treatments.

An interaction between RDP and BCVFA was observed ($P < 0.05$) for milk fat yield and energy-corrected milk (ECM). In addition, RDP and BCVFA interacted ($P = 0.05$) for ECM per unit of DMI. The interactions occurred because the variables were increased by BCVFA only when greater RDP was provided. Milk protein concentration increased with greater RDP ($P < 0.01$), but yield was not affected by RDP or BCVFA. Milk urea nitrogen (MUN) increased by greater RDP (11.4 vs. 13.5 mg/dL; $P < 0.01$) and tended to increase for BCVFA ($P < 0.10$) but had a trend of interaction between RDP and BCVFA ($P = 0.09$). The interaction tended to occur because BCVFA increased MUN further under greater RDP. Total-tract digestibilities of DM, OM, and NDF were not affected by treatments. In conclusion, supplementation of BCVFA was effective in improving milk fat yield, and ECM when RDP was sufficient. The increased MUN may indicate BCVFA increased microbial activity or mass in the rumen, which was further enhanced with greater RDP. Because total-tract digestibility of fiber was not affected, further investigation on ruminal digestibility of fiber is necessary.

Key Words: isoacids, isobutyrate, 2-methylbutyrate

2678 Changes in body measurements, blood glucose and β-hydroxybutyrate concentrations, and milk yield due to prepartum muscle reserves and branched-chain volatile fatty acid supplementation of transition dairy cattle.

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This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).

The objective of this study was to determine the effects of prepartum muscle reserve and supplementation with branched-chain volatile fatty acids (BCVFA) on body measurements, metabolic markers, and subsequent milk yield in multiparous dairy cows. Muscle reserve was assessed by ultrasound of longissimus dorsi muscle depth (LDD), and cows (n = 48) were assigned to either high muscle (HM; > 4.6 cm) or low muscle (LM; < 4.6 cm) groups based on LDD −42 d before expected calving. Cows were then randomly assigned to either control (CON) of soyhull pellets or BCVFA treatment that contained isobutyrate (30 g/d), 2-methylbutryate (15 g/d), and isovalerate (15 g/d). Treatments were top dressed during the last 42 d of the dry period, resulting in 4 combinations of groups and treatments: HM-CON (n = 13), HM-BCVFA (n = 13), LM-CON (n = 11), and LM-BCVFA (n = 11). Body weight (BW) and body condition score (BCS) were measured on the following days relative to calving −42, −35, −21, −7, 0, 7, 14, 21, 28. Weekly blood samples were taken to measure glucose and β-hydroxybutyrate (BHB) and milk yield was recorded daily for the first 4 wks of lactation. The statistical model included the fixed effects of treatment, group, time, their interactions, and the random effect of cow within treatment. Pre and postpartum, HM had greater BCS ($P < 0.0005$) than LM. Postpartum, HM had increased BW ($P < 0.0001$) compared with LM, with a tendency for BCVFA to increase BW ($P = 0.06$) compared with CON. Prepartum, BCVFA treatment and HM group tended to increase blood glucose concentrations compared with CON and LM group ($P = 0.08$ and $P = 0.10$, respectively). Postpartum, BCVFA increased blood glucose concentration ($P = 0.04$) and tended to decrease BHB ($P = 0.06$) compared with CON cows. Milk yield was 2.5 kg/d greater for HM compared with LM group ($P = 0.02$) with no difference detected for BCVFA supplementation compared with CON (38.5 vs. 36.4 kg/d; $P = 0.32$). Results indicate prepartum muscle reserve is related to metabolic status and productivity of dairy cows in the transition period. Additionally, BCVFA supplementation in the dry period may reduce BW loss, increase blood glucose and reduce BHB concentrations.

Key Words: branched-chain volatile fatty acids, transition period, longissimus dorsi muscle

2679 Derivation of the maintenance energy requirements in Jersey cows differing in body condition score (BCS).

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral).

Research in a variety of species including cattle has suggested energy required for maintenance may be affected by body condition. The objective of this study was to use indirect calorimetry and total fecal and urine collections to estimate maintenance energy and fasting heat production (FHP) of cows of differing in body condition score (BCS). Twelve multiparous dry, nonpregnant Jersey cows were assigned to one of 2 treatment groups. To construct these groups cows were fed 2 different diets for 84 d resulting in a BCS of >5 (HBCS) and a BCS <3 (LBCS) where 1 is very thin and 5 is very over conditioned. After, all were fed at maintenance (NELm, (Mcal/d) = $0.10 \times BW^{0.75}$) for 21 d followed by 4 d of experimental measures, cows were then fasted of 96 h with measures once again taken over the final 24 h. No difference was observed ($P > 0.49$) in initial body weight (BW) or BCS, averaging 509 ± 26.0 kg and 4.05 ± 0.229. At the initiation of maintenance, differences ($P < 0.01$) in BW and BCS existed (439 and 566 ± 19.0 kg BW, and 2.96 and 4.96 ± 0.134 BCS, for LBCS and HBCS, respectively). Heat production increased ($P = 0.02$) with increasing BCS (13.1 to 16.2 ± 0.55 Mcal/d), but when expressed per unit of metabolic body weight (MBW) no difference ($P = 0.30$) was observed (0.138 ± 0.0022 Mcal/d/MBW). When fasted for 96 h, total body weight loss did not differ ($P = 0.27$) averaging 28.9 ± 0.181 kg. The FHP did not differ ($P = 0.40$) averaging 0.933 ± 0.0040 Mcal/d/MBW and resulted in the following representation of maintenance; NELm, (Mcal/d = $0.0933 \pm 0.0040 \times BW^{0.75}$). Nitrogen free reaction quotient tended ($P = 0.07$) to differ (0.686 and 0.726 ± 0.0141 for LBCS and HBCS, respectively) and oxygen consumption and carbon dioxide production for protein oxidation differed ($P = 0.05$; 5.44 and 2.35 ± 0.988 and 4.52 and 1.95 ± 0.821 L/MBW). Although FHP increased with increasing BCS, FHP per unit of MBW did not differ. Although BW change was similar during fasting, differences in oxygen consumption and carbon dioxide production per unit of MBW used for protein oxidation indicates differences in the nature of body tissue utilization in cows differing in BCS.

Key Words: body condition score, energy utilization, fasting heat production

2680 A controlled energy diet was less inflammatory than a higher energy diet during the dry period in Holstein cows.

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Our hypothesis was that overfeeding energy during the dry period would be more inflammatory during the periparturient period than feeding to the cows' requirements. Pregnant multiparous Holstein cows were assigned to a controlled energy diet (LE; 1.43 Mcal/kg DM; n = 7) or a moderate energy diet (HE; 1.61 Mcal/kg DM; n = 7) at 50 d before expected parturition. All cows received one lactation diet for 42 d postpartum. Milk and DMI were measured daily; milk composition was measured weekly. Blood was sampled frequently around parturition and liver biopsies were obtained on d −14, 10 and 30 relative to calving. Liver tissue was frozen in liquid N and stored at −80°C. Total RNA was extracted from liver tissue and applied to a commercial 44,000-sequence bovine oligonucleotide microarray (Agilent Technologies Inc.). Data were analyzed in a mixed effects repeated measures model with diet and week as fixed effects and cow random. The DMI did not differ prepartum (12.8 ± 1.3 kg/d) or postpartum (18.4 ± 1.3 kg/d). Milk yield (50.1 vs. 47.5 ± 2.0 kg/d, LE vs. HE) and milk fat (4.55 vs. 4.71 ± 0.18%) did not differ ($P > 0.10$) between prepartum diets, whereas milk protein (2.93 vs. 3.16 ± 0.07%) was greater ($P = 0.01$) for cows fed HE. Blood concentrations of glucose, NEFA, BHB, cholesterol, albumin, and insulin did not differ ($P > 0.10$) between diets. Liver triacylglycerol at 10 DIM did not differ between diets ($P > 0.10$). Abundance of mRNA for *ACADVL, ACOX1, ACSL1, CPT1A, CYP4A11, AGPAT1, DGAT1, GPAM, PC,* and *PPARA* did not differ between diets according to quantitative PCR. At d 10, the microarray showed that haptoglobin, serum amyloid A3, mammary serum amyloid A3.2, and serum amyloid A2 were upregulated in HE vs. LE by 3.41-, 2.92-, 2.70-, and 2.35-fold, respectively. Other transcripts upregulated included GTPase, IMAP family member 4-like (4.48 fold), GTPase, IMAP family member 4 (2.30-fold), lactoferrin (2.15-fold), interleukin 36, α (2.13-fold), carbonic anhydrase IV (2.09-fold), carbonic anhydrase III (2.06-fold), and metallothionein 2A (2.06-fold). Twenty transcripts were downregulated by ≥ 2-fold in HE vs. LE. Despite similar performance and lipid metabolism between LE and HE, cows fed HE had greater hepatic inflammation early postpartum than cows fed LE during the dry period.

Key Words: inflammation, dietary energy, periparturient period

2681 Lactational performance and enteric methane emissions of phenotypically high and low methane emitting dairy cows fed bromoform.

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Bromoform is a bioactive compound of the red seaweed *Asparagopsis taxiformis* (AT), shown to have a strong anti-methanogenic effect in ruminants. Several animal studies demonstrated that AT could decrease enteric CH_4 emissions by up to 99%, but pure bromoform has not been evaluated in vivo. Additionally, the interaction between CH_4 inhibition and the CH_4-emitting phenotype of the cows has not been studied. The objective of the current study was to evaluate the effects of bromoform inclusion in the diets of phenotypically high and low CH_4-emitting Holstein cows. Previously 48 Holstein cows were screened for their enteric CH_4 emissions. Out of these 48 cows, 5 were selected as low [LM, 13.3 ± 0.99 g CH_4/kg dry matter intake (DMI)] and 5 as high CH_4-emitters (HM, 18.8 ± 1.94 g/kg DMI) for a crossover design experiment, with 2, 28-d periods. Cows were grouped based on lactation number, days in milk, milk yield (MY), and CH_4 yield. Cows within a group were randomly assigned to one of 2 treatments: CON (basal diet) and BROM (basal diet plus 542 mg/cow/d of bromoform or 0.02% of DMI; equivalent to 0.5% inclusion of AT in previous experiments). A 3-weeks washout period (no bromoform treatment) was allowed between periods 1 and 2. Data were analyzed using PROC MIXED of SAS with treatment, phenotype, and the interaction in the model. Production variables such as MY (31.5 ± 5.27 kg/d), DMI (22.6 ± 3.34 kg/d), body weight, and milk components did not differ ($P \geq 0.27$ between CON and BROM, and the 2 CH_4 phenotypes. Enteric CH_4 emissions were numerically higher ($P = 0.12$) in HM (430 g/d) vs. LM (368 g/d) and were lower ($P = 0.01$) in BROM (343 g/d) vs. CON (455 g/d). Similarly, CH_4 yield (g/kg DMI) was 30% lower ($P = 0.002$) in BROM compared with CON but did not differ between LM and HM (17.8 vs 18.4 g/ kg DMI) cows. There was no interaction between treatment and CH_4 phenotype for all CH_4 metrics. Overall, the inclusion of bromoform in the diet resulted in decreased enteric CH_4 emission with no negative effects on production; however, there was no difference in the response to bromoform between LM and HM cows.

Key Words: bromoform, enteric methane, phenotype

2682 Predicted methane yield and mitigation potential of 3-nitrooxipropanol on typical US diets offered to dairy cows.

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Our objective is to explore the predicted mitigation potential of 3-nitrooxipropanol (3-NOP) when added to typical diets for lactating cows across the United States (US). Three forage-to-concentrate ratios (F:C on a dry matter basis, DM) were considered: 70:30, 50:50, and 30:70, respectively. Corn silage (CS) was combined with a second forage source (F2) at 70:30, 50:50, and 30:70 for each F:C. The F2 option was either one of 3 grass-legume silage mixtures (GLS), or legume hay (LH). Twenty US regional by-product-based concentrate mixes (BP) were formulated. Inclusion rates of the BP and corn grain were flexible within the concentrate component of the diet to meet the energy (1.52 ± 0.040 Mcal/kg DM of NE_L), and crude protein (16.0 ± 0.30% DM of CP) requirements for a cow eating 24 kg of DMI/d and producing 36 kg of milk/d. Chemical composition of feedstuffs was taken from tabulated values. In total, 144 diets were formulated. Methane yield (CH_4/DMI; g/kg) for each diet was predicted using an equation by Niu et al. (2018; Eq. 42) including both animal factors (energy corrected milk, milk fat and protein contents, and body weight) and diet factors (ether extract (EE) and neutral detergent fiber (NDF), both as % DM). Across all diets, the CH_4 yield estimates ranged from 17.6 to 19.4 g/kg DMI. Mitigation potential of 3-NOP (% of reduction in CH_4 yield emission) was assessed at a fixed dosage of 70 mg/kg DM using the recent Kebreab et al. (2023) equation built from the 3-NOP dose (mg/kg of DM), and NDF, EE, and starch contents in the diet (% DM). Within the 144 diets evaluated, CH_4 yield emissions can be reduced from 8% to up to 38%. The greatest reduction was achieved from an initial CH_4 yield of 18.3 g/kg when 3-NOP is added to a Northeast diet containing 70% of concentrate (42% DM BP mix) and LH as the F2 source at 21% of the total diet (DM basis). For the given diet offered to a herd of 1,000 lactating cows and assuming a Global Warming Potential (GWP) for CH_4 of 34, we estimated that the addition of 3-NOP has the potential to decrease enteric CH_4 production up to 2,070 t of CO_2-eq per year.

Key Words: concentrates, feed additive, methane mitigation

2683 The effects of water temperature and water intake on rumen temperature of dairy cows.

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Maintaining rumen temperature (RT) in an optimal range is important for rumen microbial enzyme activity. The objective of this study was to determine the effects of water temperature (WT) and water intake (WI) on RT when WT was lower than the RT (Sep to Dec). A total of 16 lactating dairy cows, averaging (±SD) 224 ± 36 d in milk, 26.9 ± 3.72 kg/d milk yield, 732 ± 51.9 BW, 25.2 ± 2.64 kg/d DMI, and 85.3 ± 12.77 kg/d daily WI, were used. The experiment consisted of 4 24-d periods and measurements were conducted during the last 7 d of each period when cows were in tie-stalls. Cows were fed TMR based on

corn and grass silage and concentrates (CP and NDF content of 15.8% and 37% of DM) 2x/d, and water was provided ad libitum. Temperatures of tie-stall barn (measured using Netatmo smart system every 5-min) and water (measured using i-Button every 1-min) were highly correlated (r = 0.97; $P < 0.01$). The WT was predicted using environment temperature (ET) and were on averaging (±SD) 15.0 ± 1.55, 14.6 ± 1.25, 10.1 ± 0.94 and 5.8 ± 1.95°C for periods 1 to 4, respectively. The WI and RT of the cows were recorded every 15 and 10 min using GWF system and smaXtec boluses, respectively. Average (±SD) daily RT of the cows (38.8 ± 0.07°C) was positively correlated with WT (r = 0.65, $P < 0.01$). Distinct drinking events were identified (n = 1,147) to analyze how WI and WT effect RT. Variables of interest including RT recovery time (min; duration from post-drink minimum RT to RT that reaches ± 0.10°C of the pre-drink RT) and RT change (°C; deviation of pre- and post-drinking RT) were analyzed using a linear mixed model including fixed effect of WI, WT, and period, and random effect of cow. Water intake of individual drinking events positively affected RT recovery time (+3.38 min/kg WI; $P < 0.01$) and RT change (+0.14°C/kg WI; $P < 0.01$). Further, WT negatively affected RT recovery time (+1.5 min/1°C; $P = 0.02$) and RT change (+0.03°C/1°C; $P = 0.05$). Overall, WI and WT below the RT influenced RT and RT recovery time in dairy cows. Further investigation on the effect of WT and WI on nutrient digestibility is needed.

Key Words: dairy cow, rumen temperature, water temperature

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2684 Drinking water temperature preferences in crossbred dairy beef calves. L. Llonch*[1], S. Martí[1], X. Vergara[1], G. Prat[1], M. Vestergaard[2], and M. Devant[1], *[1]Ruminant Production Program, Institut de Recerca i Tecnologia Agroalimentàries, Torre Marimon, Barcelona, Spain, [2]Aarhus University, Department of Animal and Veterinary Sciences, Tjele, Denmark.*

The aim of this study was to explore which drinking water temperature (DWT) is preferred by dairy beef calves. Twenty newly weaned crossbred bull calves (75 ± 2.5 d of age) were individually allocated to roofed hutches with straw as bedding placed on concrete floor in a private farm (located in Montgai, Lleida, Spain) during December 2022. Using a preference test (PT), 2 different DWT treatments were tested: 1) ambient temperature water (AT), bucket with 5 L of water at ambient temperature; 2) hot water (HOT), bucket with 5 L of water at 35°C. The study lasted 4 consecutive days, where both DWT treatments were offered simultaneously in a 10-min PT per day following a previous 6-h period without water. The 2 treatment buckets were randomly located either left or right of the concentrate bucket and they were exchanged every day. The rest of the day, calves were fed concentrate, straw, and water ad libitum. Treatment water intake and DWT were recorded during the PT and concentrate and water intake were recorded per day. Water intake and percentage of preference per treatment were analyzed using a mixed model. Number of preferences >65% were analyzed by a chi-squared test. Ambient temperature and environmental humidity (mean ± SEM) were 4.0 ± 0.7°C and 81 ± 4%, respectively. Drinking water temperatures of AT and HOT treatments (mean ± SEM) were 9.9 ± 0.1°C and 35.6 ± 0.3°C, respectively. Water intake during PT was greater for HOT than for AT treatment (2.9 vs. 0.6 ± 0.34 L, $P < 0.01$). Also, the percentage of preference was greater for HOT than for AT treatment (76 vs. 24 ± 5.7%, $P < 0.01$), with the number of preferences >65% being more frequent for HOT than for AT treatment (79 vs. 21%, $P < 0.01$). Daily total water intake (mean ± SEM) was 7.3 ± 0.2 L/day, and daily concentrate intake (mean ± SEM) was 2.5 ± 0.1 kg DM/day. In conclusion, newly weaned dairy beef calves preferred to drink water at 35°C, a temperature like that of milk, compared with water at ambient temperature (around 10°C) during the winter. The present study should be repeated in other seasons to assess the repeatability of these findings.

Key Words: dairy beef, drinking water temperature, calves

2685 Use of accelerometer data as a proxy for assessing cow comfort on different milking machine settings. M. Browne*[1,2], P. S. Boloña[1], and J. Upton[1], *[1]Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, [2]VistaMilk SFI Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

Milking gently, quickly and completely are positively impacted through optimized milking machine settings. Increasing the milk flow-rate switch-point (flow-rate at which Automatic Cluster Removers (ACRs) detach clusters from a cows' udder) has been found to reduce milking duration without impacting on milk yield or SCC. A higher milk flow-rate switch-point may also benefit cow comfort by limiting exposure to elevated teat-end vacuum during the low milk-flow period at the end of milking. This study investigated differences in stepping/kicking during milking between 2 milk flow-rate switch-point settings (0.2 kg/min: MFR0.2 and 0.8 kg/min: MFR0.8) as measured by rear leg mounted 3-dimensional accelerometers (IceTags, Ice Robotics, Scotland). Milking and accelerometer data were collected from 37 cows on a crossover experimental design, where each cow was milked twice per day for 2 weeks on each setting. Statistical analysis was performed using the GENMOD negative binomial procedure of SAS with the response variable (IceTag step count) analyzed separately for AM and PM milkings. Independent variables included treatment, period (based on crossover), breed and parity. The IceTag step count of MFR0.2 (5.75) was higher than that of MFR0.8 (4.96, $P = 0.01$) during PM milkings. Excluding minute of cluster attachment reduced step count by 35% on MFR0.2 and 37% on MFR0.8, yet a significant difference remained between treatments ($P = 0.01$). Due to the 16:8 h milking interval, AM milkings were much longer (386 s AM vs 271 s PM, MFR0.2). A trend toward greater leg movement on MFR0.2 during AM milkings was not significant. MFR0.2 resulted in lower mean milk flow-rates ($P < 0.001$). Lower milk flow-rates tend to increase vacuum under the teat, potentially impacting cow comfort. Shorter milking intervals between AM and PM milkings resulted in lower udder fill and reduced milk flow-rate (32% lower on MFR0.2). Under low udder fill conditions, rear leg movement, as an indicator of cow comfort, reduced ($P = 0.01$) when ACR milk flow-rate switch-point increased from MFR0.2 to MFR0.8.

Key Words: milking efficiency, accelerometer, cow comfort

2686 Using changes in feeding behavior patterns to find calves at risk for diarrhea. M. C. Cantor*[1,2], A. Welk[1], M. M. Woodrum Setser[3], J. H. C. Costa[3], and D. L. Renaud[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Penn State University, College Park, PA, [3]University of Kentucky, Lexington, KY.*

The objective of this study was to develop and cross-validate an alert to identify calves at-risk for diarrhea using milk feeding data (behavior) from automated milk feeders (AMF). We enrolled calves (n = 259) from 2 facilities who were health scored daily. For alert development, calves were offered 10 or 15 L/d of milk replacer. The alerts were cross validated using different data from these facilities (127 calves all offered 15 L/d) for −2 to 1 d relative to diarrhea diagnosis. We enrolled calves that were either healthy or had a first diarrheal bout (loose feces ≥ 2 d or watery feces ≥ 1 d). Daily milk intake, drinking speed, and rewarded visits were recorded by an AMF. Relative change and rolling dividends for each behavior were calculated for each calf from the previous 2 d. Logistic regression models and receiver operator curves (ROC) were used to assess the diagnostic ability for relative change and rolling dividends in behavior to classify calves at-risk for diarrhea from −2 to 0 d relative to diagnosis. To maximize sensitivity (Se) to correctly classify calves at-risk with diarrhea, alert thresholds were based on ROC optimal classification cut-off. Diagnostic accuracy was met when the alert had a moderate ROC curve (≥0.70), high accuracy (Acc) (≥0.80), high Se (≥0.80), and very high precision (Pre) (≥0.85). For alert development, deviations in rolling dividend milk intake with drinking speed had the best performance for classifying a calf at-risk for diarrhea (10 L/d: ROC AUC = 0.79, threshold ≤ 0.70; 15 L/d: ROC AUC = 0.82, threshold ≤ 0.60). Our diagnostic criteria were only met in calves offered 15 L/d (10 L/d: Se 75%, Acc 72%, Pre 92%, specificity (Sp) 55% vs. 15 L/d: Se 91%, Acc 91%, Pre 89%, Sp 73%). For cross-validation, the best behavior alert was facility dependent. Deviations in rolling dividend milk intake with drinking speed met diagnostic criteria for one facility (threshold ≤ 0.60, Se 86%, Acc 82%, Pre 94%, Sp 50%). However, relative change in milk intake with drinking speed met diagnostic criteria for the other facility (threshold ≤ −0.35, Se 83%, Acc 83%, Pre 0%, Sp 0%). We suggest changes in milk patterns may indicate diarrhea in calves.

Key Words: precision technology, disease

2687 Computer vision-based models for estimation of respiratory rate of dairy cows using contactless videos.

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Respiratory rate (RR) serves as an important physiological indicator of animal health and welfare, for example, for dairy cows experiencing heat stress and respiratory illness. Traditional methods of RR monitoring, e.g., counting the flank movements, is labor intensive and not applicable in on-farm settings. There are growing interests in developing contactless video-based RR monitoring techniques, however, prior studies have primarily focused on estimations using short-term video clips, which limits the accuracy and utilization of these techniques given farm environment. This study aimed to develop computer vision-based models to estimate RR of dairy cows using contactless video for extended periods. The experiment involved video recording of 3 cows for 24 h each, with RR measurements collected through Embla XactTrace Respiration Belt, serving as the ground truth (GT). A digital camera (DH-SD1A404XB-GNR) was positioned at the side of the cow to capture the region around abdomen. To minimize the impact of noises, the video data were processed using a 10-point moving average filter, followed by a bandpass filter excluding frequencies outside the range of 0.2 to 2 Hz. A sliding window was used to continuously estimate the RR, with each window undergoing a fast Fourier transform analysis. The frequency with the highest amplitude was considered as the RR. To evaluate the performance of models, 5 videos with a mean length (±SD) of 32 min 32 s ± 4 min 7 s including both day and night occasions, were selected. The GT measurements for the selected videos of the 3 cows had a mean RR (±SD) of 23.4 ± 2.77 breaths per minute (bpm). The comparison between the RR from video recording vs. GT showed the effectiveness of the computer vision-based method, with mean absolute error (MAE), root mean squared error (RMSE) and root mean square percentage error (RMSPE) values of 4.08 and 4.71 bpm and 20.2%, respectively. Future investigations will aim to further enhance the performance of the model through the development of more sophisticated algorithms.

Key Words: respiratory rate, computer vision, precision livestock farming

2688 To go or not to go? Assessing anticipation for outdoor access in dairy cows.

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Regular outdoor access may enrich movement-restricted cows' lives, but how they perceive it is unclear. Measures of cognitive processes, such as anticipation, can provide information on animal emotions. Our study aimed to characterize the anticipatory behaviors of cows living in restricted movement environments when an outing experience is provided as enrichment. In 2 independently analyzed trials, 32 tie-stall-housed lactating cows were blocked by parity and DIM and divided into 2 groups. Control cows (C; n = 8 in winter (W) and summer (S)) remained inside for the duration of the experiment, and outdoor cows (O; n = 8 in W and S) were provided with 5 d/week of outdoor access for 8 weeks. The outdoor areas combined different durations of outing (1 vs 2 h) and area sizes (20, 40, 60 and 80 m^2). Exit cues, such as route preparation and halter placement in front of the outdoor cows, were set 20 min before the cows had outdoor access, and video recordings were taken during this period. The number of behavioral and ear position transitions were recorded and analyzed with 2 mixed models:1) to compare C vs. O cows with treatment, day, and their interaction as fixed effects, and cow nested within block as random effect; and 2) to test the influence of duration, area size, their interaction, and day as fixed effects, and cow nested within block as random effect. For W and S, no significant differences were found for anticipatory behaviors between C and O cows, nor for different durations and area sizes (overall mean ± SD: behavioral transitions: C: 23.9 ± 22.5, O: 20.9 ± 20.4; ear position transitions: C: 71.3 ± 22.5, O: 79.6 ± 20.4). Investigation of other behaviors before the exit (e.g., exploration, trampling, or lying) and having targeted observation periods (e.g., the first 5 min after setting exit cues) could allow for a more detailed study of the animal's behavioral expression during anticipation. Combined with other indicators such as motivation, it would provide a basis for assessing emotional states in animals.

Key Words: animal emotion, enrichment, outdoor access

2689 Comparison of bolt penetration depth by three low-cost captive bolt devices used for on-farm cattle euthanasia.

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This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).

Comparative studies are lacking to guide lower cost penetrating captive-bolt device (PCB) selection for on-farm cattle euthanasia. This study aimed to compare bolt penetration depth (BPD) of 3 low-cost PCBs used for on-farm cattle euthanasia. We hypothesized that higher cost PCBs would have a deeper BPD than the cheapest PCB tested. Seven shots per PCB-cartridge unit (9 mm Blitz-Kerner / 252J red cartridge (BK), 0.25" Jarvis BA-BOOM / 407J red cartridge (JBB), and 0.25" Matador SS3000 / 406J red cartridge (MSS)) were performed in random order into a 20% ballistic gelatin block, then in 10 cadaveric bovine heads each. The BPD was measured with an electronic caliper and by CT, respectively. For each experiment, a one-way ANOVA was performed followed by a Tukey's test to compare BPD between PCBDs (significance level: $P < 0.05$). In gelatin, mean BPD of the BK (3.3 ± 0.5cm) was significantly lower than both JBB and MSS ($P < 0.0001$). In the cadaveric bovine heads (28 females and 2 males, 7–111 mo), mean BPD was greater for the MSS (8.6 ± 0.6cm) than the JBB (7.8 ± 0.7cm; $P = 0.016$) and the BK (7.1 ± 0.5cm; $P < 0.0001$); BPD was also greater in the JBB group than the BK group ($P = 0.048$). Using cartridges with the kinetic energy recommended for adult cattle, the cheapest PCBD (BK) showed less penetrating capacity than the others, which could result in less in vivo cerebral trauma. Though these results must be confirmed in vivo, the MSS may be preferentially selected over the BK and JBB for on-farm cattle euthanasia to maximize deeper cerebral tissue disruption.

Key Words: cattle, euthanasia, captive bolt

2690 Increasing fitness for transport in cull dairy cows.

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Welfare of cull cows during transport to slaughter is a current concern in the Canadian dairy industry. Cull cows sold through auction often have a high prevalence of lameness, low body condition score (BCS), hock lesions, and udder engorgement. To evaluate whether delaying transport can address these challenges, 37 cows were selected and randomly assigned to either be fed for 60 d after being dried off (FED; n = 18) or control cows, which were sent directly to slaughter (DIRECT; n = 19). FED cows were assessed weekly for locomotion (5-point scale), BCS (5-point scale), hock lesions (3-point scale), and udder engorgement (3-point scale), while DIRECT cows were scored once, on the day before slaughter. Weights of the FED cows were determined at time of enrollment in the trial as well as measured again the day before slaughter, and DIRECT cows were weighed the day before slaughter. Simple *t*-test and chi-squared statistics were used to compare experimental groups for continuous and dichotomous outcomes, respectively. FED cows gained an average of 135.6 kg over the 60 d (SD = 75.88). FED cows had an average weight at slaughter of 839.8 kg, whereas DIRECT cows' average weight was 745.6 kg ($P < 0.05$, SD = 93.94). The FED cows' average BCS at the start of the trial was 2.4, and at slaughter was 3.5, with an average gain of 1.2 BCS points. At slaughter, proportion of udders involuted in the FED group was 44.4% (n = 8) and in the DIRECT cows, was 0% ($P < 0.05$, SD = 0.50). There were no significant differences in locomotion or hock lesions between the FED and DIRECT groups ($P > 0.05$). Due to the improved BCS and udder scores, cows fed for 60 d may be better prepared for transportation to slaughter, as well as earn producers more money in the auction ring due to increased weight and body condition.

Key Words: transport, welfare, cull

2691 Relationship between body surface temperature and shade-seeking behavior in dairy buffaloes.

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The present study aimed to investigate the relationship between physiological responses, body surface temperature, and shade-seeking behavior in Nili Ravi dairy buffaloes during summer months. The study enrolled 60 buffaloes, and each animal was observed for 3 consecutive days starting before sunrise until they moved toward the shade structures. A repeated measures ANOVA was employed to assess the changes in physiological parameters and body surface temperature between the early morning and the occurrence of shade-seeking behavior. The average temperature humidity index (THI) and heat load index (HLI) during the behavioral monitoring period (0400 to 1200 h) were 81.3 ± 6.5 and 92.9 ± 17, respectively (mean ± SD). The results revealed that there was no significant difference in core body temperature before sunrise and at the time of shade-seeking event (39.27 vs. 39.26°C; $P = 0.92$). However, the buffaloes had a slightly higher respiration rate of 3 breaths per minute at the time of shade-seeking (19.5 vs. 22.4 breaths/min; $P < 0.001$). In addition, body surface temperature, measured at the flank region, shoulder, base of the ear, and forehead was significantly higher at the occurrence of shade-seeking behavior compared with the early morning ($P < 0.001$). On average, the buffaloes sought shade when the surface temperature was 2°C higher than the temperature recorded before sunrise. Overall, the current findings suggest that body surface temperature, rather than core body temperature was strongly associated with shade-seeking behavior in dairy buffaloes. These findings could be useful in developing strategies to mitigate the effects of heat stress in dairy buffalo herds and improve animal welfare.

Key Words: dairy buffaloes, shade-seeking behavior, heat stress

2692 Evaluation of time budgets and vaginal temperature of lactating Holstein cows offered a choice of shade and sprinklers on pasture.

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This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).

Mitigating heat stress is challenging in pasture environments and knowledge on cow interactions with pasture heat abatement strategies is limited. The study aim was to evaluate cow behavior associated with both heat abatement strategies (HAS) together: 80% shade cloth (SH) and PVC sprinkler systems (SP). Cows (n = 46) were used in a companion study immediately before they entered this study where cows were separated into 2 groups and subject to a crossover design with only 1 HAS. Between studies, cows were housed in freestalls for 3 d due to severe weather. Lactating Holstein cows (n = 46) were assigned to a study pasture with both HAS. Cows stayed in this pen for a 1-d acclimation followed by 3 d of observations. A drone was deployed at 30-min intervals between 1000 to 1430 to evaluate heat abatement use (HAU). Vaginal temperature (VT) was collected at 5-min intervals with transvaginal data loggers. Steps, lying bouts (LB), and time lying (TL) were recorded with a triaxial accelerometer. Temperature humidity index (THI) data were collected. PROC GLIMMIX of SAS was used to evaluate the fixed effect of HAS and THI on HAU, VT, steps, LB, and TL. To determine HAU, data were separated into morning (1000 to 1230) and afternoon (1230 to 1430). All other analyses were separated by HAS. At morning, cattle used SH most at THI 71 and 76 ($P < 0.01$ for both) but used SP more at THI 79 ($P = 0.04$). At afternoon, cows used SP at THI 77 ($P < 0.01$) but SH at THI 80 ($P < 0.01$). Cows with the greatest VT in the afternoon used SP more than SH or no HAU ($P < 0.01$ for both). Cows using SH had decreased VT ($P < 0.01$) whereas cows with minimal HAU had greater VT ($P < 0.01$). Cows at lower THI with increased VT in both HAS and no HAU had increased lying bouts ($P < 0.01$ for all). Cows not using HAS had increased steps ($P < 0.01$) compared with cows with either HAU. At lower VT and LB, cows using both HAS as well as no HAU all spent more time standing ($P < 0.01$ for all). Given the choice, cows used both SH and SP systems on pasture. As THI fluctuates throughout the day, cows will alter HAU. The HAS used by cows on pasture influenced their VT and daily time budgets.

Key Words: heat abatement, heat stress, pasture

2693 Investigating the effect of feeding milk replacer or electrolytes on transported surplus dairy calf lying behavior.

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The objective of this study was to determine if feeding milk replacer or 2 different oral rehydration solutions (ORS) to surplus calves during a mid-transportation rest period (RP) effected lying behavior during transportation and in the days following arrival to a calf-raising facility. Surplus dairy calves (n = 87) were transported in 4 cohorts from February to July 2022 for 12 h to a holding facility, rested for an 8 h RP, and transported for an additional 6 h to a calf-raising facility. During the RP, calves were randomly assigned to 1 of 3 treatments: 2 L of milk replacer (MR; n = 29), a high sodium ORS developed for diarrhea (ORS-D; n = 29), or a high potassium ORS developed for transported calves (ORS-T; n = 29). Calves were fed 2 L of their treatment twice, upon arrival and before leaving the holding facility. IceQube accelerometers were attached to the hind left leg of each calf upon arrival to the holding

facility to record lying time, lying bouts, and motion index (index based on total step count and rate of acceleration of leg). Multivariable linear mixed models were used for analysis. The association of treatment with proportion of time spent lying (min lying/total min on the trailer × 100), lying bouts, and motion index during transportation were assessed. Daily lying time (h/d), lying bouts (no./d), and motion index were assessed for associations with treatment during transportation, and on the day of transportation (d 0) until 3 d post arrival to the calf-raiser. During transportation, calves fed ORS-T had a lower motion index than calves fed MR (−308.1; $P = 0.03$) but lying bouts were not associated with treatment. On d 0, the calves fed ORS-T had more lying bouts (+15.7; $P = 0.03$) and a lower motion index (−817.5; $P < 0.01$) than calves fed MR. On d 0, calves fed ORS-D also had a lower motion index than calves fed MR (−816.6; $P < 0.01$). There was no association of treatment with lying time on any day. These results suggest that MR fed to calves during a mid-transportation RP was associated with greater total activity compared with ORS calves, without compromising total lying time.

Key Words: calf, transportation, electrolytes

2694 Evaluating how rest periods impact calf activity following long-distance transportation.

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Recent changes to transport regulations in Canada include providing rest periods to surplus dairy calves when transported long distances. However, few studies have investigated how rest periods influence calf activity. The objective of this study was to determine how providing a mid-journey rest period (RP) impacts activity in calves transported for 16 h. This study was conducted between September 2022 to January 2023 and included 113 surplus dairy calves that were a median age of 9 d (range 7–19 d) at the time of transport from a commercial dairy farm in Ontario, Canada. Calves were enrolled 1 d before transportation and randomly assigned to one of 2 treatment groups: 1) continuous transportation by road for 16 h (58 calves), or 2) 8 h of transport, 8 h RP, then a further 8 h of transport to a single calf-raising facility (55 calves). On the day of enrollment, IceQube data loggers were attached to the left hind leg of each calf to monitor standing time, lying time, lying bouts, and steps for 2 d after unloading. Calves that received a RP were fed 2 L of milk replacer at the time of unloading for the RP and again before reloading for the second leg of transportation. Mixed models with repeated measures were built to assess how the RP influenced these activity parameters in the 2 d following transportation. The median (range) time lying on the day after arrival to the calf-raising facility was 17.49 h (9.75–23.62 h) for continuously transported calves, whereas calves that received the RP spent 18.97 h (14.88–21.30 h) lying. Additionally, calves that had received the RP spent more time lying (1.59 h, $P < 0.01$, 95% CI 0.75 to 2.43) and took fewer steps (−218.97 steps, $P < 0.01$, 95% CI −307.67 to −130.27) on the day after arrival than calves that were continuously transported. There were no significant differences in number of lying bouts d 1 after arrival ($P = 0.54$). No significant differences in lying time ($P = 0.58$), number of steps ($P = 0.82$), or lying bouts ($P = 0.79$) were found on d 2 after arrival. These findings show that providing a rest stop to calves was associated with a greater need to rest on the day after transport compared with continuously transported calves.

2695 Brush use monitoring using data integration and computer vision.

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Mechanical brushes facilitate grooming behavior and changes in brush use may indicate compromised welfare. However, existing methods for measuring brush use, including direct observation and automated techniques, are either labor-intensive or require custom hardware. In this study, we aimed to design a system for measuring individual brush use in group-housed dairy cattle by merging data from brush rotation with automated identification of cattle detected near the brush. We installed a commercial rotating brush in a pen of 24 dairy cows and collected data for 3 d. The brush was equipped with a processing unit that recorded rotation, and 2 methods were tested for individual cow identification: 1) Radio Frequency Identification (RFID) using ear tags; and, 2) computer vision using fiducial markers attached to the cow's collars (detected by a camera connected to a Raspberry Pi). Machine Learning algorithms were trained based on human observation to separately integrate data from each identification method with rotation data and output individual brush use. We evaluated the performance of supervised (Random Forest, Logistic Regression, Artificial Neural Networks) and unsupervised (K-means, Gaussian Mixture) algorithms using 2 d of data for training (66%) and one day for testing (33%). The most accurate RFID-based and Computer Vision-based methods were compared based on their agreement with the human observer's total daily brush usage for each cow in the group. The RFID-based method showed moderate agreement ($r = 0.63$, $P = 0.001$) whereas the Computer Vision-based method resulted in high agreement ($r = 0.89$, $P < 0.001$) with human observation. These findings suggest that, when integrated with data on brush rotation, the use of camera-aided fiducial markers is a promising option for accurately measuring individual resource use in group-housed dairy cattle.

Key Words: precision livestock, animal welfare, mechanical brush

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2696 Evaluation of antimicrobial combination therapy to control bovine mastitis pathogens. A. J. Moreira*[1,2], K. Araujo[2], K. Camargo[3,1], A. Assumpcao[1], N. Aulik[1], and H. Mantovani[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Universidade Federal de Vicosa, Vicosa, MG, Brazil,* [3]*Universidade Estadual Paulista, Jaboticabal, SP, Brazil.*

Antimicrobial resistance (AMR) has been recognized as an urgent global public health threat and risk factors have been linked to the heavy use of antibiotics in farm animals. Combinatorial antimicrobial therapies have been proposed as an alternative to overcome resistance. In this study, the objective was to evaluate potential synergisms between an antimicrobial peptide (PEP1) and a chemical adjuvant (CA1) against mastitis pathogens. The panel of target organisms was composed of 35 strains isolated from mastitic milk representing a range of etiological agents including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus chromogenes*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*. Synergism assays were performed using the chequerboard method to obtain the Fractional Inhibitory Concentrations Index (FICI). Cultures were grown in cation-adjusted Mueller–Hinton (MH) broth with the inoculum standardized at 10^5 cfu.mL^{-1}. Plates were incubated at 37°C for 24 h and optical density (600 nm) was monitored. FIC Indexes (FICI) were calculated as $\mathrm{FIC_{CA1}/MIC_{CA1} + FIC_{PEP1}/MIC_{PEP1}}$. A FICI of ≤ 0.5 was used as the threshold to indicate synergism. Time-dependent killing assays were performed against *K. pneumoniae* and *S. aureus* to evaluate the bactericidal efficacy of the combinations. FICI results varied from 0.1 to 0.5. The combination of 32 µg/mL (PEP1) and 1024 µg/mL (CA1) showed the best antimicrobial activity against all strains tested. When combined, the concentration of PEP1 and CA1 required to inhibit bacterial growth decreased by 4x and 16x, respectively. Some bacterial regrowth was observed after 24 h of incubation when cultures were incubated with PEP1 alone. However, this phenomenon was not observed when *S. aureus* or *K. pneumoniae* were exposed to the PEP1+CA1 combination. These results support the hypothesis of greater efficacy and complementary activities for the combined molecules. Results indicate the PE1+CA1 combination as an alternative antibiotic-free formulation to control multiple species of major mastitis pathogens.

Key Words: synergism, bactericidal activity, chequerboard

2697 Molecular characterization of *Staphylococcus aureus* isolates implicated in subclinical mastitis in Malaysian dairy herds. B. Ali*[1] and Z. Zakaria[2], [1]*Department of Biological Sciences, Sule Lamido University, Kafin-Hausa, Kafin-Hausa, Jigawa, Nigeria,* [2]*Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine, Universiti Putra, Malaysia, Serdang, Selangor, Malaysia.*

Staphylococcus aureus is a well-known contagious and opportunistic pathogen that causes both clinical and subclinical bovine mastitis globally. The sequence types (STs) of *S. aureus* isolates associated with bovine mastitis in Malaysia have not been previously studied. The aim of this study is to characterize the *S. aureus* isolates implicated in subclinical mastitis from Malaysian dairy herds. *S. aureus* isolates were recovered from subclinical mastitis across 33 (33) Malaysian dairy herds. A hand glove was used to collect the milk sample following standard procedures. Multilocus sequence and typing were carried out using a boiling method. A total of 10 (n = 10) *S. aureus* isolates were preliminarily analyzed. Six different community-associated (CA) sequence types (STs) were identified. These are ST97 (CC97) (n = 4) which is the most prevalent, followed by ST1 (CC1) (n = 2), and then ST221 (CC5) (n = 1), ST1496 (CC1) (n = 1), ST4427 (CC97) (n = 1) and ST2125 (CC1) (n = 1). Among the STs, ST2125 and ST1496 were first found in association with bovine mastitis. The most widely spread *S. aureus* ST associated with subclinical mastitis in Malaysia is identified as ST97. The molecular epidemiology of this ST along with other STs identified in this study in relation to different *S. aureus* STs around the world was defined.

Key Words: *Staphylococcus aureus*, multi-locus sequencing, mastitis

2698 Characterization of *Staphylococcus* isolates from subclinical mastitis cattle in the free state Province, South Africa. N. G. Khasapane*[1], K. Myburgh[2], Z. T. H. Khumalo[3,4], S. J. Nkhebenyane[1], and O. M. M. Thekisoe[5], [1]*Department of Life Sciences, Centre for Applied Food Sustainability and Biotechnology, Central University of Technology, Bloemfontein, South Africa,* [2]*Department of Animal Sciences, Faculty of Natural and Agricultural Sciences, Bloemfontein, South Africa,* [3]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, University of Pretoria, Pretoria, South Africa,* [4]*Clinvet International, Study Operations, Uitsig Road, Universitas, Bloemfontein, South Africa,* [5]*Unit for Environmental Sciences and Management, North West University, Potechsftroom, South Africa.*

Mastitis is an important disease in dairy industry causing major economic burden. The disease has an economic impact on farms, either directly or indirectly, through reduced milk production and quality, high culling rate, decreased reproductive performance as well as treatment and control costs. The aim of this study was to assess prevalence of Subclinical mastitis (SCM) in dairy cows and further characterize staphylococcal species isolated from cows milk in the Free State province using Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and Polymerase chain reaction (PCR) sanger sequencing. According to the threshold set by National Mastitis Council's guidelines on normal and abnormal raw milk based on SCCs, cows with SCC of 1x10^5 cells.mL^{-1} or less indicated an uninfected cow; SCC of 1x10^5 cells.mL^{-1} - 2x10^5 cells.mL^{-1} would indicate that a cow had intramammary infection in at least one or more teats; and SCC of 2x10^5 cells.mL^{-1} - 5x10^5 cells.mL^{-1} or greater indicated that the cow was infected significantly and probably had high bacterial counts. Therefore, out of 166 cows that were screened for subclinical mastitis only 55 (33.13%) were considered to have intramammary infection/subclinical mastitis at a cow level, while the quarter-level prevalence was 87/160 (54%) for intramammary infections using somatic cell counts (SCC) and California mastitis test (CMT). Thereafter from 50 bacterial culture isolates cultured, 48 (96%) were identified as Staphylococci species i.e., *S. aureus* 36 (72%*)*, *S. chromogenes* 6 (12%), *S. epidermidis* 2 (4%), *S. haemolyticus* 2 (4%) while 2 samples were mixed cultures between *S. aureus* and *S. hyicus* 2 (4%) by MALDI-TOF MS. The 16S rDNA PCR products and obtained sequences indicated that *S. aureus* was the dominant species infecting tested cows at 76%, followed by *S. chromogenes, S. agnetis, S. argenteus* and *S. devriesei* at 10%, 4%, 4%) and 1% respectively. This study has shown the presence of diverse *Staphylococcus* species infecting subclinical mastitis cows in sampled areas of the Free State province. It further indicated the importance

Table 1 (Abstr. 2699). 305 ME at dry-off (DO) and 30-day postpartum outcomes

Item[1]	305 ME at DO (kg)	*P*-value	30-d milk (kg)	*P*-value	30-d mast rate (%)	*P*-value	30-d dead rate (%)	*P*-value	30-d sold rate (%)	*P*-value
SDO	14,285	<0.001	1,198	0.068	4.30	>0.14	1.08	>0.48	1.80	>0.90
SDCT	13,092		1,161		1.30		0		1.27	

[1] SDO = standard dry-off treatment; SDCT = selective dry cow therapy.

of using various diagnostic tools for bacterial infection surveillance involved in subclinical mastitis to ensure accurate species identification.

Key Words: mastitis, raw milk, *Staphylococcus*

2699 Using milk sensor technology for determining which cows to dry off without the need for antibiotic (selective dry cow therapy).

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SomaDetect is a Light Scattering Sensor (LSS) device mounted on the milk line that collects and transmits a series of images to the cloud for analysis using proprietary machine learning to measure SCC and other parameters. With Selective Dry Cow Therapy (SDCT) still largely unused by US dairies, either because they do not use SCC testing (cost) or are concerned that excluding a dry-off antibiotic will create adverse production or cow health effects, the goal of this study was to determine if the sensors and an appropriate algorithm could choose cows for SDCT with no adverse effects compared with standard DO treatment (SDO). Cows selected for SDCT were prepped based on NMC Guidelines. Milk samples were taken for the SDCT cows only (to determine specificity of the SDCT selection), teats were re-prepped, internal teat sealant was infused, and teats were post-dipped. All other cows were prepped and infused with an antibiotic and internal teat sealant. SDCT criteria included: no daily avg. SCC >200,000 for last 60 d, no clinical mastitis for last 30 d, <2 clinical mastitis cases during lactation, and ≤24.9 kg of milk/day at DO (owner's request). There were 435 cows with 278 receiving SDO and 157 receiving SDCT. No cows in either group developed mastitis while Dry. No major pathogens were cultured in the SDCT group (Specificity = 100%), confirming that the sensors and algorithm were effective at selecting animals for SDCT. Mastitis, Mortality, and Sold Rates were not different (Chi-Square analysis). The owner's request to include animals with > 24.9 kg. for SDO selected for higher production animals (ANOVA on 305 Day ME). However, using the F-test ANOVA with a mixed model (fixed treatment and cow age and random group effects), average 30-d Cumulative Milk was not different ($P = 0.068$; Table 1). The use of the LSS devices and algorithm accurately determined animals for SDCT, saving $18.23 (no antibiotic) per cow, with no adverse effects.

Key Words: selective dry cow therapy, SomaDetect, sensors

2700 Changes in milk exosome miRNAome in association with a *Staphylococcus aureus* intramammary infection.

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Bovine-specific miRNAs have been identified as promoters of the inflammatory response to intramammary infection (IMI). Most milk miRNAs are carried by exosomes but the impact of an IMI on the expression of milk exosome miRNAs is still largely unknown. We aimed to characterize the miRNA expression profile in bovine milk exosomes, before and after an experimental IMI with 54 cfu of *Staphylococcus aureus* (*spa* type t529), to better understand localized host immune response, and to investigate the miRNA-based crosstalk between the host and the milk microbiota. Milk was sampled from 11 lactating Holsteins, 2 quarters each. Quarters were randomly assigned as infected or non-infected and both quarters were sampled before infection and then 2 weeks post-infection when the infection reached an average of 2.99 ± 0.81 log10 cfu /mL of milk/quarter. Milk exosomes were isolated, and miRNA profiles were obtained using Illumina NovaSeq 6000 small RNA technology. Bovine miRNAs and human miRNA homologs were identified using miRBase 22, and differential expression was calculated using DESeq2. Part of the non-host genome sequences were identified as belonging to *S. aureus*, indicating that the experimental infection was successful and that there is a cross-talk interaction between the pathogen and the host exosomes. Regardless of treatment, the core milk exosome miRNAome shared by all the cows consisted of 657 miRNAs, with bta-miR-148a being the most expressed. From the 1154 miRNAs identified across all samples, 106 miRNAs were identified only in the non-infected quarters, while 47 were expressed exclusively after infection. Hsa-miR-320b, bta-miR-155, bta-miR-146a, bta-miR-142–5p and bta-miR-223, which have been linked with mastitis, were downregulated with infection, while bta-miR-487b was upregulated. These results imply that miRNA abundance is potentially related to specific pathogens infection but that the mechanisms underlying the infection response require further analysis. Understanding the role of miRNAs in the process of inflammation could result in the development of rapid IMI assays based on specific host-pathogen miRNA-induced response.

Key Words: microRNA, exosomes, *Staphylococcus aureus*

2701 Staphylococcal intramammary infections in dairy cows: Prevalence at calving in first lactation cows and prevalence, incidence, and persistence over the dry period.

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First lactation cows may already have intramammary infections (IMIs) at calving, and these may be acquired during different stages of their development. The dry period (DP) is another critical "non-lactating" period for acquisition of new IMIs, which may persist until calving. The objective of this study was to describe the species-specific staphylococcal IMI prevalence at calving in first lactation cows and their prevalence, incidence and persistence over the DP in dairy cows. Quarter-milk samples were collected from each recruited cow. The first lactation

Table 1 (Abstr. 2701). The distribution of the staphylococcal IMIs in dry cows

Species	Prevalence, % (95CI) before DP	Prevalence, % (95CI) at calving	Incidence, % (95% CI) during DP	Persistence, % (95% CI)
Staphylococcus chromogenes	7.3 (6.0–8.7)	3.4 (2.5–4.3)	1.9 (1.2–2.8)	22.1 (14.6–31.3)
Staphylococcus epidermidis	3.9 (3.0–5.0)	1.8 (1.2–2.5)	1.4 (0.8–2.2)	11.8 (4.4–23.9)
Staphylococcus haemolyticus	3.9 (3.0–5.0)	0.8 (0.4–1.4)	0.7 (0.4–1.4)	1.9 (0.05–10.1)
Staphylococcus aureus	1.4 (0.9–2.2)	1.6 (1.0–2.3)	1.3 (0.8–2.0)	33.3 (14.6–56.9)
Staphylococcus simulans	1.3 (0.8–2.0)	0.8 (0.4–1.3)	0.5 (0.2–1.0)	17.7 (3.8–43.4)
Staphylococcus xylosus	0.7 (0.4–1.3)	0.8 (0.4–1.3)	0.5 (0.2–1.0)	27.3 (6.0–60.9)

cows were sampled twice at early lactation. Other cows were sampled twice before DP, and twice at early lactation. Staphylococcal species were identified using bacteriological culture followed by MALDI-TOF mass spectrometry. In total, we obtained the results for 318 quarters of 80 first lactation cows at calving and for 1762 quarters of 447 cows around DP. Most frequently isolated species among first lactation IMI were *Staphylococcus chromogenes* (10.5%, 95% CI 7.2–14.7%) and *Staphylococcus equorum* (2.1%, 95% CI 0.8–4.5%). Table 1 summarizes the results of most frequent staphylococcal IMIs in cows before and over the DP. *Staphylococcus agnetis* and *Staphylococcus capitis* were isolated solely from post-calving milk samples of first lactation cows and *Staphylococcus epidermidis*, solely from the samples from cows before and after the DP. Our study highlights that some staphylococcal IMIs are already present at first calving and others may persist over the DP.

Key Words: staphylococcal intramammary infection, dry period, MALDI-TOF

2702 Identification of functional SNPs and INDELs associated with mastitis in Holstein dairy cows using whole genome sequencing and RNA-sequencing.

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In the dairy cattle industry, mastitis infections significantly impact the profitability and health of herds. As such, there is interest in discovering new and efficient ways to reduce the incidence of mastitis. One approach is through integrating data from high-throughput transcriptomic and genomic technologies. Previous research using RNA-Sequencing (RNA-Seq) on milk somatic cell samples from Holstein dairy cows identified 3 differentially expressed (DE; FDR <0.05, |FC| > 2) mRNA isoforms (*CREBBP, HIF-1*α, and *SMARCA4)* associated with mastitis. These mRNA isoforms are involved in the immune response to infection in Holstein dairy cows. In the present study, whole genome sequencing (WGS) was completed using hair samples collected from the tail of 23 Holstein dairy cows (n = 11 healthy and n = 12 mastitic). Using the WGS data, the 3 DE functional candidate mRNA isoforms were studied more in-depth to identify variants, including single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) that were fixed and uniquely present in the healthy and mastitic groups of cows. Preliminary results identified 58 variants (26 SNPs and 32 INDELs) located within the DE mRNA isoforms. The goal of this research was to identify key structural variants present in the genome that may impact susceptibility to mastitis. In turn, this research could benefit and positively impact the dairy industry by integrating these variants (SNPs and INDELs) into genomic breeding programs and aid producers to select superior animals to breed, leading to decreased prevalence of mastitis in their herds.

Key Words: mastitis, RNA-sequencing, whole genome sequencing

2703 The effects of feeding CLOSTAT (*Bacillus subtilis* PB6) to lactating dairy cows in an automated milking system as measured by milk production, early lactation culling, mastitis incidence, and somatic cell count.

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The understanding of intestinal health and the role it plays in overall animal health has been growing over the past 2 decades. *Bacillus subtilis* PB6 has been shown to both inhibit pathogenic bacteria that can play a role in developing leaky gut and reduce gut inflammation. To evaluate the effects of supplementing *Bacillus subtilis* PB6 on early lactation culling, milk production, mastitis and somatic cell count (SCC), 600 head of Holstein dairy cows (170 DIM) located in a herd in the US Midwest equipped with an automated milking system (AMS) were enrolled in the study. At the start of the study (completed over 6.5 mo), lactating cows from 5 pens were blocked by days in milk and randomly assigned to one of 2 treatments: Control (CON; n = 269) or *Bacillus subtilis* PB6 (PB6; n = 266). Periparturient cows (n = 281) were alternately assigned to one of the 2 treatments after calving. The robotic milkers had 2 feed sources: a bin containing the base pellet (Bin 1) and a second bin (Bin 2) containing the base pellet in addition to *Bacillus subtilis* PB6 (CLOSTAT 500, Kemin Industries, Inc., Des Moines, IA), allowing a feeding rate of 0.5 g/head/day. All CON cows were fed a partially mixed ration (PMR) and the base pellet (Bin 1), and all PB6 cows received PMR with 1.8 kg of pellets from Bin 2 (substituting 1.8 kg pellets from Bin 1). Milk production and health parameters were tracked with AMS software. Continuous and categorical data were analyzed using the MIXED and Chi-Square procedures of SAS, respectively (release 9.4, SAS Institute, Inc.). Cow was treated as experimental unit and dietary treatment as fixed effect. Milk production during the first 60 DIM was greater (0.73 kg ± 4.71) for PB6 cows than CON cows ($P = 0.05$). Somatic cell count was 99,000 cells/mL ± 93,550 greater for PB6 than CON cows during the first 60 DIM ($P < 0.01$), but tended to decrease 28,000 cells/mL ± 90,166 for PB6 more than CON after 200 DIM ($P = 0.07$). Mastitis incidence in PB6 cows was 19% compared with 39% for CON cows ($P < 0.01$). Early lactation culling was not affected by treatment. *Bacillus subtilis* PB6 supplementation was shown to increase milk production during early lactation, decrease mastitis incidence and reduce SCC as lactation progressed.

Key Words: *Bacillus subtilis,* mastitis, somatic cell count

2704 Withdrawn.

2705 Management culture and mastitis on dairy farms in the United States.

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Bovine mastitis is an inflammation of the udder of dairy cows that is caused by bacteria. Mastitis remains the most important infectious disease of dairy cattle that poses a burden on dairy farmers because it results in cow and milk losses and affects the quality of dairy products. Despite progress in managing and treating mastitis, it has persisted, which may have more to do with the management culture on dairy farms than the already proven mastitis control practices such as milk and treatment protocols. Using data from a 15-mo trial research project of 124 dairy herds in Michigan (n = 59), Pennsylvania (n = 57), and Florida (n = 8), this study focuses on the influence of the management culture on dairy farms on the prevalence of mastitis and change in mastitis overtime. Herds on DHI Somatic Cell Count (SCC) in each state were stratified by herd size and then randomly assigned to either intervention or control group. The intervention consisted of on-farm evaluation of parlor and milking routines, milking practices, milking system, cow environment, monitoring and therapy of infected cows, and farm management culture. The intervention herds had additional visits to monitor employee behaviors. We interviewed both managers and employees at the beginning and end of the trial and supplemented those data with monthly DHI SCC reports for each herd. Findings show that there were significant differences in the prevalence of mastitis at the beginning of the intervention and overtime, but no significant differences in the prevalence of mastitis between intervention and control groups. The perception of mastitis as a problem on farm at baseline was negatively related to the prevalence of mastitis. Owners/managers who strongly disagree that mastitis is a problem on their farm had lower average prevalence of mastitis. We also found that owners/managers who frequently talk to their employees had lower prevalence of mastitis as compared with those who rarely or occasionally talk to them. These findings remain significantly related to the prevalence of mastitis even after controlling for state, herd size, milking protocols, treatment protocols, and housing environment. These findings highlight the importance of the management culture on dairy farms in preventing mastitis.

Key Words: mastitis, management culture

2706 Development of a bio-economic model to study mastitis.

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Mastitis is one of the most common diseases in dairy cattle. It has a high impact on farm economy, farmers' working time and antibiotic consumption. The objective of our study was to develop a bioeconomic model to study different mastitis management scenarios (e.g., selective dry cow therapy, vaccination, selective treatment in lactation). A stochastic dairy simulation model (DairyHealthSim) based on a weekly Markov chain model was used to simulate herd dynamics, reproduction, production, culling decisions, health outcomes, and management of health events. A specific module was developed for the simulation of quarter-level IMI acquisition and elimination. They are simulated for each quarter using baseline risks for each etiological agent (*Staphylococcus aureus, Streptococcus uberis, Escherichia coli, Klebsiella* spp., Non-aureus staphylococci, and streptococci). For contagious mastitis, the computed risk considers contamination between quarters of a cow and between cows in the herd. Each infected quarter moves between different states: subclinical, clinical grade 1, 2 or 3. Each state determines the impact on the quantity and quality of milk produced by the quarter, the conception rate of the infected cow and the treatment to be applied by the farmer or veterinarian. Blanket dry cow therapy with internal teat sealant was included as a baseline drying-off management scenario to which alternative management approaches will be compared (analyses ongoing). Calibration data were based on previous literature. Finally, 25 different IMI risk scenarios were defined to represent herds with various udder health situations. After running this baseline simulation 100 times for the equivalent of 15 years, several indicators were used to evaluate the model's realism: the average herd somatic cell count in simulated herds was 177,188 cells/mL. Cows had an average of 1.4 new IMI/y and an average of 0.35 clinical mastitis episodes per year. On average, each wk 18% of the cows were over 200,000 cells/mL. Cows received an average of 1.2 defined course doses of antibiotics per lactation. We developed a realistic and stable bio-economic model simulating quarter-level IMI in dairy cows. In the future this model could be used to investigate the economic impact of various mastitis management strategies.

Key Words: mastitis

2707 Computation of a nomogram to estimate the 60-day probability of death or culling due to severe clinical mastitis in dairy cows at first veterinary clinical evaluation.

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Severe clinical mastitis (CM) is a frequent disease of dairy cattle. An effective mean of predicting death or early culling despite treatment would be helpful for making early euthanasia decisions for poor prognosis cases. The objective of the study was to develop a nomogram for prediction of death or culling (non-survival) in the 60 d following a severe CM episode in dairy cows at first veterinary visit in farm settings. A nomogram is a tool to easily compute results of a multivariable model. A prospective study was conducted by 5 veterinary practices in Québec, Canada. A total of 222 dairy cows presenting a case of severe CM and examined for the first time by a veterinarian were included. Several physical and laboratory (complete blood cell count, L-lactate, cardiac troponin I, milk culture) variables were recorded. Animals were followed for 60 d after the initial veterinary visit for death or culling events. Missing data were considered as missing at random and missing values were handled via multiple imputation. An adaptive elastic-net Cox proportional hazards model was built with all predictors as explicative values. A graphical and a web-based nomogram were computed from this model. A total of 27% (n = 54) of the enrolled animals died within 60 d of the severe CM episode and an additional 27% (n = 54) were culled during the same period. Several variables were selected by the regression technique: lactation number, being a downer cow, presence of depression, capillary refilling time, ruminal frequency, dehydration level, lactates concentration, hematocrite, band neutrophils count, monocyte count, and milk bacteriology. The model was discriminative (C-index of 0.71). In conclusion, we were able to develop a nomogram predicting the survival of dairy cows affected by a severe CM episode following a first veterinary visit in farm setting. It could be used for early euthanasia decisions in animals that would not survive despite treatment. To facilitate the use of this nomogram by veterinarians, a web-based app was also developed (https://tlp-umontreal.shinyapps.io/paraclinical_nomogram/).

Key Words: mastitis, nomogram

2762 Investigating the effect of feeding electrolytes or milk replacer on nonesterified fatty acids, β-hydroxybutyrate, and glucose concentrations in transported surplus dairy calves. A. Bajus*[1], K. C. Cruetzinger[2], M. C. Cantor[1,3], D. Kelton[1], J. Wilms[4,5], M. A. Steele[5], and D. L. Renaud[1], *[1]University of Guelph, Department of Population Medicine, Guelph, Ontario, Canada, [2]University of Wisconsin–River Falls, River Falls, WI, [3]Penn State University, Department of Animal Science, University Park, PA, [4]Trouw Nutrition, Amersfoort, the Netherlands, [5]University of Guelph, Department of Animal Biosciences, Guelph, Ontario, Canada.*

The objective of this study was to determine the effect of feeding milk replacer (MR) or 2 different oral rehydration solutions (ORS) to surplus calves during a mid-transportation rest period (RP) on blood metabolites indicative of energy and fat mobilization. Surplus dairy calves (n = 65) were transported in 4 cohorts from February to July 2022 for 12 h to a holding facility, rested for an 8 h RP, and transported for an additional 6 h to a calf-raising facility. During the RP, calves were randomly assigned to 1 of 3 treatments: MR (n = 20), a high sodium ORS developed for diarrhea (ORS-D; n = 22), or a high potassium ORS developed for transported calves (ORS-T; n = 23). Calves were fed their 2 L treatments twice, once upon arrival and again before leaving the holding facility. At the holding facility, blood samples were taken after unloading and before reloading before feeding treatments. Blood samples were also taken after arrival to the calf-raising facility, after unloading, and at 24 h and 48 h. Concentrations of nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) were assessed at a commercial laboratory. Glucose concentrations were analyzed immediately with an i-STAT Alinity hand-held analyzer. Linear mixed models were used to assess the impact of treatment on NEFA, BHBA, and glucose concentrations. At reloading, calves fed the ORS-T had greater concentrations of NEFA (+0.1 mmol/L; $P = 0.05$, 0.0 to 0.2) and BHBA (+24.9 μmol/L; $P = 0.05$, 0.1 to 49.7) than calves fed MR. At arrival to the calf-raising facility, calves fed the ORS-T and ORS-D both had greater concentrations of NEFA (ORS-T: +0.1 mmol/L; $P = 0.03$, 0.0 to 0.2; ORS-D: + 0.2 mmol/L; $P < 0.001$, 0.1 to 0.3) and BHBA (ORS-T: 34.8 μmol/L; $P < 0.01$, 10.1 to 59.4; ORS-D: +36.1 μmol/L; $P = 0.005$, 10.7 to 61.5) than calves fed MR. There were no differences in glucose concentrations between the treatment groups. The results of this study suggest that feeding milk replacer during a mid-transportation RP can minimize fat mobilization and energy expenditure compared with feeding oral electrolyte solutions.

Key Words: calf, transportation, electrolyte

Breeding and Genetics 3: Advances in Methods for Genetic Improvement

2708 Single-step genome-wide association analysis with *P*-values for large genotyped populations. N. G. Leite, M. Bermann, S. Tsuruta, I. Misztal, and D. Lourenco*, *University of Georgia, Athens, GA.*

Most of the methods used for GWAS can account for population structure but do not consider phenotypes for non-genotyped individuals. Single-step methods can combine information on genotyped and non-genotyped individuals because of the use of a joint pedigree and genomic relationship matrix. Single-step GWAS can be utilized in large genotyped populations; however, only SNP effects and variance explained were possible in such a case. A significance test based on p-values was available only for small genotyped populations, i.e., up to 50k, depending on the model. This is because p-values rely on the prediction error variance (PEV) for each SNP effect, which is backsolved from the prediction error covariance for animals, requiring the inverse of the left-hand side (LHS) of the mixed model equations. When using more than 50k genotyped animals, single-step genomic BLUP (ssGBLUP) methods rely on a sparse representation of the inverse of the genomic relationship matrix (G-1) computed with the APY algorithm. In APY, genotyped animals are split into core and noncore, and recursions on the core animals are used to compute G-1 at a low cost. With APY, the PEV of SNP effects relies only on the information of core animals, varying from 4k to 6k in pigs and chickens and 10k to 15k in cattle. Instead of using the inverse of the LHS, we approximated the PEV for core animals, then computed PEV and obtained p-values for SNP effects. We obtained similar GWAS resolution between the inverse and approximation using 50k genotyped animals, 1.5M animals in the pedigree, and 850k phenotypes. We then ran ssGWAS with p-values using 450k genotyped animals. As expected, a better resolution was observed when using 450k genotyped animals compared with 50k. Having single-step GWAS for large genotyped populations is feasible and allows including all available data in association studies.

Key Words: significance test, single-step genomic BLUP, QTL mapping

2709 Improving the efficiency of heritability estimation with genomic information—Method R. M. K. Hollifield*, J. Hidalgo, F. Bussiman, D. Lourenco, and I. Misztal, *University of Georgia, Athens, GA.*

Estimating heritabilities with large genomic models is computationally expensive and often impossible. For feasible heritability estimation, the amount of data can be reduced by taking samples or using generation intervals; however, this could introduce biases. The purpose of this study was to test and compare heritability estimation with genomic information using AIREML and a modification of Method R. A simulated population was generated using QMSim and contained 10 generations of 5,000 animals each. Each animal contained a record for a trait with a heritability of 0.3, a phenotypic variance of 10.0, and was genotyped at 50k SNP. Method R works by regressing EBV calculated with the whole data set (EBV_w) on EBV calculated from a partial data set (EBV_p). In this study, we used GBLUP and, therefore, GEBV, and the partial data set was created by removing the last generation of data. Predictive ability was also tested by finding the correlation between adjusted phenotypes and $GEBV_p$. It is expected that the intercept (b_1) of this regression will equal one, and predictive ability will be maximized when the correct heritability is used. We estimated the heritability for several intervals with varying numbers of consecutive generations. In every scenario, predictive ability increased as more data were used and maximized with the simulated heritability. However, the predictive abilities for all intervals and at all heritabilities tested did not differ more than 0.01, suggesting predictive ability is not the best indication for heritability estimation. For Method R, b_1 was one when all generations were used and deviated from one when fewer generations were used, suggesting Method R works best with more data. For the whole data set, the heritability was estimated as 0.30 ± 0.00, 0.26 ± 0.01, and 0.30 ± 0.04 for the simulated values from QMSim, AIREML with genomics, and Method R with genomics, respectively. Method R improved timing by 83%, reducing the time from 9.5 h to 1.6 h on average. Method R can be used to estimate heritabilities with large genomic information at a low cost when many generations of animals are present.

Key Words: linear regression, predictive ability, variance components

2710 Converting linear breeding values to probabilities for health traits in dairy cattle. J. Hidalgo*[1], S. Tsuruta[1], D. Gonzalez[2], G. de Oliveira[2], M. Sanchez[2], A. Kulkarni[2], C. Przybyla[2], G. Vargas[2], N. Vukasinovic[2], I. Misztal[1], and D. Lourenco[1], [1]*Department of Animal and Dairy Science, University of Georgia, Athens, GA,* [2]*Zoetis Genetics and Precision Animal Health, Kalamazoo, MI.*

Phenotypically expressed as binary, health traits are evaluated using threshold models assuming normally distributed underlying liabilities. Breeding values obtained in the liability scale are transformed into probabilities to express the phenotype of interest and are used to rank animals easily. Threshold models require significant computing time and face convergence challenges because they imply a nonlinear system of equations. To overcome these challenges, we can use linear models because the solutions from both models are highly correlated (≥0.95); however, linear breeding values are on a different scale, needing to be scaled to the liability scale to be transformed and used as probabilities. The objective of this study was to derive a formula to approximate the breeding values on the linear to liability scale. The proposed formula

Table 1 (Abstr. 2710). Number of records, incidence, heritabilities in liability and linear scales, and correlations between genomic estimated breeding values (liability-linear scales) and probabilities for health traits

					Correlation	
Trait	Records (in millions)	Incidence	h^2_{linear}	$h^2_{liability}$	GEBV	Probabilities
Mastitis	6.3	0.27	0.09	0.05	0.98	0.98
Displaced abomasum	5.1	0.02	0.09	0.01	0.96	0.96
Lameness	5.0	0.14	0.08	0.03	0.96	0.96
Ketosis	3.6	0.06	0.08	0.02	0.95	0.95

is: $\text{GEBV}_{\text{liability}} \approx \text{GEBV}_{\text{linear}} / [\sigma^2_{\text{e\_linear}} * (1\text{-}\lambda)]^{0.5}$, where λ is given by the ratio $h^2_{\text{linear}} / h^2_{\text{liability}}$. A dairy cattle population with a pedigree of 6M animals (1.7M with genotypes) and phenotypes for 4 health traits were used to test the approximation (Table 1). Univariate threshold and linear analyses were performed. Spearman rank correlations were used as metrics of the agreement between probabilities derived from threshold or linear models. The correlations were ≥0.95 and constant when transforming GEBV to probabilities (Table 1). Distributions of breeding values (not shown) in the probability scale obtained from the threshold and linear models almost entirely overlapped for all the traits but displaced abomasum, a trait with very low incidence. The overlapping distributions indicated an accurate approximation of breeding values on a linear to liability scale. The proposed transformation enables using linear models to analyze binary traits.

Key Words: threshold models, binary traits, liability

2711 Approximation of reliabilities for random-regression single-step genomic best linear unbiased predictor.

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Random-regression models (RRM) are used for dairy cattle genetic evaluations in many countries. The output of a genetic evaluation with RRM is an estimated breeding value (EBV) for a specific function of the additive genetic random regression coefficients, say, 305-d milk yield and its corresponding reliability. The reliability of an EBV in RRM is calculated from the inverse of the coefficient matrix of mixed model equations (MME). Without genomic information, the reliabilities of an RRM are approximated by weighing different information sources while processing the data and pedigree files twice. These approximation methods were successful for non-genomic evaluations because of the sparse structure of the inverse of the numerator relationship matrix. This advantage is lost when genomic information is included by using single-step genomic best linear unbiased predictor. Current methods to approximate reliabilities in ssGBLUP are not easily extended to RRM. Thus, the objective of this study was to develop an efficient method to approximate reliabilities for RRM with ssGBLUP. The method we proposed works as follows: (1) approximate 305-d reliabilities without genomic information, (2) back-solve the reliabilities to obtain effective record contributions (ERC), (3) obtain the reliabilities of a genomic best linear unbiased predictor (GBLUP) model using the ERC as weights, (4) remove double-counting of pedigree information for genotyped animals, and (5) propagate the genomic gain to non-genotyped animals using selection index theory. The method was tested with Uruguayan Holstein data. The data set had over 800,000 animals from which 4000 were genotyped. The correlation between the approximated reliabilities and the exact ones calculated from the inverse of the MME was 0.97. The slope and the intercept of the regression were 1.01 and 0.04, respectively. The reliabilities for all the animals were approximated in less than 15 min. Our method can be scaled for large-scale genomic evaluations by replacing the GBLUP model with a marker-effects model or by using the Algorithm for Proven and Young.

Key Words: random regression models, genomics, single-step

2712 Improved accuracy of a dynamic programming model to optimize replacement and insemination decisions for dairy cattle.

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Objective of this work was to develop a dynamic programming model with improved accuracy to support economically optimal replacement and insemination decisions for dairy cattle. Decisions on replacement and insemination now and in the future are made to maximize future cash flows. In most published research, dairy cattle are characterized by a limited number of discrete states, a combination of at least lactation number, stage of lactation, reproduction status, and level of milk production. Improvements in accuracy were made along 2 lines. First, in the developed model, new traits were added such as semen type and breed, feed efficiency, body weight, genetic merit, fertility and forced culling. Time step is a week. Dairy cows are replaced by calving heifers. Dairy heifers are replaced if purchased heifers are cheaper than the cost of raising. Insemination decisions are made for both dairy cows and heifers, including delay of inseminations, and choice of semen. However, the state space remains limited and may not always accurately represent an animal in the herd. Therefore, a second improvement is that a separate dynamic programming model is executed for each animal. This allows for every animal to have her own predicted milk yields, fertility, risk of health events, feed intake, etc. in the current parity. Cash flows in wk 1 of future lactations are calculated and stored by the developed traditional model. When the current parity transitions into the next parity, the best fitting future cash flows from wk 1 are chosen. Decisions regarding replacement and insemination are supported by keep values and insemination values. Preliminary results with animal records from multiple dairy farms show high sensitivity of optimal decisions to predicted milk production, which is amplified when milk prices are high. The conclusion is that the model is a step forward toward accurate optimization of replacement and insemination decisions for dairy cattle. However, accurate prediction of future animal performance is also necessary to make better decisions.

Key Words: profit, economics, culling

2713 Utilizing relationship value (R-value) to reduce inbreeding in the Canadian dairy cattle population.

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The increasing rate of inbreeding poses a threat to genetic diversity and tends to negatively affect economically important traits, such as those related to production, health and fitness. This potentially reduces the return on investment of producers. To simultaneously make genetic improvements, control inbreeding, and maintain genetic diversity, measures need to be taken to quantify genetic relationships to help with selection strategies and mating decisions. The genetic relationship value (R-value) represents the number of expected alleles identical by descent shared between an individual and a reference population. Therefore, the aim of this study was to estimate the pedigree-based genetic relationship (R-value) value across the Canadian Holstein cattle population and across herds. The data set used for the across population (within and across herds) R-value estimation consisted of 980 active sires (currently contributing to the Canadian dairy population) and 116,666 active cows from 3,924 herds born from 1999 to 2017 and 2006 to 2020, respectively. Additionally, the R-value estimation across herds was based on herds with 10% of their active cow population genotyped, resulting in a data set composed of 31,936 cows, 947 sires and 1,177 herds. All data were provided by Lactanet Canada and analyses were carried out using the PEDIG software with the par2 function for relationship estimation.

Results of the across population R-value showed that the active sire population had an average relationship ranging from 1% to 8.5% with the active cow population. Across herds, the average sires' relationship with cows ranged from 0.43% to 32.38%. In conclusion, our results showed that active sires' relationship with active cows had some variation. If these sires were randomly mated to the population of active cows, their progeny would show a range of inbreeding coefficient from 0.5% to 4.25%, which demonstrates the importance of planning the matings using R-value as a tool. Future work will incorporate genomic information so that farmers can make an informed decision during mating and selection processes to manage inbreeding.

Key Words: dairy cattle, relationship value, inbreeding

2714 Pedigree and genomic adjustments for single-step genomic BLUP applied to residual feed intake. A. Legarra*[1], M. J. Vandehaar[2], R. J. Tempelman[2], J. E. Koltes[3], H. M. White[4], K. A. Weigel[4], R. Baldwin[5], P. VanRaden[5], F. Peñagaricano[4], J. Santos[6], and K. L. Parker Gaddis[1], *[1]US Council on Dairy Cattle Breeding, Bowie, MD, [2]Michigan State University, East Lansing, MI, [3]Iowa State University, Ames, IA, [4]University of Wisconsin, Madison, WI, [5]USDA, Beltsville, MD, [6]University of Florida, Gainesville, FL.*

Use of VanRaden's genomic relationship matrix (G) requires, in principle, use of allele frequencies at the foundation of the pedigree ("base allele frequencies"). Use of ssGBLUP implies that genomic relationships of non-genotyped individuals are predicted from G using pedigree relationships in the numerator relationship matrix (A). This requires that both G and A describe relationships from the same base population, a requisite often called "compatibility." Three major methods to achieve compatibility are: (1) use of base allele frequencies ("base"); (2) use of observed allele frequencies across all genotyped animals, followed by a correction to force same average BV and same genetic variance ("tuning"); and (3) use of (fictitious) 0.5 allele frequencies followed by corrections of pedigree relationships to match observed genomic relationships through the theory of metafounders ("MF"). In theory the best alternatives are "base" for single base populations if these are known, and "MF" for multiple base populations or if base allele frequencies are difficult to estimate. Here we compare the 3 options in a subset of 9,000 Holstein individuals phenotyped for Residual Feed Intake, and a corresponding pedigree of 56,000 individuals, of whom 13,000 have genotypes. Base allele frequencies were estimated from old genotyped animals using findhap.f90. We modeled a single base population or metafounder, with gamma = 0.235 estimated by Maximum Likelihood. The correlations (r) and regressions (b) of genomic relationships G on pedigree relationships A are shown in Table 1. The best option was "MF" followed by "base" and then by "tuning." No method has regression slopes of 1 as might be expected. Correlations among GEBVs were: "base"-"tuning": 0.89, "base"-"MF": 0.998, "tuning"-"MF": 0.90, showing that the resulting GEBVs do differ depending on the different choices although, in this data set, "base" and "MF" resulted in nearly identical results. Heritability estimates were 0.16 and nearly identical in all cases.

Key Words: feed intake, single-step genomic BLUP, relationships

Table 1 (Abstr. 2714). Correlations (r) and regressions (b) of genomic relationships G on pedigree relationships A

Item	Base	Tuning	Metafounder
Diagonal elements			
Correlation	0.59	0.01	0.81
Regression slope	0.82	0.02	1.02
Off-diagonals			
Correlation	0.79	0.66	0.95
Regression slope	0.82	0.59	1.11

2715 Quality and value of imputing gene tests for all animals. J. R. OConnell*[1], P. M. VanRaden[2], and E. O. O. Ogwo[2], *[1]University of Maryland Baltimore, Baltimore, MD, [2]USDA Animal Genomics and Improvement Laboratory, Beltsville, MD.*

Genomic selection is driven by genotyping arrays designed for uniform coverage of the genome because most quantitative trait loci (QTLs) underlying the heritability of the trait are unknown. Labs have improved the arrays since 2014 with custom content by adding selected QTLs discovered from whole-genome sequencing (WGS) and high-effect markers from higher-density arrays. Which years and arrays include QTLs affects the number of animals genotyped and accuracy of imputing QTL for other genotyped animals. Breed differences, missing data rates, and error rates were investigated for 8 QTL gene tests currently imputed for all genotyped animals of 5 breeds plus crossbreds. Gene content for each gene test was predicted for non-genotyped relatives using methods like those used in single-step genomic evaluation, allowing potential direct selection across all animals. Some QTL have economic merit not yet included in national selection indexes such as 1) polled mutations near 1:2578598 (chr:position on ARS-UCD1 map) that suppress horn growth, improve animal welfare, and reduce farm labor, 2) β-casein allele (a2) at 6:84451299 in a milk protein gene that may improve digestibility, and 3) K-casein alleles near 6:84451299 that can increase cheese yield. Other QTLs mainly affect traits already in selection such as 4) DGAT1 QTL at 14:611019 affecting fatty acid metabolism, percentages, and yields of fat and protein, 5) BGHR QTL at 20:31888449 with large effect on protein percentage, and 6) the ABCG2 QTL at 6:36599640 with largest effect for Net Merit in Holsteins, but the favorable allele is now nearly fixed. Many other QTL have recessive lethal effects. For the 8 QTL studied, Mendel error rates were low except for polled in Jerseys and DGAT1 in most breeds. Imputation errors resulted in smaller effects for DGAT1 than the nearby flanking SNPs that are present on most arrays. Because some valuable gene tests are sold by labs rather than delivered with array genotypes, freely imputed QTLs could benefit breeders and progress. Decreasing costs of WGS data will increase power of QTL discovery, and more QTL genotypes should increase imputation accuracy, prediction accuracy, and economic gain.

Key Words: gene test, imputation, dairy cattle

2716 Standardizing lactation yields from national data with age-parity-season-region corrections for fair comparisons across individual cows and environments. A. Miles*[1], P. VanRaden[1], J. Hutchison[1], G. Fok[1], and M. Schutz[2], *[1]Animal Genomics and Improvement Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, [2]Department of Animal Science, University of Minnesota, St. Paul, MN.*

Multiplicative adjustment factors to standardize milk, fat, and protein lactation yields for age, parity, calving season, geographical region, milking frequency, and previous days open were last updated in 1994. Since then, the national animal model has estimated new adjustments within each 5-year period but those have not been publicized or summarized until now. New estimates were obtained using 101.5 million milk, 100.5 million fat, and 81.2 million protein lactation records from 1960 to 2022 in a multi-trait animal model. The pedigree file included 91.3 million animals of all dairy breeds and crossbreds. Along with breeding values for those animals, the model included 392 unknown parent groups, 39.9 million permanent environmental effects, 11.3 million herd by sire interactions, and regressions on pedigree inbreeding and heterosis. New age-parity factors were estimated within each period-breed combination and new season factors were estimated within each period-region combination. The final adjustment factor then multiplies the 2 new age-parity and season-region factors with the original previous days open and milking frequency factors to produce a single multiplier. We observed that age-parity factors are reduced in recent decades, indicating faster maturity rates. The new factors standardize records to 36 mo and second parity instead of mature equivalent, which was already the policy in genetic evaluations since 2005, to bring averages of standardized records much closer to actual herd averages. Seasonal effects are now estimated within 5 regions defined by each state's average climate zone scores. Within each region the seasonal differences are smaller in recent decades, suggesting that improved heat abatement and management is decreasing the effect of the environment on lactation yields. These new factors were designed for application to lactation records in the national database and will be distributed to cooperators who also use standardized records to encourage the widespread adoption of a uniform approach to fair comparisons of lactation yields.

Key Words: standardized lactation yields, mature equivalent

2717 Combined effects of milking intervals and frequencies.

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Dairy herd income depends on actual production, whereas the merit of individual cows can be better compared using standardized performance. Adjustment factors for milking interval estimate actual 24-h yield from morning or evening data (AM-PM records) but have not standardized to the higher yield the cow would have produced if milked at even intervals of 12 and 12 h. By contrast, daily yields with 3 milkings (3X) are standardized downward to the expected twice daily (2X) yield. Smooth, nonlinear functions can standardize for both number of milkings and milking intervals to account for increases in yield from 2X to 3X, 4X, etc., decreases from 1X milking, and decreases from uneven milking intervals. Both adjustments can be computed using a constant c to the power of -N for cows with N equally spaced milkings per day as NX factor = $(1 - c^{-N})/(1 - c^{-2})$. Division by $1 - c^{-2}$ simply sets the 2X factor to 1.0 for the standard milking frequency of 2. Constant c is obtained using numerical methods from a known 3X factor such as 1.11, which gives c = 2.5. Interval and frequency factors use i milking intervals as fractions of the 24-h day as weights (w_i = interval / 24) and equivalent milking frequencies (N_i = 24 / interval) that are not necessarily integers. Daily yield is then estimated as $\sum w_i * N_i$. For adjusting and reporting multi-day average yields, the combined factors are summed across days and then divided by the number of days (or partial days). The proposed function fitted well to actual data from 7,544 Holstein cows with differing milking intervals and could help when comparing cows within or across herds. For example, 3X takes 50% more milking labor than 2X and gives 9–14% more yield and components. A parlor milking 24 h and 3X / day would expect 11% less milk if dropping to 2X with 12-h intervals and a further loss of 4% with 8- and 16-h intervals by moving the morning and evening shifts closer. Standardizing records for differing intervals can more fairly compare cows, provide helpful management advice in herds milking with differing intervals or with robots, and improve genetic evaluation.

Key Words: milking frequency, milking interval, standardized records

2718 ABS Health Index's impact on commercial cow's longevity, disease incident rates, and herd economics.

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In April 2020, the ABS Health Index (AHI) was released as an economically weighted composite index. Traits included were ABS's propriety evaluations for Mastitis, Metritis, Ketosis, Heifer Survival, and Twinning Rate plus CDCB's evaluations for Displaced Abomasum, Hypocalcemia, and Retained Placenta. Including AHI in a custom index lets producers decide how much emphasis to place on cow health in their genetic goal to minimize health-related economic losses and improve their herd. This study was developed to analyze AHI's impact in commercial herds by analyzing ABS Real World Data on > 2.74 million daughters for longevity and incidence rates for 5 health traits (mastitis, metritis, ketosis, abortion, and twining rate) across 3 lactations and > 1.3 million calf records for heifer death rate. Animals were from > 2000 farms, spread over 20 countries, and from 11,752 AI sires. Incidence of conditions and related cost were tracked across parities and compared for bulls grouped in quartiles according to their AHI value. Sires in the top AHI quartile (average AHI = 118) had 60.9% of daughters performing in lactation 2 and 33.8% in lactation 3, while those in the bottom quartile (average AHI = 85) had 52.5% and 23.1% daughters in lactation 2 and 3 respectively. Daughters from sires in the top AHI quartile had on average a 2% lower incidence versus sires in the lowest quartile for the analyzed health traits. The total incidence cost of health conditions was applied to a 1,000 head herd example to determine that using top quartile AHI sires would have a positive $42,000 yearly economic impact from improved female health versus if sires in the bottom quartile had been used. Therefore, including AHI in a custom index will allow for selection of sires that fit a customer's genetic goals and produce healthier and long-lasting daughters that will translate into a more profitable and sustainable farm.

Key Words: health, genetics, profitability

2719 Integrating foreign information into single-step evaluations in US Holsteins.

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Interbull collects EBV based on BLUP from member countries and combines them into MACE proofs. After excluding the information from its own country to avoid double-counting, each country can use these proofs as external information in its evaluation system with the expectation of increased reliability. The EBV from BLUP is affected by genomic preselection at a level specific to each country, and each country may apply specific adjustments to account for the biases. If genomic preselection is not adequately accounted for, the use of external information may reduce reliability. Two methods have been considered for incorporating external information into single-step GBLUP (ssGBLUP) evaluation. The first method is based on Bayesian priors while accounting for base change. The second method treats the external information as pseudo-phenotypes with heterogeneous residual variances. Fixed effects in the second approach can account not only for the base but also for the different amounts by preselection by year, possibly nested within the country of origin. The 2 methods were applied in ssGBLUP using US Holstein data. A total of 42.4M phenotypes for milk fat and protein yields recorded from Jan 2000 to June 2020 were available for 18.3M cows. Pedigree information was recorded on 24.5M animals, of which 1.2M were genotyped. A 3-trait repeatability model was applied to a complete (reduced) data set with phenotypes of cows born from

1992 to 2018 (2014). A group of 3,584 bulls was used to validate the scenarios based on the coefficient of determination (reliability) and the coefficient of the regression (b1) of daughter yield deviation on GEBV. Preliminary results showed that both methods were equivalent, but the increase in reliability for US bulls was minimal because the external information was a small fraction of the domestic data.

Key Words: external information, MACE, single-step genomic BLUP

Forages and Pastures 1

2720 Chemical composition, ruminal fermentation, in vitro digestibility and gas production parameters of several grasses planted in greenhouse. A. Jafari*[1], H. Behrozpour[2], H. Fazaeli[3], and R. Mohammadi[4], *[1]Department of Animal Science, Yasouj University, Yasouj, Iran, [2]Department of Animal Science, Yasouj University, Yasouj, Iran, [3]Animal Science Research Institute, Karaj, Iran, [4]Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Tabriz, Iran.*

This experiment was conducted to investigate the chemical composition, ruminal fermentation, digestibility and gas production parameters of selected genotypes of 4 cool season grasses (including: *Festuca arundinacea*, *Bromus inermis*, *Dactylis glomerata* and *Phalaris aquatica*) in greenhouse condition. The experiment was performed in Randomized Complete Block Design with 4 treatments and 3 replications in greenhouse of Agricultural Biotechnology Research Institute of Iran (ABRII), Tabriz. Samples were taken from the mentioned species in the vegetative stage and their chemical compositions were determined by using conventional methods. The fermentability and gas production potential of samples were measured by gas test method with 200 mg of each sample at 2, 6, 8, 12, 24, 48, 72 and 96 h after incubation. Fermentation parameters and digestibility were calculated by using the produced gas at 24 h after incubation. Data analysis was performed as Duncan test ($\alpha = 0.05$). There was no significant difference between species on dry forage yield, crud protein percentage, non-fibrous carbohydrates and neutral detergent fiber ($P > 0.05$). The percentage of acid detergent fiber were the highest in *Festuca arundinacea* and there was no significant in other species ($P < 0.05$). The amount of short chain fatty acid, organic matter digestibility, and microbial protein synthesis content in *Bromus inermis* and *Dactylis glomerata* were better than other 2 species ($P < 0.05$). The dry matter intake were the highest in *Phalaris aquata* and *Bromus inermis* ($P < 0.05$). The highest of GP at 24 h was in the *Bromus inermis* and *Dactylis glomerata* ($P < 0.05$) and at 48 h it was not significant between species. Based on the results, *Bromus inermis* and *Dactylis glomerata* had higher nutritional value than other 2 species for planting in greenhouse condition.

Key Words: grasses, greenhouse, in vitro

2721 Evaluation of pasture biomass from cool-season pastures in the Midwestern USA with satellite imagery, herbage mass clippings and an electronic plate meter. B. J. Heins*, L. Dourado Clemente, and K. T. Sharpe, *University of Minnesota, Morris, MN.*

Evaluation of pasture biomass measurements aids producers in knowing the availability of cool season grass in pasture for cattle grazing. The objective of this study was to compare satellite technology with the rising plate meter and forage biomass clippings as a method of pasture monitoring in the Upper Midwest of the USA. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN. The grazing periods were from May 2021 to October 2021 and May 2022 to September 2022. The pasture system was composed of cool-season perennials. Grazing height and forage availability were measured weekly in 9 pastures with a Jenquip pasture plate meter (Jenquip, Feilding, New Zealand). Pastures ranged in size from 2.55 ha to 9.7 ha. Satellite images were from Planet Labs PBC (San Francisco, CA) and average normalized difference vegetation index (NDVI) values were calculated weekly for the area inside each pasture. Pearson correlations were from PROC CORR of SAS 9.4 and determined associations of forage biomass from the satellite imagery, rising plate meter, and herbage mass clippings. Forage biomass was analyzed with PROC MIXED and included the fixed effects of pasture (9 pastures) and week with repeated effects. Across the summer grazing season for both years, mean forage biomass was 2,966 kg DM/ha (range was 2,025 to 3,794 kg DM/ha; $P < 0.05$) from the plate meter, 2,507 kg DM/ha (range was 1,761 to 3,213 kg DM/ha; $P < 0.05$) from NDVI satellite images and 2,234 kg DM/ha (range was 1,543 to 3,588 kg DM/ha; $P < 0.05$) from the herbage mass clippings. The correlations for specific pastures of the rising plate meter and the satellite image NDVI ranged from 0.06 to 0.37 ($P < 0.05$) in 2021 and 0.21 to 0.59 ($P < 0.05$) in 2022. Correlations were greater with optimum forage availability in the pastures. Alternative methods to calculate the biomass of pastures may provide more advantages for farmers to determine the grazing management of pastures.

Key Words: rising plate meter, pasture, satellite image

2722 Agrivoltaic effects on forage biomass and forage nutritive value. S. L. Portner[1], B. J. Heins*[1,2], E. S. Buchanan[2], and M. H. Reese[2], *[1]Department of Animal Science, University of Minnesota, St. Paul, MN, [2]West Central Research and Outreach Center, University of Minnesota, Morris, MN.*

Agrivoltaics combines solar energy and agricultural production in one location. The objective of the study was to evaluate biomass and nutritive value of forages and grain crops grown under different solar energy sites. Seven forage species, 3 mixes of grass and legume species, as well as 4 grain crops were planted underneath a 30-kW solar site (30kW), a 50-kW solar site (50kW) and 1 control site without solar panels (CON) in May 2022 with 4 replicates per site. Forage crops included alfalfa, field peas, meadow fescue, orchard grass, red clover, brown midrib sorghum-sudan grass, white clover and 3 meadow fescue, orchard grass, and legume mixes with either alfalfa, red clover, or white clover. Grain crops included corn, oats, soybeans, and wheat. Biomass samples were harvested at appropriate maturity levels for feeding to lactating dairy cows or harvesting grain. Samples were sorted for botanical composition and analyzed for nutritive value. Biomass, dry matter, and nutritive value were analyzed with PROC Mixed of SAS with the fixed effects of site (30kW, 50kW, or CON), crop nested within site, and cutting (1st or 2nd) and the random effect of replicate nested within site. Forages produced less biomass ($P < 0.05$) for the 30kW (564 kg/ha) and 50kW (446 kg/ha) sites compared with CON (1,100 kg/ha). The forages at the 50kW site had greater ($P < 0.05$) crude protein (25.8%) than the 30kW (21.4%) site and CON (20.9%) site. The forages at the 50kW site had greater ($P < 0.05$) total-tract neutral detergent fiber digestibility (57.1%) than the 30kW (52.5%) site and CON (51.0%) site. Additionally, the forages at the 50kW site had greater ($P < 0.05$) calcium (1.05%) compared with the 30kW (0.75%) site and CON (0.84%) site. Forage biomass and nutritive value varied across solar site because of the amount of sun exposure.

Key Words: Agrivoltaics, forages, pasture

2723 Effects of wilting duration, microbial inoculation, and ensiling duration on oat silage quality. L. Lima*, L. Mu, F. Amaro, C. Niño-de-Guzman, S. Farooq, K. Arriola, H. Sultana, and D. Vyas, *University of Florida, Animal Science Department, Gainesville, FL.*

The objective of this study was to evaluate the effects of wilting duration (WD), microbial inoculation (MI), ensiling duration (ED) and all possible 2- and 3-way interactions on aerobic stability, fermentation parameters, microbial counts and in situ ruminal dry matter degradability (DMD) of oat silage. Study design was randomized complete block design with a 2 × 2 × 2 factorial arrangement of treatments and 5 replicates per treatment. Treatments were 2 levels each for WD (48 h vs. 72 h), MI (Control vs. Inoculant), and ED (30 vs. 90 d). Harvested forage was allowed to wilt for 48 or 72 h. Wilted forage was chopped and treated with either MI (1.5×10^5 cfu of *Lentilactobacillus buchneri* LB1819 and *Lactococcus lactis* O224/g of wet forage) or distilled water. Forages were ensiled in buckets for either 30 or 90 d. Silage samples were analyzed for aerobic stability, ammonia, pH, and microbial counts. For in situ ruminal degradability, dried silage samples were incubated in 2 ruminally cannulated cows at several incubation time points (0, 2, 4, 8, 16, 24, 48 and 72 h). Data were analyzed using the GLIMMIX procedure of SAS for main effects of WD, MI, ED, and their interaction. Aerobic stability was increased for silage ensiled for 90 d and improvement was greater with long WD (WD × ED; $P < 0.01$). Low WD increased ammonia-N (NH_3-N) concentrations while high WD decreased NH_3-N concentrations after 90 d compared with 30 d ED; (WD × ED; $P = 0.03$). Microbial inoculation increased LAB counts ($P < 0.01$); however, MI had no effects on aerobic stability ($P < 0.01$). Silage DM increased with longer WD ($P < 0.01$) and longer ED ($P < 0.01$). Microbial inoculant decreased DMD and effective degradability of DM while increased the fraction C of DM ($P < 0.01$). The interaction WD × ED was observed for fraction A and B of DM ($P < 0.01$); however, the interaction MI × ED was also observed for fraction A ($P < 0.01$). Oats ensiled for longer duration improved aerobic stability while short WD results in lower silage pH.

Key Words: Degradation kinetics, Silage, Winter grass

2724 Effects of curing extent on the phytoestrogen levels of red clover hay and silage across storage phases.

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Our objective was to assess the effects of insufficient (WET) or ample (CUR) curing on the phytoestrogen levels of red clover silage (29.4 and 45.3% DM) and hay (65.1 and 89.1, respectively). Measurements were taken at the start of storage (STRT), after 14 d (MicA), and once storage processes had stabilized for hay and silage (50 and 78 d, respectively; LATE). Hay was packed into mini-bales (~500 fresh g; ~232 kg DM/m^3) and silage into mini-silos (19.5 L; ~214 kg DM/m^3). Data were analyzed as a randomized complete block design (5 blocks) with a 2 (curing extents) x 2 (storage methods) x 3 (storage phases) factorial. Differences were declared at $P \leq 0.05$. The concentration of formononetin was higher in silage vs. hay at STRT (4,624 vs. 2,893 ± 175 mg/kg of DM, respectively), MicA (5,383 vs. 2,110), and LATE (5,666 vs. 2,569). Also, more formononetin was found in WET vs. CUR silage (5,841 vs. 4,608.1 ± 154 mg/kg of DM, respectively). However, no differences were observed between WET vs. CUR hay ($\bar{x}$ = 2,524). At STRT, biochanin A was higher in silage vs. hay (3,105 vs. 2,012 ± 139 mg/kg of DM, respectively). A similar trend was observed at MicA (3,233 vs. 1,057), and LATE (3,295 vs. 1,228). In addition, more biochanin A was observed in WET vs. CUR silage (3,793 vs. 2,628.9 ± 127 mg/kg of DM, respectively). The opposite was observed in WET vs. CUR hay (1,219.5 vs. 1,644.9). The genistein concentration was higher in silage vs. hay at STRT (149 vs. 120 ± 8.36 mg/kg of DM, respectively), MicA (246 vs. 62.6), and LATE (264 vs. 72.9). Also, the concentration of genistein was higher in WET vs. CUR silage (236 vs. 202.3 ± 7.21 mg/kg of DM, respectively) but the opposite was observed in WET vs. CUR hay (72.8 vs. 97.3). In the case of daidzein, hay had a higher concentration of this phytoestrogen than silage (12.8 vs. 6.67 ± 0.43 mg/kg of DM, respectively). In conclusion, red clover silage maintains higher levels of formononetin, biochanin A, and genistein than hay during curing period and across the storage phase. Also, adequate curing is more beneficial for silage than hay in terms of decreasing the phytoestrogen levels.

Key Words: red clover, formononetin, biochanin A.

2725 Loss of fermentation acids in silages affected by drying temperature.

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The volatile compounds in silages are partially or totally lost during the drying process inherent with dry matter (DM) determination. The objective of this work was to study the losses of fermentation acids in beet silages dried at different temperatures. Beets (*Beta vulgaris*) were co-ensiled with 7 effluent absorbing feeds (dried sugar beet pulp, pressed sugar beet pulp, maize silage, grass silage (×2), rapeseed meal, and hay) and sampled after 6 mo ensiling in round bales. Silages were dried in duplicate until constant weight by freeze-drying (72h) or forced-air oven at 50 and 60°C for 24–25 h, and 70, 80 and 100°C for 6–7 h. Original and dried samples (100 g of substrate in fresh matter) were extracted by blending with 1 L of deionized water followed by centrifugation. Volatile fatty acids (VFA) and L-lactic acid were analyzed, and pH was measured. Data were statistically analyzed as a mixed model (Proc Mixed of SAS) in which temperature (T), sample (S), and T×S were fixed effects. Reference DM concentration was chosen to be 100°C. The original samples without drying showed that acetic acid content of the different silages varied between 13 and 58 g/kg DM and L-lactic acid content between 18 and 62 g/kg DM, while pH ranged from 3.5 to 4.2 and the presence of other VFA was minor. Drying resulted in significant ($P < 0.001$) losses (relative to content in original undried sample) of 52.6, 81.5, 82.2, 79.2, 80.8, and 85.1% for acetic acid and 0.5, 16.5, 18.5, 14.6, 16.1, and 28.4% for L-lactic acid at freeze-drying, 50, 60, 70, 80, and 100°C, respectively (mean values of all the silages analyzed). In addition, sample effect was significant ($P < 0.001$) for acetic and L-lactic acids losses and the pH values of dried samples varied between 3.46 to 4.31, increasing at 100°C and decreasing with FD ($P < 0.05$). Interactions were found between silage type and drying temperature ($P < 0.001$), with greater relative losses of acetic acid in silages that originally had a higher content. In conclusion, the drying process affect the drying losses of fermentable compounds and the present results could serve to update equations for corrections of silage DM.

Key Words: silage, fermentable acids losses, dry matter determination

2726 Association between fragility and analytical parameters of various forages.

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Forage fragility represents one of the limitations of the peNDF system. Objectives of the following experiments were to assess the relationship between various analytical parameters and forage fragility, measured as grinding energy (GrE). In Experiment 1, 28 forages were analyzed for DM, total N, starch, NDF, ADF, ADL, 24h NDFd, total phenols (TP) and non-tannic phenols (NTP). Initial particle size (IPS) was determined after grinding the samples with a 2-cm screen knife mill. GrE was measured using an ultra-centrifugal and a hammer mill, with a 1-mm screen, and final particle size (FPS) was determined. Difference between the 2 mills was determined using a *t*-test for 2 dependent samples. Multiple regressions were used to predict observed GrE measurements from the set of parameters obtained. Energy measurements ranged from 34.9 to 356 J/g with the ultra-centrifugal mill consistently using less energy than the hammer mill ($P < 0.001$). The independent variables selected were able to explain 70 to 90% of the GrE and IPS consistently accounted for most of the variation, showing the difficulty of having a homogeneous IPS. Forage fragility did not depend on the fiber quantity only, but also on the different quality and interaction of its components. In Experiment 2 GrE was measured on 34 forages. In vitro NDFd was obtained from 0 to 240 h. Correlations between GrE and NDFd at various time points and NDF rates of digestion (kd) were calculated and the best predictors among fiber, its components and digestibility values were selected. The results showed high negative correlations of NDFd and kd with GrE ($P < 0.001$). In particular, 18 and 24 h NDFd had the highest correlation values (−0.62 and −0.81) across samples and species, showing the important relationship between forage fragility and fiber digestibility. The IPS, FPS, cellulose (ADF-ADL) and kd explained up to 75% of the variation. Grinding energy can be used to rank forages to account for different fragility. Forages peNDF might then be adjusted to account for different fragility. Diets for dairy cows would then need to be adjusted as well to reflect the fragility values.

Key Words: grinding energy, physically effective neutral detergent fiber, particle size

2727 Evaluation of a portable X-ray fluorescence device for a sustainable mineral nutrition in dairy herds.

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Imprecise supplementation of macro and micro-minerals in dairy herds induces nutritional imbalances, economic losses, and environmental impacts. The mineral content of ruminant's basal diet is variable and not analyzed in routine due to the high cost of traditional analytical methods performed in laboratories. Proposing an innovative hand-held analytical tool for cheap and immediate mineral analysis at the farm, will give the opportunity to perform a precise mineral supplementation. The objective of this work was to validate the analysis of selected minerals (Ca, P, K, Mg, Na, S, Zn, Cu, Mn, Fe and Mo) in 89 forages with a portable device based on X-Ray Fluorescence (XRF) technique in comparison to wet chemistry method. Twenty samples of grass, 21 of hay, 11 of haylage, 21 of maize silage and 16 samples of grass silage were collected all over Auvergne-Rhônes-Alpes area in France, then dried and ground. Forage mineral content was directly determined by XRF, in parallel, the corresponding samples were analyzed by ICP-AES. The relationship between XRF and ICP-AES methods was given by the coefficient of determination (R^2) resulting from the linear correlation established between XRF and ICP-AES results. Mean absolute error (MAE) between ICP and XRF was also calculated. Globally, the correlation between portable XRF and ICP-AES methods was dependent on the chemical element. Magnesium and sodium, due to XRF physical limits, were poorly ($R^2 = 0.02$ for Mg) or not at all detected (Na). Copper, iron and sulfur contents were quantified by the portable XRF, R^2 were respectively 0.70, 0.78 and 0.94 and MAE were respectively 13%, 24% and 8%. Because of its very low concentration, ranging between 0.4 and 3.7 mg/kg DM, molybdenum was a challenge for the portable XRF analysis ($R^2 = 0.49$, MAE = 61%). For the other minerals, R^2 ranged between 0.89 and 0.96 and MAE between 6 and 18%. Although, the portable XRF analysis showed globally promising results to quantify mineral content in forage, further analytical development is needed for molybdenum

Key Words: antagonists, precision, sustainability

2728 Lactational performance of dairy cows fed diets based on corn silage with varying organic matter digestibility.

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Penn State's Organic Matter Digestibility Index (OMDI) is a quality measure for corn silage (CS) based on laboratory analyses of rumen degradability of its major nutrients. An experiment was conducted to evaluate the effects of diets formulated using CS varying in OMDI on the productive performance and enteric gas emissions of dairy cows. Following a 2-wk covariate period, 48 Holstein cows averaging (±SD): 129 ± 53 d-in-milk (DIM), and 44 ± 7 kg/d milk yield (MY) were assigned to 1 of 4 treatments in a 10-week randomized complete block design experiment. Cows were blocked based on lactation number, DIM and MY. Treatments were diets based on CS included at 50% of diet dry matter (DM); inclusion rate of all other feed ingredients was similar among diets. Corn silages A, B, C and D were, respectively, 44.3, 43.3, 44.1 and 44.9% DM and had (% of DM): neutral-detergent fiber, 32.9, 33.8, 31.9, and 29.5; starch, 38.7, 38.6, 39.7 and 41.2; and OMDI 59.0, 62.7, 65.6 and 60.6. Feed intake and milk production were recorded daily and milk samples for component analysis were collected every other week during the experimental period. Enteric CH_4 emission was measured using the GreenFeed system. Data were analyzed using the PROC MIXED of SAS and an orthogonal polynomial contrast to evaluate a linear effect of CS OMDI. Block and block × treatment were random effects. Treatment did not affect MY or energy-corrected MY (ECM). Dry matter intake (DMI) decreased numerically ($P = 0.11$) but feed efficiencytended to increase linearly ($P = 0.08$) with increasing CS OMDI. There was no effect of increasing CS OMDI on milk components concentration or yields. There was also no effect of increasing CS OMDI on bodyweight (BW) or BW change. Treatment did not affect daily CH_4 emission (338 g/d; SEM = 14.0) or emission yield (per kg DMI) and intensity (per kg MY or ECM yield). When CS makes up 50% of dietary dry matter, differences of 2.9 to 6.6% in its OMDI had no effect on MY, ECM, and enteric CH_4 emission, but feed efficiency tended to increase linearly with increasing CS OMDI.

Key Words: corn silage, organic matter digestibility index, performance

2729 Replacing conventional concentrates with sprouted barley or wheat: Effects on milk production in dairy cows.

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Feeding sprouted grains may improve nutrient digestibility and reduce manure nutrient excretion. Therefore, our objective was to evaluate the impact of replacing conventional concentrates with sprouted barley or wheat on milk production efficiency in high-producing cows. Twenty-four multiparous Holstein cows (105 ± 23 d in milk [DIM]; 3.25 ± 1.33 lactations) were used in a study with a replicated 3 × 3 Latin square design. Following a 2-wk covariate period, cows were fed 1 of 3 experimental diets: a total mixed ration (1) without sprouted grains (CON), or with (2) 10% sprouted barley (STB; dry matter basis) or (3) 10% sprouted wheat (STW) for 21 d experimental periods. Diets were formulated to be isoenergetic and isonitrogenous (16.1% CP, 22.2%

starch, and 32.3% aNDF) with sprouted grains replaced for ground corn, soybean meal, canola meal, and dextrose. Sprouted grains were grown using a semi-automatic hydroponic system developed by Renaissance Ag (Vineyard, Utah) and harvested after 6 d of growth, chopped, and then mixed with other ingredients. Data and sample collection occurred during the final 3 d of covariate and experimental periods. Data were analyzed using PROC MIXED with square, period, treatment, square × treatment as fixed effects and cows as random effects. Feeding sprouted grains did not modify yields of milk or energy-corrected milk (ECM); however, dry matter intakes (DMI) were lower for STB and STW, relative to CON ($P < 0.001$ and $P = 0.07$, respectively). Feed efficiencies were greater for STB, relative to CON (1.49 vs. 1.43 for milk yield/DMI; 1.85 vs. 1.73 for ECM/DMI; $P < 0.01$). Milk urea-N concentrations were greater for STW, relative to CON ($P = 0.04$). Yields and concentrations of milk components (i.e., fat, true protein, and lactose) were not impacted by treatment. Control cows numerically consumed 4 more gallons of water/d than cows fed sprouted grains. Rumination and physical activity were not impacted by treatment. Our results indicate that sprouted barley as an alternative feed to traditional concentrates can improve milk production efficiency in lactating cows.

Key Words: dairy cow, sprouted grain, milk production

2730 Ruminal fermentation, urinary purine derivatives excretion, and enteric methane emissions in grazing dairy cows fed an extruded flaxseed-based supplement. M. A. Rahman*, K. V. Almeida, D. C. Reyes, E. A. Cruz, A. L. Konopoka, M. A. Arshad, and A. F. Brito, *Department of Agriculture, Nutrition, and Food System, University of New Hampshire, Durham, NH.*

We aimed to evaluate the effect of a flaxseed-based feed supplement (LinPRO-R) on ruminal fermentation, urinary purine derivatives excretion (PD), and enteric CH_4 emissions using 18 multiparous and 2 primiparous Jersey cows (128 ± 52 DIM) in a randomized complete block design during the grazing season. Cows grazed a mixed grass-legume pasture (herbage allowance = 15 kg of DM/cow daily) overnight and received a partial total-mixed ration (pTMR) during the day. The pTMR were formulated to contain (DM basis) 37.5% mixed, mostly legume baleage and 62.5% of a soybean meal/ground corn-based concentrate mash. Cows were randomly assigned to 1 of 2 diets: (1) pasture plus pTMR (control = CTRL) or (2) pasture, pTMR, and 6% LinPRO-R (LIN). Ground corn and soybean meal were replaced with LinPro-R in the LIN diet. Diets were formulated to be isonitrogenous and to yield a 60:40 forage to concentrate ratio. Pasture averaged 17.5% CP and 53% NDF, and pTMR 9.7% CP and 15% NDF. The experiment lasted 12 wk with 2 wk for a covariate period followed by 3 sampling periods during wk 4, 7, and 10. Two GreenFeed units were used to measure gaseous emissions throughout the experiment. Spot urine samples were collected 5 times over 3 d, and ruminal fluid was collected once via stomach tubing during each sampling period. Data were analyzed using the MIXED procedure of SAS with repeated measures over time. No treatment effects were observed for total VFA concentration (mean = 90.8 m*M*), and the molar proportions of acetate (mean = 71%), propionate (mean = 15%), butyrate (mean = 11%), and the acetate:propionate ratio (mean = 4.68). Likewise, treatments had no effect on the excretion of urinary N, allantoin, uric acid, and total PD. Production of CO_2 (mean = 10,885 kg/d) and enteric CH_4 (mean = 350 g/d), as well as CH_4 yield (mean = 15 g/kg of DMI) and CH_4 intensity (mean = 10.5 g/kg of ECM) did not differ with feeding CTRL vs. LIN. In conclusion, LinPRO-R fed at 6% of the diet DM had no effect on ruminal fermentation profile, urinary PD excretion, and enteric CH_4 emissions in grazing dairy cows.

Key Words: flaxseed, greenhouse gas, pasture

2731 Effects of grass species on cell wall components and milk production of dairy cows. D. Sousa*[1], M. Murphy[2], A. Larsson[1,2], R. Hatfield[3], J. Takahashi[4], W. Richardt[5], and E. Nadeau[1], [1]*Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Skara, Sweden,* [2]*Lantmännen Lantbruk, Malmö, Sweden,* [3]*US Dairy Forage Research Center, Madison, WI,* [4]*Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Umeå, Sweden,* [5]*LKS mbH, Lichtenwalde, Germany.*

Silages from tall fescue (TF), meadow fescue (MF) and timothy (TI) harvested at first and second cut were used as experimental treatments in the present study. Dietary forages were composed by 40% from the first cut and 60% from the second cut of each species. Diets were formulated to have similar forage NDF concentrations to allow the cows to eat the experimental diets equally. The nutrient composition (g/kg of DM) of the diets (TF, MF and TI) were NDF (359, 360 and 363, respectively), CP (183, 181 and 176, respectively), starch (177 for TF and MF and 190 for TI) and crude fat (34 for TF and TI and 33 for MF). Forty-eight dairy cows were blocked by lactation number, DIM, and ECM and randomly assigned into one of 3 treatments containing 16 cows each. Cows received the experimental diets continuously for 7 weeks and samples were collected on wk 3, 5 and 7. Individual DMI was recorded continuously and milk yield twice a day throughout the experiment. Milk samples were collected during 3 consecutive days twice a day in each sampling week for milk composition analysis. Data were analyzed using MIXED procedure of SAS (ver. 9.4) with silage and week as fixed factors, and block and cow nested within block as random factors. Week was considered as repeated measures. Pairwise comparisons were analyzed according to Tukey's test when a significant F-value occurred ($P < 0.05$). Timothy silage showed greater concentrations of NDF, ADF and ADL compared with TF and MF. Tall fescue showed similar in vitro NDF digestibility but greater concentration of lignin monomers, *p*-coumarate and the sum of *p*-coumarate and ferulate compared with TI. Cows receiving TI had greater milk yield and ECM than cows receiving TF. Milk yield and ECM of cows fed MF did not differ from cows fed TF or TI. Cows receiving TF showed the lowest milk protein yield and the highest milk urea N. Feed efficiency tended to be greater for cows fed TI compared with cows fed TF and MF. In conclusion, replacing TI or MF by TF in diets of dairy cows can compromise milk production, which can be related to components of the lignin.

Key Words: meadow fescue, tall fescue, timothy

2732 An assessment of virtual fence technology for dairy heifer grazing. C. Holohan[1], F. O. Lively[1], R. K. Ogden[2], L. M. Bauman[2], H. Gümüs[3], D. M. Jaramillo[2], G. Arnott[4], R. Palme[5], and M. S. Akins*[2,6], [1]*Agri-Food and Biosciences Institute, Hillsborough, Co. Down, Northern Ireland,* [2]*USDA Institute for Environmentally Integrated Dairy Management, 2615 Yellowstone Drive, Marshfield, WI,* [3]*Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey,* [4]*School of Biological Sciences, Queens University Belfast, University Road, Belfast, Northern Ireland,* [5]*Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria,* [6]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Virtual fencing (VF) is a technology which enables grazing livestock to be managed without the use of a physical fence. The system typically comprises a mobile phone application (through which the user sets the VF boundary) and a GPS-enabled neck collar. When the animal approaches the VF, it receives an audio warning to turn around. This is followed by an electric pulse if it proceeds beyond the VF. There is significant potential for VF technology to facilitate managed grazing on US farms, however there is a requirement that such technologies are

reliable and adhere to high animal welfare standards. Using Nofence VF collars, the objective of this study was to evaluate the ability of VF to contain grazing cattle and to determine possible impacts on animal welfare compared with conventional electric fencing. Sixty-four postbred Holstein heifers were blocked by age and bodyweight and randomly assigned to one of 2 fencing treatments; virtual fencing (VF; n = 32) and electric fencing (EF; n = 32). Eight groups of 8 heifers were assessed across 2 consecutive cohorts, from July until September 2022, with each cohort lasting 28 d. All heifers were first habituated to electric fencing over a 2-d period before moving to pasture, and then given a further 5 d to acclimatize to their pasture. Following this, VF heifers entered a 7-d virtual fence training period. They were then managed for the remaining 2 weeks of the study using a front and rear virtual fence while the EF heifers were similarly managed using electric fence wires. Measurements included weekly fecal cortisol metabolites (FCM), liveweight gain (LWG), and VF data (audio cues and electric pulses). The study found that VF was capable of maintaining the heifers within their allocated area. Heifers learned to correctly respond to the audio cue after an initial learning period, with some variation between animals. Mean FCM was similar in EF (18.0 ng/g) and VF (18.38 ng/g; $P > 0.05$), while LWG did not differ significantly between treatments ($P > 0.05$). Results suggest that VF can be an effective and welfare-friendly technology for managing grazing dairy heifers.

Key Words: dairy, pasture, technology

Joint Growth and Development and Physiology and Endocrinology—General Orals

2733 Cow-calf relationships of endocrine and metabolic parameters immediately after parturition in Holstein animals. A. L. Freihofer, R. M. Bruckmaier, and J. J. Gross*, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Besides the metabolic and health status of cows at parturition, intrauterine conditions, calving process, and colostrum feeding may affect endocrine and metabolic pathways in the neonate. Forty-six clinically healthy cows without dystocia and their calves were enrolled. Blood samples were collected from cows [4 h postpartum (pp)] and calves (4, 12, and 24 h pp). Calves were fed colostrum from their dams immediately after blood sampling at 4 and 12 h pp. Concentrations of glucose, nonesterified fatty acids (NEFA), and prolactin (PRL) were measured in plasma of cows. In calves, parameters related to the lipid metabolism (NEFA, phospholipids (PL), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C)), and concentrations of immunoglobulin G (IgG) and glucose were measured. Pearson correlation coefficients among parameters measured in cows and calves were calculated. Unless stated otherwise, presented correlations were significant ($P < 0.10$). At 4 h pp (i.e., before colostrum feeding), maternal glucose was positively correlated with glucose (r = 0.29) and NEFA in calves (r = 0.25). Plasma NEFA in dams were negatively correlated with fat metabolism [phospholipids (r = −0.31), HDL-C (r = −0.32)] and plasma IgG (r = −0.28) in calves at 4 h pp. Positive correlations between glucose of dams and calves were identified (12 h pp: r = 0.26; 24 h pp: r = 0.45). Maternal NEFA were positively associated with calf lipid metabolism at 24 h pp [PL (r = 0.44), TC (r = 0.39), LDL-C (r = 0.37), HDL-C (r = 0.36, $P = 0.11$)]. Primarily positive and significant correlations were detected between maternal PRL and lipid metabolism in calves [NEFA (12 h pp: r = 0.26), PL (12 h pp: r = 0.31; 24 h pp: r = 0.57), TC (24 h pp: r = 0.62), LDL-C (24 h pp: r = 0.48), HDL-C (24 h pp: r = 0.69)]. In conclusion, the metabolic status of cows after parturition is partly associated with the neonate's metabolism before its first feeding, whereas later associations between cow and calf are likely due to colostrum feeding.

Key Words: cow, calf, metabolic status

2734 Predicting age at puberty of dairy heifers based on biometric body features extracted from 3D images during the preweaning phase. A. Negreiro*, A. Vang, T. Bresolin, R. E. P. Ferreira, G. J. M. Rosa, L. L. Hernandez, and J. R. R. Dorea, *University of Wisconsin–Madison, Madison, WI.*

Dairy heifer replacement costs represent a significant investment for dairy operations, and these costs can be further compounded when animals fail to provide a financial return due to poor reproductive performance. Although genetics and early-life nutrition are known to influence conception rates after puberty, predicting the future reproductive health in calves is a challenging task. However, computer vision systems offer a non-invasive method for extracting body biometrics that can provide insight into individual animal physiology. This study aimed to predict the age at puberty (AP) of dairy heifers using body biometrics collected through computer vision during the preweaning phase. Top-down view depth images of 124 Holstein calves (1 to 6 wk of age) were collected on 6 consecutive weeks (50 images per week) resulting in 300 images per calf. The images were segmented to isolate calf's body, and the masks were applied to each depth image. Body biometrics, including body area, volume, length, width, eccentricity, circularity, extent, and perimeter, were extracted from the depth images, and a Random Forest algorithm was trained to predict AP. We used a nested 5-fold cross-validation technique for model training, tuning, and validation. Puberty was determined by blood plasma progesterone levels above 1ng/mL for 2 consecutive weeks. The model was trained to classify animals that reach puberty before or after an average age of 335 d. All analyses were performed in Python using the open-source package Scikit-image and frameworks Tensorflow and Keras. The precision, recall, and F1-score for predicting AP were 0.56, 0.84, and 0.67, respectively. Despite the moderate prediction performance, there is great potential of using image features from body biometrics as predictors of AP given the large time gap of approximately 300 d between the last data point collected and the AP. Other analytical approaches and additional data from subsequent periods of the preweaning phase may enhance the prediction quality.

Key Words: computer vision, dairy heifer, reproduction

2735 Computer vision-based body weight estimation in group-housed calves. D. Sun*[1], R. van der Tol[1], L. Webb[2], K. van Reenen[3,2], and M. Afonso[4], [1]*Agricultural Biosystems Engineering Group, Wageningen University and Research, Wageningen, Gelderland, the Netherlands,* [2]*Animal Production Systems Group, Wageningen University and Research, Wageningen, Gelderland, the Netherlands,* [3]*Livestock Research, Wageningen University and Research, Wageningen, Gelderland, the Netherlands,* [4]*Biometris Group, Wageningen University and Research, Wageningen, Gelderland, the Netherlands.*

Body weight (BW) is a robust indicator of growth performance and health for calves. A regular BW measurement could support the identification of deviations from an estimated growth curve, allowing for timely adjustments in feeding and assistance in health monitoring. Recent studies suggested that deep learning based algorithms can be applied to 3-D images for BW estimation. This study aimed at selecting an algorithm that can accurately estimate BW using features related to body size obtained from RGBD top-view images in calves. Holstein-Friesian veal calves (n = 228; 17 ± 4 d of age at arrival) were housed in 6 large pens, each with an automated milk feeder and a RGBD camera (Intel RealSense Depth Camera D435, Intel Corporation, Santa Clara, California) aiming down from 1975mm height. The images were obtained when calves visited the feeders. Scaled-based BW was used as gold standard, measured 3 times per month throughout the 6 mo fattening period. First, a deep learning model maskRCNN was used for background subtraction to select calves' data. Then, 250 RGB images were selected for annotation (i.e., back length and neck width) of which 200 images were used for training, 50 images for validation. Sanity check showed that features were obtained with an average precision of 90% on the validation data set for back length and neck width.

Table 1 (Abstr. 2735). Performance of body weight prediction models

Method	Median relative error	Mean relative error
Linear regression	0.11	0.15
SVM	0.09	0.13
Random forest	0.10	0.13
XGBoost	0.11	0.15

With these features 4 BW models were trained using one-hundred-and 20 annotated RGB images: linear regression, support vector machine (SVM), random forest, and XGBoost. The results (Table 1) show that SVM had the lowest median relative error and mean relative error. We conclude that SVM is most promising among the included BW models. Future work will include exploring new features that better present the information on BW (e.g., the curviness of skin due to adipose tissue or subcutaneous fat) that can be added to the mentioned models.

Key Words: body weight, continuous monitoring

2736 Evaluation of growth and health costs of dairy calves raised in individual, pair, or group housing compared to dairy calves raised with dams. K. Sharpe*[1], B. Goncalves Da Costa[2], M. Endres[2], and B. Heins[1,2], *[1]West Central Research and Outreach Center, Morris, MN, [2]University of Minnesota, St. Paul, MN.*

The purpose of this study was to investigate growth at weaning and incidence of treatments for scours and respiratory issues of pre-weaned dairy calves raised in individual (I), pair (P), group (G), or dam- raised (D) housing systems. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN. Three-hundred forty-five Holstein and crossbred calves were assigned to housing treatments during 3 fall calving seasons and 2 spring calving seasons from September 2020 to November 2022. The I, P, and G raised calves were enrolled at 3 d and fed 10 L of whole milk per d. Calves raised by their dam were isolated together for 3 d and introduced to a larger cow-calf group where they nursed ad libitum. All calves were weaned at 63 d. Independent variables were the fixed effects of breed group, birthweight, season of calving, and treatment group. Pen within season was a random effect. Hip height for I, P, G, and D calves was not different ($P > 0.05$). The D calves had similar heart girth to G calves, but larger ($P < 0.05$) heart girth (114.1 cm vs. 111.1 and 110.6, respectively) than I and P calves. The D calves had higher ($P < 0.05$) average daily gain (1.15 kg/d vs. 0.98, 0.96, and 0.99 kg/d, respectively), higher ($P < 0.05$) weaning weights (112.1 kg vs. 99.7, 99.2, and 102.8 kg, respectively), and higher ($P < 0.05$) total gain (74.1 kg vs. 61.9, 60.9, and 64.3 kg/d, respectively) than I, P, and G calves. The D calves had more ($P < 0.05$) calves triple their birthweight (52.9%) than I, P, and G calves (12.8, 14.4, and 15.5%, respectively). The G calves had higher ($P < 0.05$) health costs ($5.11/calf) than I, P, and D calves ($1.23, $1.16, and $1.54, respectively). The G calves had a higher ($P < 0.05$) incidence for treatment of scours (20.2 vs 7.0 and 9.4%, respectively) than I and D calves. The G calves had a higher ($P < 0.05$) incidence for treatment of respiratory issues than I, P, and D calves (15.5 vs. 3.5, 1.1, and 3.5%, respectively). Results from this study indicate there may be growth advantages to raising calves with their dams during the pre-weaning period.

Key Words: group, pair, dam-raised

2737 Intake and growth parameters of dairy calves dosed with rumen inoculum from mature donor cows of varying milk production efficiency. D. J. Nelson*[1,2], K. F. Kalscheur[2], G. I. Zanton[2], M. S. Cox[1], and G. Suen[1], *[1]University of Wisconsin–Madison, Madison, WI, [2]US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Modulating the rumen microbial community before its establishment affords an opportunity for influencing downstream growth and productivity in dairy cattle. The objective of this experiment was to evaluate whether inoculating pre-weaned dairy calves with rumen microbiota from donor cows of different milk production efficiency (MPE) affected intake and growth from birth to calving. Two lactating Holstein cows with comparable production of energy-corrected milk were selected as rumen content donors throughout the study; each cow was previously identified as either the high MPE (HE) cow or low MPE cow (LE). Donor cows were fed the herd diet. Female Holstein dairy calves (n = 60) were randomly assigned at birth to one of 3 treatment cohorts (n = 20) over a 24-wk time span. The donor cow identified as HE provided inoculum for cohort 1, while the donor cow identified as LE provided inoculum for cohort 2. Cohort 3 (control) inoculum was prepared by autoclaving rumen content from each of the 2 donor cows in equal parts. Inoculum (50 mL) was administered by oral gavage to each calf within 3 d of birth and subsequently at d 14, 28, and 42. Intake, body weight (BW), and skeletal body frame measurements including heart girth, wither height, hip height, hip width, and body length were recorded weekly for the first 9 weeks after birth and at age 6 mo, 12 mo, and within 3 d post-calving to quantify growth and efficiency. Data were analyzed using mixed procedures in SAS. Calves entered the study averaging 39.9 kg BW. Repeated dosing of rumen inoculum from HE vs. LE did not affect BW, intake, or average daily gain ($P > 0.10$). Average daily gain from wk 1 to 9, wk 9 to 6 mo, 6 to 12 mo, and 12 mo to post-calving averaged 0.78, 0.96, 0.90, and 0.61 kg/d, respectively. Skeletal body frame measurements from birth to calving also did not differ ($P > 0.10$) among treatment groups. These results demonstrate that repeated dosing of rumen inoculum from HE and LE cows into neonatal calves did not affect growth parameters from the preweaning period through first calving.

Key Words: heifer, growth, milk production efficiency

2738 Characterization of mature body weight and heifer maturity at first conception and first calving in Quebec Holstein herds. R. A. Molano*[1,2], O. Brassard[1], L. Laflamme-Michaud[2], E. Charbonneau[2], and D. E. Santschi[1], *[1]Lactanet, Canadian Network for Dairy Excellence, Ste-Anne-de-Bellevue, QC, Canada, [2]Université Laval, Quebec, QC, Canada.*

Current growth and nutritional recommendations for dairy heifers are based on a size-scaling approach using mature body weight (MBW) as reference. In practice a population estimate for MBW is often used at the herd basis. The objective of this observational study was to estimate the MBW and evaluate the degree of maturity attained at first conception (FCo) and first calving (FCa) in Quebec Holstein herds. A convenience sample of 73 herds with 126 ± 15.5 cows were visited at least once between April 2021 and May 2022. During visits a sample of heifers from birth up to 2 d prior calving (n = 2212), first lactation cows (n = 204, 60.9 ± 3.6 DIM) and mature cows (n = 358, 4.2 ± 0.1 lactations and 136.8 ± 15 DIM) were measured with a weight tape to estimate their BW (6493 records). An ANOVA was used to test the influence of herd on MBW. A growth curve as a function of age was computed for the entire population and each herd using a 4-parameter logistic model. Considering the age at FCa (24.2 ± 0.2 mo) and a 276 d gestation, the age at FCo and their respective BW were calculated. The equation describing growth was solved by age to determine age at a given BW. Maturity targets (MT) at FCo and FCa were considered to be 55% and 82–85% of MBW, respectively. The overall mean MBW was 767 ± 3.6 kg. Mature BW was influenced by herd ($P < 0.01$) and ranged from 662 to 892 kg among herds. Overall FCo and FCa were achieved at 482 and 663 kg, respectively. The MT for FCo was attained at 12.9 mo and, if implemented, heifers would weigh 632 kg at FCa. In 77% of herds FCo occurred over the MT (62.8 ± 0.6% MBW). In 66% of herds FCo occurred over one estrous cycle later (74.9 ± 5.1 d) relative to their attained FCo MT (13.0 ± 0.2 mo). Only in 20% of herds heifers attained the MT at FCa. Heifers arrived under (77.2 ± 0.8% MBW) and over (88.7 ± 0.5% MBW) matured at FCa in 38 and 42% of herds, respectively. Most of visited herds would benefit of monitoring MBW and heifer BW to adjust their breeding and rearing practices. Overall, achieving FCo according to the MT would ensure adequate maturity at calving while reducing age at FCa by 2 mo.

Key Words: heifer, weight, maturity

2739 *Salmonella enterica* serovars and resistant genes in calves and maternity pens from Mexico. S. Barrera, S. Vázquez-Flores*, M. De Donato, and C. Lucio, *Tecnológico de Monterrey, Querétaro, México.*

The main objective of this cross-sectional study was to determine the genetic identity of *Salmonella* spp. in maternity areas relating to close-up cows, calves, and bedding. Samples were taken directly from the rectum of asymptomatic calves <60 d of age, asymptomatic close-up cows, and maternity floors with Q-swabs. 55 dairies were sampled for a total of 378 samples in 13 states of Mexico. The overall prevalence was 63.6% with 57 *Salmonella enterica* isolates in total, whereas dairies with more than 1,000 cows showed 73.1% prevalence. Samples were cultured, serotyped, amplified, sequenced, and analyzed. Relative risk analysis (MedCalc), Pearson correlation coefficient, and Nominal Logistics (JMP 17.0, SAS Institute) were performed. Serogroups were E (50%), C (25%), G (15%), and B (10%). Stratified analysis indicated that 10 calves were found positive 7–49 d, in 8 dairies (n = 127), where the most frequent serovars were Give, Newport, and Cubana; 16 close-up cows were positive (116 samples), in 14 dairies with the serovars Montevideo, Anatum, Meleagridis; 31 isolates were positive from 134 sampled maternity areas, accounting for 25 positive dairies ($P < 0.05$) where the serovars were Give, London, Reading, Anatum, Agona, Meleagridis, Havana, Montevideo, and Cubana. Resistant genes were determined, where a calf showed 8 in the Newport serovar in San Luis Potosí, followed by manure bed with 6 in a London serovar from Baja California, and 5 in a Give serovar from Tlaxcala. Coahuila showed the amplest variety of resistant genes (8) in manure bedding. Ciprofloxacin, ampicillin, amikacin, gentamicin, and levofloxacin were the highest in resistance ($P < 0.05$). This study indicates that the main sources of *Salmonella* spp., are maternity areas, where periparturient shedders are contaminants and perpetuate the pathogen. The resistant genes are the highest in calves, followed by the type of floor, with the main antibiotics used for diarrhea showing the highest resistance.

Key Words: calves, *Salmonella*, serovars

2740 Peripheral blood mononuclear cell mitochondrial enzyme activity in calves indicates average daily gain, future lactation performance, and survival. A. M. Niesen* and H. A. Rossow, *University of California, Davis, Davis, CA.*

Mitochondria are central to metabolism and are the primary energy producers for all biosynthesis. The objective of this study was to determine if the mitochondrial enzyme activity rates of peripheral blood mononuclear cells (PBMC) in calves are associated with ADG, lactation performance, and survival. Twenty-three Holstein and 23 Jersey heifer calves were enrolled at 1 dairy, blood and body weight data were collected at 1, 2, 8, 36, 52 wk and 2 y of age. Respiratory and fecal scores and treatment number were recorded for the first 30 d of life. Milk production data were collected from herd management software on surviving animals (10 Holsteins, 16 Jerseys). Mitochondrial isolation and enzyme activities for citrate synthase, complex I, complex IV, and complex V were determined using kits from Abcam (Cambridge, MA). Data were analyzed using GLM and the Logistic procedure of SAS (Version 9.4, Cary, NC). Multivariate regression analyses were conducted to determine if calf mitochondrial enzymatic activity was associated with ADG and lactation performance. Average daily gain parameters (prewean, 9 mo, 12 mo and 2 y ADG) were regressed on calf enzymatic activity rates with prewean health indices (respiratory and fecal score, treatment number, hematology) as covariates with the criteria for inclusion at $P \leq 0.05$. Milk production parameters (milk yield, fat yield, solids yield, ECM, 305ME and relative value) were regressed on enzymatic activity and prewean health indices (respiratory and fecal score, treatment number). For both breeds, mitochondrial enzyme activities and prewean health were correlated to all ADG and milk production parameters ($R^2 \geq 0.64$, and $R^2 \geq 0.47$, respectively). Logistic regression analyses were performed to determine if early life enzymatic activity impacted survival outcomes in the herd. Calves in the lowest quartile for complex V enzyme activity at 1 wk had 13.5 greater odds of being culled or dying before their first lactation. These findings suggest that predictions of cow performance could be improved by considering the impact of early life mitochondrial enzymatic activity and prewean health indices.

Key Words: mitochondria, calves, survival

2741 The impact of maternal liver glutathione concentration on offspring growth performance and health during the neonatal period. A. Flavia Souza Lima*, G. Goncalves Begalli, M. H. Oliveira, R. Chaves Barcellos Grazziotin, J. Halfen, and J. Osorio, *School of Animal Science, Dairy Science, Virginia Tech, Blacksburg, VA.*

The objective was to investigate the effects of maternal liver GSH concentration on offspring's growth performance, metabolism, and antioxidant status during the neonatal period. Twenty-five Holstein dairy calves were monitored from birth until 21 d of age. Calves were born to dams classified according to prepartal hepatic GSH concentrations in either high GSH (HGSH; n = 10, 1.43 ± 0.1 m*M*) or low GSH (LGSH; n = 15, 0.51 ± 0.1 m*M*). All calves were housed in individual calf hutches and received 3.8 L of colostrum (brix ≥ 21%) from their respective dams within 4 h after birth. Then, they received 2 L of the same colostrum. Calves received 2 L of milk replacer (24% CP, 20% fat, 15% DM) 2x/d on 1 and 2 d after birth and 3 L of the same milk replacer 2×/d until 21 d. The calves received ab libitum starter grain (21% CP) from 3 d and had free access to water. Milk replacer and grain intakes were recorded daily. Body weight (BW) was recorded at birth and then weekly. Fecal scores, respiratory scores, and rectal temperatures were recorded daily. Blood samples were collected at 0 (before colostrum), 2, 7, 14, and 21 d to evaluate biomarkers of oxidative stress and metabolism. Statistical analysis was performed using the MIXED procedure of SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. There was a trend for a greater ($P = 0.06$) birth BW (47.6 vs 44.7 kg) in HGSH calves compared with LGSH. In contrast, calves born to HGSH dams had lower ($P < 0.01$) BW compared with LGSH during the trial. This effect was associated with a lower ($P = 0.02$) ADG (0.32 vs 0.45 kg/d) in HGSH calves compared with LGSH. Maternal BCS change from −3 to −1 wk relative to calving tends ($P = 0.06$) to be lower for LGSH than HGSH (+0.1 vs −0.1 BCS). Overall, our results suggest that prepartal liver GSH has a positive effect on calf birth BW, which could also be influenced by positive maternal BCS change. Lower birth BW in LGSH calves could have triggered a compensatory mechanism to improve postnatal growth rate.

Key Words: neonatal period, oxidative stress, glutathione

2742 Adipogenesis is modulated by depot-specific extracellular matrix microenvironment in adipose tissue of dairy cattle. J. F. Fiallo Diez*[1], C. G. Flesher[2], A. P. Tegeler[1], T. C. Michelotti[1], M. N. Hoque[4], B. Bhattarai[4], L. S. Florez[1], O. J. Benitez[1,3], G. Christopher[4], and C. Strieder-Barboza[1,3], [1]*Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX,* [2]*Department of Medicine, University of Pennsylvania, Philadelphia, PA,* [3]*School of Veterinary Medicine, Texas Tech University, Amarillo, TX,* [4]*Department of Mechanical Engineering, Texas Tech University, Lubbock, TX.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral).
Adipose tissue (AT) expands through both hyperplasia and hypertrophy. During adipogenesis, adipocyte progenitors (AP) proliferate and then accumulate lipids, influenced by local AT microenvironment. Increased adipogenic capacity (AC) is desirable as it relates to metabolic health, especially in transition dairy cows. Our aim was to elucidate depot-specific AC and how extracellular matrix (ECM) affects adipogenesis in subcutaneous (SAT) and visceral (VAT) AT of dairy cows. Flank SAT and omental VAT samples were collected from 10 lactating dairy cows in a local abattoir and digested via collagenase to obtain the stromal vascular fraction (SVF) and mature adipocytes. AC was assessed by adipocyte sizing (K2 Cellometer and H&E; minimum 100 cells/depot), AP (CD45-CD31-) frequency in SVF via flow cytometry, and *ADIPOQ* gene expression (qPCR; 2 technical replicates) in in vitro adipocytes. Viscoelasticity of SAT and VAT was assessed by rheology and then decellularized for native ECM isolation. Data were analyzed in GraphPad 9 using paired *t*-test and one-way ANOVA. SAT AC was greater than VAT, as indicated by increased AP abundance, increased magnitude of adipocyte *ADIPOQ* expression during differentiation, and higher adipocyte lipid accumulation as shown by an increased proportion of larger adipocytes. Rheologic analysis revealed that VAT is stiffer than SAT, which led us to hypothesize that differences between SAT and VAT AC are mediated by depot-specific ECM characteristics. Thus, we studied depot-specific ECM-adipocyte crosstalk using a 3D model with native ECM (decellularized AT). SAT and VAT AP were cultured and differentiated into adipocytes within depot-matched and mis-matched ECM for 14d, followed by *ADIPOQ* expression analysis. SAT ECM rescued adipogenesis of VAT adipocytes by increasing *ADIPOQ* expression, while VAT ECM impaired adipogenesis in SAT cells. Our results demonstrate that SAT is more adipogenic than VAT and suggest that divergences between SAT and VAT metabolic function are partially mediated by the depot-specific ECM microenvironment. APs and the ECM are potential targets for modulating AT function in dairy cows.

Key Words: adipogenesis, progenitors, extracellular matrix

2744 Cryopreservation and resuscitation of bovine duodenum tissues and enteroids cultivation methods. K. Nishihara*[1], K. Wood[1], L. L. Guan[2], and M. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Developing a methodology for intestinal tissue cryopreservation and resuscitation may help researcher's use of enteroids as an in vitro model to study gut functions. The objective of this study was to develop a method for intestinal tissue cryopreservation and resuscitation for enteroid cultivation. Two different types of tissues, fresh duodenum tissues (n = 3, from Angus steers) and duodenum tissues cryopreserved in 90% fetal bovine serum (FBS) and 10% dimethyl sulfoxide (DMSO) (n = 3, from Holstein calves), were collected to develop enteroids. Crypts were isolated by 2 m*M* EDTA/phosphate-buffered saline from both fresh and cryopreserved tissues and embedded in basement membrane extract. Embedded crypts were seeded in a 24-well plate and cultured in IntestiCult Organoid Growth Medium (Mouse) with inhibitors cocktail and Primocin. The upper of crypts isolated from both fresh and cryopreserved tissues became sealed, and crypts formed enteroids within 24 h. Primary (passage 0) enteroids showed budding crypt domains during 7 d of cultivation. After 7 d of cultivation, enteroids were passaged in a new 24-well plate. Passage 1 enteroids also formed enteroids within 24 h after passage and showed budding crypt domains during 7 d of cultivation. The 2-dimensional (2D) area of enteroids was obtained from each animal during d 1 to 7 in passage 0 and 1 and analyzed using generalized linear mixed model, respectively. The 2D area of enteroids derived from both tissues increased during d 1 to 7 in passage 0 and 1. There were significant passage and effects on the 2D area of enteroids derived from both tissues ($P < 0.05$, respectively), and phalloidin which binds to F-actin in the brush border microvilli was distributed in the luminal side of enteroids. In conclusion, a cryopreserved solution consisting of FBS and DMSO is useful for bovine intestine cryopreservation and resuscitation for enteroid cultivation. This method allows researchers to investigate intestinal function and health in the laboratory using enteroids derived from tissues collected from cattle.

Key Words: bovine enteroids, cryopreservation, intestine tissues

Reproduction 1

2745 How long is the estrous cycle of dairy cows? J. Denis-Robichaud*[1], A. P. Oliveira[2], M. H. C. Pereira[2], K. G. Pohler[3], R. L. A. Cerri[1], and J. L. M. Vasconcelos[2], *[1]Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, [2]Department of Animal Production, São Paulo State University, Botucatu, Brazil, [3]Department of Animal Science, Texas A&M University, College Station, TX.*

The objectives of this study were to compare the length of the estrus cycle in lactating Holstein cows following artificial insemination or placebo, and to explore potential risk factors associated with delayed luteolysis. We enrolled cows from a commercial herd in Brazil in this randomized controlled trial, from October 2016 to August 2017. Cows were enrolled in a synchronization protocol (using progesterone devices and estradiol). On the day of insemination (d0), cows that were identified in estrus with the activity monitoring system were randomly allocated to either a normal artificial insemination (AI) or an empty semen straw (PBO) with a 3:1 ratio. Ultrasound was used to assess the presence of a CL on d17, 24, and 31, and pregnancy on d31. We combined automated estrus monitoring information and CL presence to determine the cycle length. Cows that became pregnant following AI were excluded from the analyses. The cycle length was categorized as short (<17 d), normal (17–23 d), long (24–30 d), and very long (≥31 d). We compared the proportion of cows in each category between the AI and PBO groups using a Bayesian cumulative ordinal mixed model. We assessed parity, season, days in milk, daily milk production, body condition score, uterine size score, and presence of a corpus luteum (CL) at enrollment as risk factors for delayed luteolysis (long or very long cycle) using Bayesian mixed logistic regression models. Results are presented as odds ratio (OR) and 95% credible intervals (BCI). Data from 876 inseminations (AI: n = 616, PBO: n = 260) was collected, for which 12% had a short cycle, 31% had a normal cycle, 19% had a long cycle, and 38% had a very long cycle. There was no difference in the odds of being in longer estrous cycle categories for the AI compared with the PBO group (OR = 0.92, 95% BCI = 0.76–1.10). Inseminations during summer had 1.89 (95% BCI = 1.25–2.96) times greater odds of being followed by delayed luteolysis than during winter. If a CL was present at enrollment, the odds of delayed luteolysis were 1.66 (95% BCI = 1.05–2.60) times greater than if there was not. The proportion of cycles longer than 24 or 31 d was similar in the AI and PBO groups, suggesting most are not due to embryonic death. Moreover, environmental and hormonal factors were associated with delayed luteolysis.

Key Words: delayed luteolysis, risk factors

2746 Use of automated monitoring devices to characterize estrous cyclicity postpartum in lactating dairy cows. P. M. G. Peixoto, L. Factor, A. Mirzaei, R. C. Chebel, and R. S. Bisinotto*, *University of Florida, Gainesville, FL.*

Objective was to evaluate the predictive value of estrus events collected using automated monitoring devices (AMD) to determine estrous cyclicity postpartum based on the presence of corpus luteum (CL). Lactating Holstein cows (n = 2,025) fitted with a collar-mounted AMD for monitoring of activity and rumination, had their ovaries evaluated using transrectal ultrasonography (US) at 29 ± 3 and 43 ± 3 d in milk (DIM). Only cows without a CL ≥20 mm at 29 ± 3 DIM were evaluated at 43 ± 3 DIM. Overall, 69.9% (n = 1,416/2,025) of cows had a CL ≥20 mm by 43 ± 3 DIM and were classified as estrous cyclic. Estrus events identified by the AMD system, included information on DIM and heat index (HI), and pen movements per cow were recorded. Sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive values (NPV), and accuracy (Ac) of using estrus events to identify estrous cyclic cows were calculated, considering all HI values, according to the following criteria: at least one estrus event by 43 ± 3 DIM (Est43), multiple estrus events 43 ± 3 DIM (MEst43). Within each category, analyses considered either all estrus events (All) or excluded estrus events concurrent with a pen movement (M0d) or that followed a pen movement by 1 (M1d) or 2 (M2d) days. Predictive value of HI for determination of ovulation was assessed by receiver operating characteristic (ROC) curves using the LOGISTIC procedure of SAS. Heat index had a low predictive value on estrous cyclicity with All (AUC = 0.54), M0d (AUC = 0.52), M1d (AUC = 0.50), and M2d (AUC = 0.50). Other test characteristics are listed in the table below. In conclusion, a low predictive value was found using AMD to determine cyclicity in cows based on estrus events, and heat index up to 43 ± 3 DIM.

Key Words: anovular, anestrus, estrus detection

2747 Repeatability of intensity and duration of estrous expression in lactating Holstein cattle. A. M. Bega*[1], T. A. Burnett[2], A. M. L. Madureira[2], J. Denis-Robichaud[1], C. Baes[3], and R. L. A. Cerri[1], *[1]University of British Columbia, Vancouver, BC, Canada, [2]University of Guelph, Ridgetown, ON, Canada, [3]University of Guelph, Guelph, ON, Canada.*

The objective of this study was to analyze the repeatability of estrous intensity and duration detected by an automated activity monitor (AAM).

Table 1 (Abstr. 2746). Test characteristics comparing the use of automated monitoring devices (AMD) with transrectal ultrasonography to determine cyclicity in cows based on estrus events at 43±3 DIM

Category	Event	Cyclic[1]	Non-Cyclic	Sensitivity	Specificity	PPV[2]	NPV	Accuracy
Est43	All	1,053	972	0.58	0.62	0.78	0.39	0.59
	M0d	922	1,103	0.52	0.70	0.80	0.39	0.57
	M1d	883	1,142	0.50	0.72	0.81	0.38	0.57
	M2d	846	1,179	0.48	0.73	0.80	0.38	0.55
MEst43	All	322	1,703	0.18	0.89	0.79	0.32	0.39
	M0d	245	1,780	0.14	0.92	0.79	0.31	0.37
	M1d	205	1,820	0.11	0.93	0.79	0.31	0.36
	M2d	176	1,849	0.10	0.93	0.77	0.31	0.35

[1] Cows predicted as Cyclic or Non-Cyclic based on estrus events, independent of the HI value, recorded by the AMD system per group.

[2] Positive Predictive Value = PPV; Negative Predictive Value = NPV.

Cows were enrolled at calving, and postpartum exams were performed to identify retained placenta and metritis. Cows were equipped with a leg-mounted AAM within 2 weeks of parturition, and the system was checked for alerts twice daily. An estrus alert was determined as a relative increase in the cow's physical activity (index) that exceeded the threshold set by the manufacturer. The peak index was defined as the highest index during estrus, and the duration was the total time a cow's index exceeded the threshold value. The ovaries were examined on alert with an ultrasound on d 0, 1, 2, and 7 after the alert to assess ovulation success. On the day of estrus, we assessed body condition score (BCS), lameness, and uterine score. We used mixed linear regression models with a random intercept for cow and parity to allow the mean behavioral expression to vary among individuals and lactation. Models also included fixed effects for variables associated with peak and duration (DIM, lameness, milk production, retained placenta, and season). The repeatability of each estrus characteristic was calculated as the proportion of the variance explained by a given random effect after controlling for the included fixed effects. A total of 2,152 estrous events were recorded from 554 lactating cows. The peak index ranged from 63 to 1,422 (median = 250), and the duration ranged from 4 to 30 h (median = 12). While controlling for DIM, lameness, uterine score, milk production, retained placenta, and season, we found that 20.5% (95% CI = 18.6 - 22.5) of the variation in estrus peak activity and 11.4% (95% CI = 10.7 - 13.1) of the variation in estrus duration could be attributed to individual differences. We also found that only 2.1% (95% CI = 0.7 - 4.4) and 2.5% (95% CI = 0.7 - 5.8) of the variation in estrus peak activity and duration, respectively, could be attributed to parity. Estrus expression characteristics could be used to phenotype individual reproduction characteristics.

Key Words: estrus, activity-monitor, phenotyping

2748 Likelihood of conception based on health status and estrus intensity in dairy cows.

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Our objective was to quantify the associations between disease events before insemination and estrus intensity level at insemination, and the likelihood of conception (P/AI). Quantification of these associations may affect on-farm decision making such as the voluntary waiting period, choice of type and breed of semen, and culling. All data were collected from a certified organic Holstein dairy herd in the western USA, from 2019 to 2021. Disease and reproduction data were obtained through DRMS, Raleigh, NC. Activity data were obtained through IceRobotics, Stirling, UK. Our final data set contained 72,368 inseminations and their outcomes (0,1) from 13,558 cows. Relative estrus intensity (steps per hour before insemination compared with the cow's baseline) was available for 16,972 inseminations. Health status was categorized as mastitis (before 1,425 inseminations), metabolic (330), reproduction (1,169), other disorders (591), unknown (29,499), 2 diseases (19,118), or at least 3 diseases (12,347). Cows without these events were declared healthy (7,889). We used Proc Logistic in SAS with parity, breeding number, and season as covariates. Variables of interest were health status and relative estrus intensity. All variables were categorized. We used the Area Under the Receiver Operating Characteristic curve (AUC) as measure of goodness of fit. Overall P/AI was 32%, ranging from 36% in the first lactation to 26% in fourth and greater lactation. For first inseminations after calving, least squares means P/AI were 39% (healthy cows), 37% (mastitis), 19% (metabolic), 16% (repro problems), 29% (other diseases), 28% (2 different diseases), and 24% (≥3 different diseases). Model AUC was 0.60. Using all inseminations, P/AI was 33% (never sick), 27% (last sick ≤60 d ago), 28% (last sick 60 to 120 d ago), and 27% (sick ≥120 d ago). Model AUC was 0.58. For first inseminations, P/AI associated with relative estrus intensity was 15% (<200%), 26% (<400%), 31% (<600%), and 26% (≥600%). Model AUC was 0.60. These results show important and long-lasting associations between health events, estrus intensity and P/AI that should inform decisions such as sire choice and voluntary waiting period.

Key Words: disease, activity, conception rate

2749 Effect of inducing luteolysis 5 or 6 d after the first GnRH on estrous expression and fertility in a modified timed-AI program for dairy heifers.

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The aim of this study was to determine the effect of delaying the induction of luteolysis in 1 d on expression of estrus and fertility in a modified timed-AI program for dairy heifers. Holstein heifers (n = 418) were randomly assigned into 2 treatments, PG5P (n = 208) and PG6P (n = 210). All heifers were pre-synchronized with a $PGF_{2\alpha}$ (Pre-PGF; 0.5 mg of cloprostenol) 2 d before the first GnRH (100 µg) of the program (G1). Heifers in PG5P received $PGF_{2\alpha}$ (PGF1) 5 d later, while heifers in PG6P received PGF1 6 d later. All heifers received an additional $PGF_{2\alpha}$ 1 d after PGF1 to ensure complete luteolysis. Detection of estrus was performed by tail chalk daily for 3 d after PGF1. Heifers detected in estrus by 2 d after PGF1 were inseminated on the same day and did not receive further treatments. The remained heifers received a final GnRH (G2) and AI 3 d after PGF1, if observed in estrus or not. Heifers were inseminated with sexed semen (n = 408) or beef semen (n = 4). Ovarian ultrasound was performed 2, 3 and 6 d after PGF1 to determine ovulation and ovulatory follicle size. Binary and continuous variables were analyzed using logistic and linear regression, respectively. The proportion of heifers detected in estrus tended ($P = 0.09$) to be greater for PG6P than PG5P (83.4% vs. 75.9%). More heifers in PG6P were in estrus 2 d after PGF1 than in PG5P (29.5% vs. 17.1%; $P < 0.01$). Even though the final ovulatory response was similar ($P = 0.12$) between treatments (PG6P – 94.4%; PG5P – 89.9%), more heifers in PG6P ovulated by 3d after PGF1 than in PG5P (26.5% vs. 12.4%, $P < 0.01$). Ovulatory follicle size did not differ between groups (PG6P – 15.8 ± 0.1 mm; PG5P – 15.8 ± 0.1 mm, $P = 0.86$). Overall P/AI 32 d after AI was 49.6% and was not different among treatments (PG6P – 49.5%; PG5P – 49.8%, $P = 0.78$). In summary, delaying time of luteolysis tended to increase overall expression of estrus up to timed-AI and increased estrus 1d before G2; however, it did not improve P/AI. Overall fertility of both treatments was satisfactory as timed-AI program using sexed semen in dairy heifers.

Key Words: heifer, timed artificial insemination, fertility

2750 Effect of doubling the dose of gonadorelin hydrochloride at the first GnRH of a CIDR Synch program on ovulation rate and pregnancies per AI in Holstein heifers.

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The objective of the study was to determine the effect of doubling the dose (100 µg vs. 200 µg) of gonadorelin hydrochloride (Factrel, Zoetis Inc.) at the first GnRH of a CIDR Synch program on ovulation rate and pregnancies per AI (P/AI) in first service in Holstein heifers. A total of 1,308 Holstein heifers were randomly allocated between 2 treatments to receive either a 100 µg (2CC = 655) or 200 µg (4CC = 653) dose at the first of GnRH (G1) of a CIDR Synch protocol (CIDR and GnRH (G1) – 6d – CIDR removal and PGF – 1d – PGF – 2d – TAI). A subset of 396 heifers had their ovaries scanned, and blood samples were collected to measure the serum concentration of progesterone (P4) at GnRH (G1) and 6 d later. The P4 concentrations were categorized as Low (<3ng/ml) or High (≥3ng/ml). Statistical analysis was performed using the GLIMMIX procedure of SAS. Ovulation rates were greater ($P < 0.01$) for heifers receiving 4CC than 2CC at G1 (54.7% vs. 42.8%). Ovulation rates were greater ($P < 0.01$) for Low P4 than High P4 at G1 (60.5% vs. 37.0%). However, there was not an interaction ($P = 0.26$) between treatment and P4 (Low P4 2CC = 57.3% vs. High P4 2CC = 28.2%; Low P4 4CC = 63.7% vs. High P4 4CC = 45.8%). The ROC curve analysis indicates that P4 concentrations at the time of G1 treatment could predict ($P < 0.01$) the ovulatory response, although the area under the curve was only 0.6. There was no effect of treatment on PAI (2CC = 61.2% vs. 4CC = 60.7%). In summary, low P4 concentration and increasing the dose of GnRH at G1 positively impacted ovulation response in Holstein heifers. However, there was no interaction between treatment and P4 on ovulation, and a subsequent impact of GnRH dose on PAI was absent.

Key Words: conception rates, ovulatory response, estrous synchronization

2751 Effect of delaying induction of ovulation on ovarian function of lactating dairy cows synchronized with Double-Ovsynch and inseminated after detected estrus or timed AI. A. L. Laplacette[1], C. Rial*[1], D. Duhastchek[1], M. M. Perez[1], M. L. Stangaferro[2], M. J. Thomas[2], and J. O. Giordano[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Dairy Health and Management Services, Lowville, NY.*

Our objective was to evaluate the effect of delaying induction of ovulation by 24h on ovarian function of cows synchronized with a Double-Ovsynch (DOv) protocol and AI at detected estrus (AIE) or timed AI (TAI). Lactating Holstein cows (n = 357) were blocked by parity, and randomly assigned to a DOv protocol with GnRH(GTAI) at 56 and TAI at 72h (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-1d-PGF-32h-GnRH-16h-TAI; G56 n = 179) after induction of corpus luteum regression (CLR) before scheduled TAI or GnRH at 80 and TAI at 96h after induction of CLR (G80 n = 178). Cows detected in estrus by leg-attached automated activity monitors after induction of CLR were AIE without GnRH. Transrectal ultrasonography and progesterone (P4) concentrations in blood were used to evaluate ovarian function. Binary data were analyzed by logistic regression and continuous data by ANOVA. At induction of CLR, there were no differences ($P > 0.10$) between G56 and G80 for largest follicle diameter (LFol; 13.9 ± 0.3 vs 13.6 ± 0.3 mm), cows with a CL visualized by ultrasound (CLUS; 97.2 vs 98.3%), P4 > 1ng/mL (CLP4; 93.6 vs 93.3), CL number (1.7 ± 0.1 vs 1.7 ± 0.1), and P4 (4.8 ± 0.2 vs 4.6 ± 0.2 ng/mL). For cows from both treatments (G56 = 2; G80 = 48) that were AIE, 100% had CLR, P4 was 0.11 ± 0.0 ng/mL, and LFol was 17.7 ± 2.4 mm at AI. At GTAI (cows AIE excluded), there were no differences ($P > 0.10$) between G56 and G80 for cows with CLR (97.0 vs 95.9%), LFol (16.1 ± 0.2 vs 16.4 ± 0.3 mm), cows with LFol > 15 mm (78.4 vs 74.2%) and P4 (0.2 ± 0.5 vs 0.2 ± 0.5 ng/mL). At GTAI, there were no differences ($P > 0.10$) between G56 and G80 for ovulatory response (96.6 vs 94.8%) and ovulatory follicle size (16.1 ± 0.2 and 15.9 ± 0.2 mm). At 6d post-AI, the same proportion of cows that received TAI had a CLUS for G56 and G80 (97.1 vs 94.8%, $P = 0.26$) but more ($P = 0.02$) cows had CLP4 for G80 (94.2%) than G56 (87.2%). Also, at 6 d post-AI CL volume (7,471 ± 287 vs 7,424 ± 339 mm3) and P4 (1.8 ± 0.1 vs 1.9 ± 0.1) did not differ ($P > 0.10$). Delaying induction of ovulation by 24h did not affect ovarian function of cows that received TAI after a Double-Ovsynch protocol when cows were allowed to be inseminated at detected estrus before the scheduled TAI.

Key Words: ovulation, estrus, synchronization

2752 Prepartum acetylsalicylic acid in high-priority cow groups: Effects on cow health and reproductive performance. E. Jimenez*[1], P. Zarei[1], J. Spring[1], M. Dailey[1], C. Zheng[1], J. Lection[2,3], M. Martinez[1], E. Hovingh[1], and A. Barragan[1], *[1]Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA, [2]Intercollege Graduate Degree Program in Integrative and Biomedical Physiology, Penn State University, University Park, PA, [3]Department of Animal Science, Penn State University, University Park, PA.*

The objective of this study was to assess the effects of pre-partum administration of acetylsalicylic acid on the incidence of diseases and reproductive performance in high-priority cow groups (i.e., over conditioned cows [BCS ≥ 3.75 pts.], primiparous cows, and cow with calving disorders [i.e., stillbirth, dystocia, and/or twins]). At 14 d before the expected calving date, cows (n = 404) and heifers (n = 160) were blocked by body condition score (optimal = 3–3.5; high ≥ 3.75) and parity (nulliparous; parous), and randomly allocated to one of 2 treatment groups: 1) ASA (n = 286): receive one oral administration of acetylsalicylic acid (4 boluses; 480 grain/bolus; 125 g/d); or 2) PLC (n = 278): receive one oral treatment with gelatin capsules filled with water. Subclinical ketosis, defined as BHB of ≥ 1.2 mmol/L, and clinical metritis, defined as precense of red-brownish watery fetid vaginal discharge, were assessed weekly for the first 21 ± 3 DIM. Clinical disease events in the first 60 DIM and reproductive performance by 150 DIM were collected from on-farm computer records. The data were analyzed using MIXED, GLIMMIX and LIFETEST procedures of SAS as a randomized complete block design. Over conditioned cows treated with ASA had lower incidence of subclinical ketosis at 7 ± 3 DIM compared with ASA optimal body condition cows (Over conditioned ASA = 32.28 ± 5.26%; Optimal body conditioned ASA = 56.48 ± 9.08%; $P = 0.04$). Primiparous cows treated with ASA tended to have higher subclinical ketosis incidence at 21 ± 3 DIM compared with primiparous cows treated with PLC. Cows and heifers in the ASA group tended to have lower incidence of clinical metritis at 7 ± 3 DIM compared to PLC cows. There was no difference in the incidence of clinical diseases events between treatment groups. A larger percentage of primiparous cows treated with ASA became pregnant in the first service compared with PLC primiparous cows (ASA = 56.14 ± 7.75%; PLC = 32.1 ± 4.93%; $P = 0.008$). Similarly, primiparous cows treated with ASA required less days to become pregnant compared with primiparous PLC cows (ASA = 106.45 ± 3.48 d; PLC = 124.12 ± 3.81 d; $P = 0.004$). These findings suggest that treatment with pre-partum acetylsalicylic acid may have positive effects on postpartum cow health and reproductive performance in high priority cow groups.

Key Words: prepartum acetylsalicylic acid, subclinical ketosis, reproductive performance

2754 Efficacy of an automated technology at detecting early postpartum estrus events: Can we detect resumption of cyclicity? S. Borchardt[1], T. A. Burnett[2], J. L. Plenio[3], R. S. Conceição[4], R. L. A. Cerri[4], and A. M. L. Madureira*[2], *[1]Clinic of Animal Reproduction, Faculty of Veterinary Medicine, Berlin, Germany, [2]University of Guelph, Ridgetown, ON, Canada, [3]Freie Universität Berlin, Institute*

for Veterinary Epidemiology and Biostatistics, Berlin, Germany, [4]University of British Columbia, Vancouver, BC, Canada.

The objective of this study was to evaluate the efficacy of an automated activity monitor (AAM) at detecting early postpartum estrus events. A total of 192 lactating cows (primiparous = 73 and multiparous = 119) were enrolled in this study. Cows were continuously monitored by a collar-mounted AAM early postpartum (7 to 30 DIM). Transition cow health was assessed early postpartum. Calving was classified as assisted (forced extraction of a calf) and unassisted (normal calving). Retained fetal membrane (RFM), metritis (MET), hyperketonemia (KET), mastitis (MAST) and milk production were recorded. Estrus events were alerted by the AAM using the standard company thresholds. Blood samples, from the coccygeal vein, were collected at 15, 18, 21, 24, 28 and 30 DIM for progesterone (P4) analysis. Resumption of cyclicity was considered when P4 concentration was $\geq$1ng/mL in any collection day. Cows were considered anovular when P4 concentration was <1ng/mL on all collection days. Cows were classified as: True Positive – P4 $\geq$ 1ng/mL and at least one estrus alert; False Positive – P4 < 1ng/mL and at least one estrus alert; True Negative – P4 < 1ng/mL and no estrus alerts and False Negative – P4 $\geq$ 1ng/mL and no estrus alerts. Statistical analyses were performed by ANOVA using the linear mixed regression models (GLIMMIX) on SAS. The specificity and sensitivity of the sensor to detect cows which had resumed cyclicity were 84.0% and 34.1%, respectively. A total of 42.2% of cows had a false negative whereas only 15.9% of cows had a false positive estrus alert. Cows that were not cycling were more likely to have RFM ($P = 0.03$), MET ($P < 0.001$) and have an assisted calving ($P = 0.02$). There was no effect of milk production ($P = 0.56$), parity ($P = 0.72$), MAST ($P = 0.45$), KET ($P = 0.63$) on the resumption of cyclicity. None of the health events recorded in this study had and an effect on the proportion of cows that had a false negative event. In conclusion, the sensor has a high specificity for detecting anovular cows, but it has lower sensitivity, and thus not effective at detecting cyclic cows, perhaps due to silent estrus early postpartum.

Key Words: resumption of cyclicity, automated activity monitor, transition cow health

2755 Development and demonstration of lateral-flow immunoassays for determination of the pregnancy and ovarian physiological status of cows. C. Rial*[1], I. Hussain[2], D. Erickson[2], J. Branen[3], and J. O. Giordano[1], *[1]Deparment of Animal Science, Cornell University, Ithaca, NY, [2]Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, [3]BioTracking, LLC, Moscow, ID.*

Our objective was to develop and evaluate lateral-flow immunoassays (LFIA) for determination of pregnancy and ovarian physiological status of cows based on concentrations of pregnancy-specific protein B (PSPB) and progesterone (P4). Test strips were developed for P4 (LFIA-P4) and PSPB (LFIA-PSPB). Strips for P4 had a sample, conjugate, spacer, and absorbent pad, fluorophore anti-P4 conjugates, and a nitrocellulose membrane with P4-BSA and anti-mouse antibody. After adding plasma and buffer, strips were read in a fluorescence microscope to estimate P4 concentrations based on test-to-control line signal (T/C ratio). The PSPB strip was made of nitrocellulose membrane with polystreptividin, anti-mouse antibody, Europium-anti-PSPB conjugates, and biotin-PSPB. After adding buffer and plasma in a 96-well plate, strips were dipped to initiate flow for estimation of the T/C ratio. The T/C ratio was linearly associated with P4 ($R^2 = 0.98$) and PSPB ($R^2 = 0.97$) concentrations. Blood was collected and transrectal ultrasonography (TUS) done 29–35 d post AI in lactating Holstein cows (n = 50) to identify pregnant cows (PG; PSPB $\geq$ 2 ng/mL or embryo visible by TUS) and cows with a functional corpus luteum (CL; P4 $\geq$ 1 ng/mL or CL diameter $\geq$ 15 mm) with LFIA-P4 and LFIA-PSPB. Referent P4 concentrations were from RIA and ELISA for PSPB. Agreement between concentrations from the LFIA and RIA for P4 (kappa = 0.81; 95%CI 0.6–1.0) and the LFIA and ELISA for PSPB (kappa = 0.9; 95%CI 0.7–1.0) was high. Compared with TUS, accuracy (Acc) of LFIA-P4 for CL detection was 92% [100% sensitivity (Se); 72% specificity (Sp)] and compared with RIA, Acc of LFIA-P4 was 96% (96% Se; 100% Sp). For PG detection, LFIA-PSPB Acc vs TUS was 90% (100% Se; 72% Sp), and Acc was 96% (95% Se; 100% Sp) vs ELISA. Combining LFIA-P4 and LFIA-PSPB outcomes, correctly classified 90%(45/50) of cows in PG/CL, NoPG/CL, and NoPG/NoCL groups compared with P4 RIA and PSPB ELISA as referent. We conclude that the current LFIA strips could be used to predict the presence of a functional CL or pregnancy based on plasma concentrations of P4 and PSPB.

Key Words: lateral flow immunoassay, progesterone, pregnancy

2756 Genomic analysis of virulence and antimicrobial resistance of *Escherichia coli* isolated from cows with metritis. A. Garzon[1], C. Basbas[1], B. Weimer[1], C. Schlesener[1], N. Silva-del-Rio[1,2], B. Karle[3], F. Lima*[1], and R. Pereira[1], *[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, [2]Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Tulare, CA, [3]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Orland, CA.*

The pathophysiology of metritis in dairy cows is complex and is not entirely understood. *Escherichia coli (EC)* plays a major role in a cascade of events that affect the prevalence and severity of the disease. Endometrial pathogenic *EC* has been identified as adapted to the endometrium and leading to uterine disease. Next-generation sequencing has shown the diverse uterine microbiome of cattle, however, disease causality for metritis has not been completely understood yet. There is limited information using whole-genome sequencing (WGS) approaches to characterize virulence factor (VF) and antimicrobial resistance (AMR) genes in EC from cows with metritis. This project aimed to investigate the association between the prevalence of specific AMR and VF genes in EC with the animal health status related to uterine infection. Using a cross-sectional study design, uterine swabs from 162 postpartum cows between 3 and 21 DIM were collected from 25 dairy farms in CA. Cows were categorized into one of 3 clinical presentation groups before enrollment using a metricheck: metritis (MET), watery, red-brown, and fetid vaginal discharge (VD); purulent VD (PUS) non-fetid purulent or mucopurulent VD; and control cows, (CTL) with either no VD or a clear VD. Bacterial isolates (162) were retrieved from the uterus using a doble-guarded swab. PERMANOVA analyses were conducted to investigate differences between the antimicrobial drug classes or the virulence factor categories among health status (control, pus, metritis), and univariate analyses were used at the gene level for both AMR and VF genes and health status. The pangenome of uterine *EC* did not differentiate among health groups. The most abundant AMR genes found at the drug class level were aminocoumarin (23%), β-lactams (12%), and fluoroquinolones (10%), without differences at the gene level. The most abundant virulence genes found in isolates belong to the adherence (55%), and the effector delivery system class (27%). fimH was a core gene on the pangenome, therefore it was not associated with health status. Although our study did not find any differences at the gene level between the EC from healthy and metritis cows, the difference in disease development may have been because of genomic variants of VF or AMR genes more than the gene presence or absence

Key Words: whole-genome sequencing, cattle, antibiotic resistance

Ruminant Nutrition Symposium: Dairy Nutrition to Improve Feed Utilization—Recognizing the Contributions of ADSA Fellow Dr. Bill Weiss

2757 How understanding variability in feedstuffs improves feeding practices. W. P. Weiss* and N. R. St-Pierre, *Ohio State University, Wooster OH.*

Software used to formulate diets do not incorporate variability in nutrient composition of feeds and requirements. Formulating diets using mean composition of ingredients to meet the mean inputs of a pen is suboptimal. The best dietary safety factors (SF) depend on economics and on the variability in both requirements and nutrient composition. Accurate estimates of true variation in nutrient composition can be used to derive best SF. Good data are available on the total variation in feed nutrients such as minerals and crude protein, but total variation includes that caused by the observer (sampling and analytical variation) and true variation. Based on limited data, observer variation comprised 40 to 70% of the total variation in nutrient composition of feeds and TMR. For many feeds, observer variation exceeded true variation, making a good population mean more accurate than a single sample. For feeds such as forages and many byproducts, multiple samples within a farm are needed to estimate true variance. This number can then be used when formulating a diet. For example, if the true SD for CP in distillers grains is known, reducing the entered CP by 1 SD unit ensures that ~67% of the time the distillers grains will provide at least as much CP as expected. Assuming good TMR making techniques, the variation in nutrient composition of a TMR should be less than the variation in its ingredients. Mathematical techniques exist to estimate SD of a TMR based on ingredient true variation and inclusion rates. With this information, one can determine the inclusion rate of an ingredient with high variation (which are often inexpensive) that will not adversely affect TMR variation. We have essentially no information on variability of nutrients calculated for the total diet (e.g., NEL and MP). Because multiple components go into calculating these nutrients, variation is likely less than what is observed for feed-based nutrients. Because NEL and MP are nutrients most likely to limit milk production, accurate estimates of their variation would allow for more accurate energy and protein SF which should reduce feed costs and increase efficiency.

Key Words: variation, composition

2758 Improvements in diet formulation and evaluation of energy for dairy cattle. P. J. Kononoff*, *University of Nebraska-Lincoln, Lincoln, NE.*

The net energy system estimates the intake of useable energy and allows comparison to the requirements of maintenance, growth, and production yielding insightful estimates of allowable production. Methods to measure or predict energy in dairy feeds have undergone evolution after the OARDC energy equation was outlined 40 years ago. This summative representation has evolved to its current use by predicting net energy for lactation (NEL) from the chemical feed fractions and pairs this information with derived digestion coefficients and subtracts the aggregate from metabolic fecal components. The NEL values of feeds in NASEM (2021) are not fixed but derived from the energy content from the digestible portion of NDF, CP, fatty acids, and starch with remaining material being described as residual organic matter. NEL represents energy of the total diet and not a sum of individual feeds. Methods to account for more feed-based sources of variation and associative animal effects have been investigated and identified. In parallel with these, adjustments in animal requirements have kept in pace with experimental observations. These include a 25% increase in maintenance energy, a more dynamic estimate of energy needed to support fetal growth, likely a result of genetic improvements, an increase in the conversion of NEL from 0.64 to 0.66. Supporting all of these advancements is use of simplistic but laborious digestion experiments which may vary in design in interpretation and novel experimental designs. Future advances in the NEL system should attempt to shed light on variable efficiencies of nutrient use. Recent interest and attention related to cattle nutrition and the environment has spurred application of methods to measure losses of energy which vary in application, accuracy, and precision; all of which will affect consideration of integration into future energy systems. In the future the NEL system will undoubtedly be enhanced with improved measure of nutrients and growing understanding of their availability and effects on the body.

Key Words: net energy of lactation, feed, digestion

2759 Dairy nutrition to improve feed utilization—Recognizing the contributions of ADSA Fellow Dr. Bill Weiss beyond prevention of metabolic diseases: Feeding transition dairy cows for optimal performance. A. Tebbe*, *Purina Animal Nutrition LLC, Shoreview, MN.*

High producing dairy cows are metabolically challenged during the transition period. This is because rapid increases in nutrient requirements for fetal growth, colostrum synthesis, and high milk solids yield often surpass metabolizable intake from the diet. When dietary supply fails to meet demands, cows mobilize nutrients from internal stores including bone, muscle or adipose tissues to meet requirements. However, transition cows can still experience a high incidence of metabolic diseases due to a lack of or excessive nutrient mobilization from body stores. Minimizing the incidence of metabolic diseases is key to improving cow health, and increasing peak milk and milk yield over an entire lactation. Thus, most nutritional research with transition cows has focused on strategies to prevent metabolic diseases (e.g., hypocalcemia, acidosis, ketosis). Most nutritional strategies have focused on the prepartum cow, and these strategies (e.g., controlled energy diets, negative DCAD diets) are commonly implemented on commercial dairies. Less research on the postpartum period is available, but as average herd size increases, grouping and feeding postpartum cows separately to improve health and production will become more common. Current postpartum research suggests increasing metabolizable protein concentration and improving amino acid balance of the postpartum diet improves milk solids yield. Increasing dietary energy in the postpartum period has had mixed results depending on the source (i.e., fat vs. starch), fermentability of the starch source, and fatty acid profile. Production responses when manipulating the postpartum diet may also depend on the prepartum diet composition and parity. Understanding the interactions of diet, parity, and the supply of nutrients between pre- and postpartum diets will be important to optimizing transition cow health and production.

Key Words: postpartum nutrition, metabolizable protein and energy supply, diet and parity interactions

2760 Mineral absorption and how that impacts mineral requirements. J. Goff*, *Iowa State University, Ames, IA.*

Macrominerals include the cations Ca, Mg, Na, and K and the anions Cl, P, and S. The requirement for absorbed mineral is the sum of tissue demands for maintenance, lactation, pregnancy and growth. The amount of mineral to be supplied by the diet to reach these absorbed mineral requirements is affected by the relative availability of the form of the mineral in the diet. Various sources of mineral will have differing coefficients for absorption. Most minerals utilize 2 methods of crossing the intestinal epithelial barrier. Specific ionized mineral transporter proteins/channels facilitate absorption of these minerals. The 2nd mechanism is by passive diffusion down a concentration and / or electrical gradient into the interstitial space below the absorptive epithelium and into the blood. The blood concentrations of the macrominerals are often tightly controlled and the homeostatic mechanisms in place to maintain a stable concentration of one mineral can affect the absorption or excretion of another mineral. The relative amounts of the minerals in the blood have a large impact on blood acid-base balance as well. The trace minerals, Cu, Zn, Mn, Fe, Co, Se, and I also have specific carrier mechanisms. More important to trace mineral absorption in ruminants is the negative impact the rumen environment has on solubility and bioavailability of trace minerals. Specific examples of each of the above mineral considerations will be presented for discussion.

Key Words: macrominerals, absorption, homeostasis

ADSA-Graduate Student Competition: Dairy Foods—Poster

1000M Impact of gas ultrafine bubbles on the efficacy of antimicrobials for removing fresh (3-day) and aged (30-day) *Bacillus subtilis* biofilms on dairy processing surfaces. P. Unger*, A. S. Sekhon, S. Sharma, A. Lampien, and M. Michael, *Washington State University, Pullman, WA.*

Ultrafine bubbles (UFB) technology is a novel concept in food safety that has the potential to improve the potency of commonly used antimicrobials to eliminate biofilms on dairy processing surfaces. This study investigated the impact of incorporating gas [air, carbon dioxide (CO_2), and nitrogen (N_2)] UFB on the potency of chlorine (Cl_2; 50, 100, and 200 ppm) and peracetic acid (PAA; 20, 40, and 80 ppm) antimicrobial (AM) solutions against fresh (3-d) and aged (30-d) *Bacillus subtilis* biofilms on polypropylene, silicone, and stainless steel surfaces. The *B. subtilis* biofilms were statically grown on polypropylene, silicone, and stainless steel coupons (2.54 × 7.62 cm) at 25°C for 3 d or 30 d, by immersing in *B. subtilis*-inoculated brain-heart infusion broth. On d 3 or 30, all coupons were rinsed in deionized water and allowed to air dry for 5 min. Coupons were then treated by submerging in AM solutions with and without UFB for 1 min and then were swabbed into Dey-Engley neutralizing broth and enumerated on brain-heart infusion agar. The log reductions for the respective antimicrobial treatments were calculated by subtracting posttreatment biofilm bacterial populations from untreated biofilm bacterial populations. Data were analyzed using 2-way ANOVA, and Tukey's test was used to determine significant differences among mean values at $P \leq 0.05$. Incorporation of CO_2 UFB in AM solutions resulted in significantly greater log reductions (2.2–3.7 log cfu/cm^2) of fresh and aged *B. subtilis* biofilms on polypropylene, silicone, and stainless steel compared with AM solutions without UFB on the respective surfaces (1.2–2.5 log cfu/cm^2). This study also demonstrated that the incorporation of 200 ppm Cl_2 (2.6–3.8 log cfu/cm^2) or 80 ppm PAA (2.7–4.4 log cfu/cm^2) resulted in significantly greater log reductions of *B. subtilis* biofilms on fresh and aged polypropylene, silicone, and stainless steel surfaces compared with 50 ppm Cl_2 (1.0–2.1 log cfu/cm^2), 20 ppm PAA (0.9–2.1 log cfu/cm^2), and 40 ppm PAA (1.6–3.2 log cfu/cm^2).

Key Words: ultrafine bubbles, *Bacillus subtilis*, biofilms

1532T Production of lactose-derived oligosaccharides under high temperature and low pH using β-galactosidase from *Lactobacillus helveticus* OSU-PECh-4A. S. Ruiz-Ramírez* and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Galacto-oligosaccharides (GOS) are a highly valued and well-known class of prebiotics with structures and functions comparable to those of human milk oligosaccharides (HMO). They are catalyzed by β-galactosidases, from which those isolated from probiotic strains have caught more attention because of the safety of the probiotic source. However, using those β-galactosidases for GOS production results in yields lower than 50% due to the enzyme's lack of stability at high temperatures. This study aims to utilize the purified β-galactosidase from *Lactobacillus helveticus* OSU-PECh-4A, a lactic acid bacterium (LAB) with probiotic properties, to synthesize GOS from lactose using sodium phosphate at acidic pH and high temperature. To achieve that, native β-galactosidase containing a specific activity of 20U/mg was added to 300 g/L of lactose solution (pH 4.5). The solution was incubated at 50°C for 24 h; samples were taken every 2 h and heated at 99°C for 5 min to stop the reaction. The GOS production was detected and quantified by high-pressure liquid chromatography coupled with a charged aerosol detector (HPLC-CAD). Results show that GOS produced by the β-galactosidase from *L. helveticus* was detected after 1 h of reaction. After 8 h, more than 50 ± 2% of lactose was hydrolyzed, and a maximum of 295 ± 10 μg/mL GOS primarily with a degree of polymerization (DP) of 3, comparable to 6'galactosyl-lactose, were synthesized under 8 h. Nonlactose disaccharides were also detected after 12 h, and it is hypothesized that the increase in lactose concentration at 12 h is due to the overlapping of peaks and structure similarity among these disaccharides. These results could directly reflect the potential of the enzyme to hydrolyze lactose efficiently and to produce highly valued GOS at high temperatures and acidic pH, making it optimal to use on dairy matrices.

Key Words: β-galactosidase, galacto-oligosaccharides, *Lactobacillus helveticus*

1002M Enhanced probiotic potential of *Lactobacillus kefiranofaciens* bdgo1 co-cultured with *Kluyveromyces marxianus* for application in dairy products. B. D. González-Orozco*, E. Kosmerl, R. Jiménez-Flores, and V. B. Alvarez, *The Ohio State University, Columbus, OH.*

Lactobacillus kefiranofaciens bdgo1 is a microorganism isolated from kefir grain that was previously shown to have probiotic characteristics. Additionally, it is the principal microorganism responsible for kefiran production, an exopolysaccharide (EPS) with vast technological and nutritional properties. Interkingdom interactions are found in natural food products such as kefir and kombucha. Previous literature has shown that co-culturing lactic acid bacteria with yeast can enhance their metabolic activities and promote resistance to environmental stressors compared with monocultures. The hypothesis is that the coculture of *L. kefiranofaciens*-bdgo1 with *Kluyveromyces marxianus* could improve its probiotic properties through increased survival during digestion, adhesion to intestinal cells, and improved anti-inflammatory activity on intestinal cells. Furthermore, the antibacterial activity and EPS production of the coculture was investigated. Survival across digestion phases was assessed by the INFOGEST digestion model and plate count technique. Adhesion and immunomodulation after inflammation induction with heat-killed *Salmonella typhimurium* LT2 cells (HKSC) were performed in Caco-2 cells. The EPS production was assessed by HPLC and by phenol-sulfuric method and the antibacterial activity of cell-free supernatant by well diffusion assay. All cell culture experiments were performed at least 3 times in triplicate for the coculture and respective monocultures and analyzed using one-way ANOVA at $\alpha = 0.05$. The EPS production experiments were performed in triplicate, and the results were analyzed by Student's *t*-test for the coculture and monoculture condition. The coculture of *L. kefiranofaciens* with *K. marxianus* significantly increased survival during simulated digestion by 2-fold and adhesion to epithelial cells by 1.3-fold. The latter may be attributed to the observed increase in EPS production in coculture which could play a role in protecting the microorganisms from the severe gastric tract conditions. Cell-free supernatant of *L. kefiranofaciens*-bdgo1 in mono and coculture portray antibacterial activity against indicator microorganisms. The results of the study warrant further research into the application of cocultures of *L. kefiranofaciens* bdgo-1 and EPS in functional/symbiotic dairy products.

Key Words: coculture, exopolysaccharides

1544T Evaluating structural changes in β-lactoglobulin induced by supercritical CO_2 treatment of whole milk powder resulting in a reduction in antigenicity. R. Venkatram[1], I. García-Cano[2], S. de Lamo Castellvi[1], L. Rodriguez-Saona[1], and R. Jiménez-Flores*[1], *[1]The Ohio State University, Columbus, OH, [2]National Institute of Medical Sciences and Nutrition, Mexico City, Mexico.*

Cows' milk allergy (CMA) is a common phenomenon in early childhood (<5 years of age) with an average occurrence of 4.9%. The prevalent allergen in cows' milk is β-lactoglobulin (β-LG). The objective of this study was to modify the antigenicity of β-LG in whole milk powder using $ScCO_2$. Whole milk powder was treated using food-grade CO_2 at temperatures of 63 and 75°C, and operating pressures between 100 - 300 bar. Proteins in whole milk powder were examined using SDS-PAGE and ELISA. Orbitrap Fusion LC/MS-MS and periodic staining were performed to confirm post translational modifications in β-LG. Changes in secondary structure of β-LG isolated before and after treatment, was studied using UV-Vis absorption, intrinsic and extrinsic fluorescence, circular dichroism and Fourier transform infrared spectroscopy. The SDS-PAGE of treated samples yielded fuzzy β-LG bands indicative of an increase in molecular weight, presumably due to chemical changes of the protein, and demonstrated a maximum of 71.13 ± 0.29% decrease in the band intensity of β-LG under treatment conditions of 75°C/300 bar for 30 min (*t*-test; $P < 0.05$). The ELISA tests proved that $ScCO_2$ treatment specifically and significantly affected the antigenicity of β-LG with a reduction of 42.9 ± 2.83% and 54.75 ± 2.43% at 63°C/200 bar and 75°C/300 bar, respectively (*t*-test; $P < 0.05$). Orbitrap fusion detected presence of fatty acids and sugar moieties bound to β-LG, and the latter was confirmed by periodic staining. Secondary structure analysis revealed interactions with fatty acids and sugars affected the surface hydrophobicity and structural composition of β-LG while also highlighting significant variations between heat and supercritical treated samples. This study demonstrates 2 approaches to mitigate β-LG antigenicity, via fatty acid binding and lactosylation as shown by chemical changes to β-LG on supercritical treatment. Thus, $ScCO_2$ treatment appears to be a promising approach with low environmental impact to overcome CMA and presents a great opportunity for further research.

Key Words: supercritical CO_2, antigenicity, β-lactoglobulin

1004M Impact of milk serum protein and lactose removal on the properties of ultrapasteurized protein beverages. K. Ow-Wing*[1], M. A. Drake[1], and D. M. Barbano[2], *[1]North Carolina State University, Raleigh, NC, [2]Cornell University, Ithaca, NY.*

Milk serum proteins (SP) denature and produce sulfur off-flavors, while thermal degradation of lactose decreases beverage whiteness. Consumers are interested in lactose free and minimal ingredient beverages. Our objective was to simultaneously remove SP and lactose from skim milk using microfiltration (MF) and determine the impact of thermal processing [direct steam injection ultrapasteurization (UP)] on chemical, physical, and sensory properties of 3.4, 7.5, and 10.5% milk protein beverages. A 0.1-µm pilot scale ceramic MF was run at 50°C with continuous diafiltration (DF) at 3.4% protein to achieve >95% SP and >97% lactose removal from 350 kg of skim milk. The micellar casein retentate was then concentrated to produce a 10.5% protein, 97% lactose reduced micellar casein concentrate (MCC), and 3 protein concentrations were achieved by dilution with DI water. This process was replicated twice with different batches of skim milk. Physical and sensory properties were evaluated, and differences were determined by ANOVA with means separation. The volume of DF water required to achieve approximately 97% lactose removal was 2.5× the milk volume at a flux of 60 kg/m^2 per hour. The starting milk pH (21°C) and freezing point were 6.75 and −0.540°H, respectively. The final pH of the 3.4, 7.5, and 10.5% protein beverages were 7.18, 7.25, and 7.40, respectively at 21°C. In addition to lactose, other low molecular weight solutes were removed and the final freezing point of the 10.5% protein concentrate was −0.020°H. L-value was increased by MF ($P < 0.05$) and UP ($P < 0.05$), and this increase in whiteness was confirmed by sensory analysis. The MF process decreased yellowness (b*value) and following UP, beverages remained less yellow than the skim milk. Apparent viscosity increased with increasing protein ($P < 0.05$). Protein beverages processed by UP had no detected sulfur or eggy flavor. Sensory viscosity and chalkiness of beverages increased with protein and UP ($P < 0.05$). With higher protein content and UP, the liquid protein beverages were more white in appearance and more bland in flavor than skim milk, making MF milk an ideal starting material to produce new flavored shelf-stable beverages.

Key Words: protein beverage, lactose free

1005M Bacteria enumerated by laboratory pasteurization count in organic raw milk are predominantly Gram-positive sporeformers and Gram-positive cocci. R. Lee*[1], R. Evanowski[1], H. Greenbaum[2], M. Wiedmann[1], and N. Martin[1], *[1]Cornell University, Ithaca, NY, [2]University of Southern California, Los Angeles, CA.*

Laboratory pasteurization count (LPC) enumerates thermoduric bacteria and is a parameter used to assess raw milk quality. Although there is currently no regulatory limit for LPC, LPC data are being used by dairy producers and cooperatives to designate quality premiums and troubleshoot bacterial contamination issues. While some studies have reported that organisms quantified by LPC predominantly represent Gram-positive cocci, there is limited knowledge on the types of organisms enumerated by LPC in contemporary organic raw milk. To address this gap, US organic raw milk samples (n = 94) were assessed using LPC, and bacterial isolates were characterized. All unique surface colony morphologies and 5 distinct subsurface colonies per sample were isolated and characterized. The LPC ranged from below detection (<0.70 log cfu/mL) to 4.1 log cfu/mL (mean = 1.5 log cfu/mL). Among 382 isolates characterized by 16S rDNA sequencing, 53, 44, and 3% were identified as Gram-positive sporeformers (GPS), other Gram-positives, and Gram-negative cocci, respectively. Isolates identified as GPS were predominantly *Bacillus* (169/382), and other Gram-positives were predominantly *Brachybacterium* (55/382) and *Kocuria* (46/382). The proportions of GPS in raw milk samples with LPC of ≤1 log cfu/mL, 1 to 2 log cfu/mL, and ≥2 log cfu/mL were 0.57, 0.50, and 0.48, respectively, showing an inverse trend between increasing LPC and proportion of GPS. In individual raw milk samples, the proportion of GPS ranged from 0.0 to 1.0. Hence, without isolate characterization, LPC alone does not provide reliable information on bacterial populations present. We thus characterized all isolates using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). Although our data indicated agreement between 16S and MALDI-TOF MS for 65.8% of isolates at the genus level, the remaining isolates were either mischaracterized or resulted in no reliable identification using MALDI-TOF MS. This suggests that further optimization of this method is needed to allow for characterization of thermoduric organisms commonly found in raw milk.

Key Words: thermoduric, laboratory pasteurization count

1006M Impact of ultrafiltration temperature on the physical and sensory properties of skim milk. T. P. Truong*[1], A. J. Hernandez[1], M. A. Drake[1], and D. M. Barbano[2], *[1]North Carolina State University, Raleigh, NC, [2]Cornell University, Ithaca, NY.*

Bound versus soluble mineral content of milk changes as a function of milk temperature, and that may affect mineral removal during ultrafiltration (UF) of skim milk. Differences in mineral content may affect physical and sensory properties. Our objective was to determine the effect of removal of lactose (removal to <0.1% lactose) and low molecular weight solutes by UF at 7 or 50°C on physical and sensory properties of UF skim milk at 3 different protein concentrations (3.4, 7.5, and 10.5%) after 3 different heat treatments (no heat; high temperature, short time; and autoclave).Two batches of pasteurized skim milk were split and UF with diafiltration was conducted at 7 or 50°C. Physical and sensory properties were evaluated by 3-way ANOVA (UF temperature, protein concentration, heat treatment). The mean flux at 7 and 50°C were 24 and 49 Kg/m^2/h, respectively, at a 2.5X concentration factor. The lactose content of the 3.4, 7.5, and 10.5% UF skim was < 0.1%. More calcium and phosphorous were removed by cold UF than hot UF (9 and 6%, respectively; $P < 0.05$), while the freezing points of all beverages were close to 0°C and not different ($P > 0.05$). All lactose-removed beverages were heat stable with high temperature, short time and autoclave heat processing, but were not stable (i.e., formed a gel during processing) with direct steam injection ultrapasteurization (142°C for 3 s). Color (L, a, b*) values were not influenced by UF temperature, protein concentration, or heat treatment ($P > 0.05$), but all lactose-removed beverages appeared whiter than the original skim milk ($P < 0.05$). Apparent viscosity ranged from 2.7 to 27 Pa·s and increased ($P < 0.05$) with protein concentration and heat treatment. Compared with the starting skim milk, all lactose-free beverages at all protein concentrations were more white and bland in flavor (lower sweet aromatic and milky flavors, lower sweet and salty tastes; $P < 0.05$) than the starting skim milk. There was an increase in viscosity with increased protein concentration that was consistent with apparent viscosity of the beverages. Ultrafiltration can effectively remove lactose to produce lactose-free milk. Ultrafiltered skim milk processed at either hot or cold temperatures with <0.1% lactose remaining provides an ideal starting material to use for protein beverage innovation.

Key Words: Ultrafiltration, temperature, protein beverages

1007M Consumer acceptance of protein beverage ingredients: Less is more.

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An array of added ingredients is used in protein beverage formulations. These ingredients may not be desirable to consumers. Our objective was to determine consumer perception of ingredients in protein beverages. An online survey was conducted with protein beverage consumers (n = 422). Maximum difference scaling and projective mapping were applied to determine the relative acceptance of ingredients based on their functional role (protein source, sweetener, stabilizer, thickener). Kano analysis and importance scales were used to assess ingredient and package messaging. Subsequently, focus groups were conducted (n = 25 consumers). Survey data were evaluated by univariate and multivariate statistics. A journey map and benefits hierarchy were generated from focus groups. Consumers placed the highest importance on the amount of protein followed by protein type in protein beverages. Plant protein, whey protein, and milk protein were most appealing, while soy protein, collagen, and casein/caseinates were less appealing ($P < 0.05$). Natural sweeteners (agave, monk fruit, cane sugar) were the most appealing sweeteners ($P < 0.05$). Fibers and starches were more appealing than gums (carrageenan, gellan gum; $P < 0.05$). Stabilizers were the least desirable class of beverage ingredients, with sodium and potassium phosphates the least desirable ($P < 0.05$). In regards to the package of a protein beverage, consumers placed the greatest importance on recognizable ingredients and plain language ($P < 0.05$). Consumers placed the least importance on local and GMO-free product claims ($P < 0.05$). Consumers have a lack of clarity on categorization of specific ingredients as "clean label" but prefer protein beverages made with minimal, familiar ingredients. There is opportunity to increase the acceptability of dairy protein beverages by reformulating beverages to include fewer and more familiar ingredients. Functional proteins, such as those derived from dairy, may have opportunities to exclude undesirable ingredients (stabilizers, thickeners) from the label.

Key Words: protein beverages, clean label

1008M The role of storage on the physical and sensory properties of aseptic milk.

D. C. Cadwallader*[1], Y. Liu[1], D. M. Barbano[2], and M. A. Drake[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Numerous studies have demonstrated that US consumers prefer the flavor of high temperature, short time pasteurized milk versus ultrapasteurized (UP) milk. Little research has addressed aseptic milk (shelf stable), which has distinct flavor and color from UP milk, although they receive a similar heat process. Our objective was to compare the effects of storage temperature on the sensory and chemical properties of aseptic milk. Milk (1%) was processed (142°C, 3 s) on 2 different dates at a commercial facility and aseptically filled into 946-mL brick containers. Containers (n = 192) were collected each day and assigned to 4°C or 21°C storage. The 4°C group was pulled from the production line and cooled to 4°C using ice, and the 21°C group was ambiently cooled. Containers were sampled initially, after 2 weeks, and monthly (n = 12). Kjeldahl total nitrogen, noncasein nitrogen, nonprotein nitrogen, particle size, neutral and sulfur volatiles (SPME GCMS/MS), viscosity, dissolved oxygen, instrumental color, furosine, and descriptive sensory analysis were conducted at each time point. ANOVA with means separation was applied to evaluate the role of storage time and temperature on physical and sensory properties. Kjeldahl total nitrogen and nonprotein nitrogen did not change with storage, whereas noncasein nitrogen increased ($P < 0.05$) slightly over 7 mo at both temperatures. Concentrations of volatile sulfur compounds (hydrogen sulfide, carbon disulfide, dimethyl disulfide, dimethyl trisulfide) and sulfur or eggy flavor intensity decreased with time ($P < 0.05$) but remained higher ($P < 0.05$) in milks stored at 4°C than those at 21°C. By mo 3, milks stored at 21°C had higher concentrations of Maillard reaction compounds (furaneol, maltol, benzaldehyde, 2/3 methylbutanal) than milks stored at 4°C, consistent with a distinct caramelized/stale flavor by descriptive analysis. Instrumental whiteness (L-value) decreased and yellowness (b*-value) increased ($P < 0.05$) at both temperatures, but changes were larger for 21°C compared with 4°C. Viscosity and dissolved oxygen increased marginally over time but were not affected by temperature ($P > 0.05$). To improve aseptic and UP milk flavor, either a new thermal or mechanical process is needed that does not produce sulfur-eggy flavors or the compounds that are responsible for these flavors need to be removed from milk.

Key Words: milk, flavor

1009M Synergistic interaction of milk fatty acid composition and butter making conditions could produce a softer butter.

C. Nelson*[1], M. A. Drake[1], and D. M. Barbano[2], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Consumers desire softer butter. Milk fatty acid (FA) composition varies with feeding, season, and stage of lactation. Our objectives were (1) to determine the variation in melting within different temperature ranges of milk fats by differential scanning calorimetry (DSC) and (2) to determine if temperature of cycling of milk fats of different fatty acid compositions would change the final melting characteristics. Forty isolated individual cow milk fats were selected to provide a wide range of FA composition, and melting curves (−20 to 50°C) were determined. Selected milk fats were heated to 50°C to erase thermal history and cooled to 1°C in the DSC. Next, they were heated from 1 to 50°C at 2°C/min to establish initial melting profiles with no temperature cycling. Subsequently, milkfats were temperature cycled as follows: held for 3 min at 50°C to erase thermal history, cooled from 50 to 1°C at 10°C/min, held for 3 min at 1°C, heated from 1°C to 25°C at 2°C/min (to melt lower melting solid fat), held for 10 min at 25°C (to allow liquid and crystal reorganization within the structure), cooled from 25°C to 1°C at 2°C/min to solidify higher melting fat, held for 30 min at 1°C to equilibrate, and heated from 1 to 50°C at 2°C/min to measure the final melting characteristics after temperature cycling. During both the initial and final heating steps from 1 to 50°C, the percentage of milk fat that was liquid at 15°C was determined for each sample. Analyses were replicated in duplicate, and ANOVA with means separation was applied to determine differences among treatments. Milk fats with a higher percentage of de novo (DN) FA and DN + mixed origin FA had more fat melted at 15°C than milk fats higher in C18:1. After temperature cycling, the amount of milk fat liquid at 15°C was positively correlated ($P < 0.05$) with increased relative % of DN FA (sum of C4 to C14) in the milk fat, whereas the amount of fat liquid at 15°C was negatively correlated ($P < 0.05$) with increased relative percentage of C18:1. An in-line temperature cycling step in butter making after churning and before packaging for a high DN FA milk fat could produce a softer butter.

Key Words: butter, firmness

1010M Changes in milk protein functionality at low temperature and low rennet concentrations. M. Hamouda* and P. Salunke, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Rennet enzyme drastically changes the functionality of milk proteins and is used for the manufacture of cheese or rennet casein powder. Over the years, technologies have been developed to produce various products by resolubilizing milk proteins using emulsifying salts when these ingredients are used in the formulation. If the controlled rennet action to modify the milk proteins is carried out without making milk protein insoluble, new products can be developed with altered functionality. The purpose of this study was to evaluate the influence of low-concentration rennet on the chemical, rheological characteristics, and protein fractions of skim milk (SM) at 4 ± 1°C. The rennet enzyme (800 IMCU) was diluted (1:10,000) times. Skim milk was divided into 4 lots of 500 mL, and diluted rennet was added at different levels at 4 ± 1°C. The rennet (mL/100 mL) was added to SM at 4 ± 1°C which included: Control (no rennet), T1 (0.001 mL/ rennet), T2 (0.01 mL rennet), and T3 (0.1 mL rennet) treatments and incubated for 24 h. The sampling was done at 0, 1, 2, 6, 12, and 24 h, and the SM after incubation time was heated to 73°C/16s to denature the rennet enzyme. Samples were evaluated for total protein (TP), non-protein nitrogen (NPN), non-casein nitrogen (NCN), pH, capillary gel electrophoresis (CGE), and rheology. Obtained data were statistically analyzed using ANOVA and mean comparison was done using Tukey. The results showed that curd was not formed during storage time. The NPN and NCN of SM samples increased significantly ($P < 0.05$) as incubation progressed however, no significant difference was observed in pH and TP values. Rheological results showed that rennet addition led to decrease storage modulus (G') values of SM compared with the control, however, there were no changes ($P < 0.05$) in loss modulus (G'') and viscosity values. The CGE data indicated, increasing the rennet concentration had a significant ($P < 0.05$) effect in decreasing κ-casein and as the incubation time progressed, κ-casein breakdown was higher at higher levels of rennet usage. To conclude, low concentrations of rennet at low storage temperatures reduced κ-casein indicating casein modification and the milk proteins remained soluble and milk can be processed further.

Key Words: rennet, skim milk, milk protein functionality

1011M Preliminary studies on the development of a simple water adsorption-based approach to measure the solubility of milk protein powders. S. Roy* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Milk protein concentrate (MPC) is a protein-rich ingredient derived from skim milk by ultrafiltration/diafiltration followed by spray drying. Complete rehydration of MPC is one of the important functionalities for utilizing in different product applications. There are several methods for measuring solubility e.g., centrifugal separation of the soluble and insoluble particles, real-time measurement of particle size, NMR-based transverse relaxation, ultrasound-based, etc. These methods are time consuming, need specific instruments, and skills. The study aimed to develop a method based on rapid water adsorption kinetics using an easily available water activity meter. A water activity meter (HC2-AW-USB probe) and a custom-built measurement chamber to accommodate water and MPC powder separated by a water vapor permeable mesh were used. The powder-to-water ratio and the data acquisition settings for continuous measurement of water activity were optimized. To validate the method, 3 batches of fresh MPC85 were procured from a commercial manufacturer and stored at 25°C and 50°C for up to 9 weeks. Solubility index and focus beam reflectance measurement were used as reference methods. Each week, a representative sample of powder and water was placed in the measurement chamber and water activity was continuously recorded for 30 min at 25°C. The change in water activity was plotted against time to derive the area under curve (AUC). The AUC was compared for powders each week for both temperatures and analyzed by repeated measures design. It was lowest for fresh powders at 16.75 min-1 and started to increase significantly ($P < 0.05$) from week-4 for 25°C powder samples (18.24 ± 0.10 min-1) and reached 20.66 ± 0.30 min-1 on week-9. However, the AUC significantly ($P < 0.05$) increased and peaked within week-1 from 16.75 ± 0.51 min-1 on day-0 to 19.86 ± 0.64 min-1 for the 50°C stored samples. The principal component analysis also showed a distinct separation based on the age of the powders. Overall, a water adsorption-based approach can be a useful technique for developing a predictive model to measure the solubility of high protein dairy powders.

Key Words: water activity, solubility, milk protein concentrate

1013M A preliminary study on using electrical resistance tomography (ERT) as a tool to detect the stability of high-protein dairy beverages. B. Zaitoun* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Electrical resistance tomography (ERT) is a novel, fast, and cost-effective method that uses measured conductivity in constructing tomograms by the back linear projection technique. This study aimed to assess the ERT in the linear probe in detecting sedimentation in high-protein beverages (HPB) during storage. To validate the method, HPB containing 8% protein (wt/wt) was formulated using 2 lots of milk protein concentrate (MPC85) and other ingredients. The formulation was homogenized, filled in 13.7 oz glass bottles, capped, and retort sterilized (121°C 15 min), then stored at room temperature for up to 225 d. Four bottles were randomly selected for the ERT measurement at each testing point. The fresh sample was used as a reference to relate the changes in conductivity measurement and tomograms during storage. Using ERT software, the sensing area of the probe was divided into zones 1 to 4, representing the top to the bottom layer of the beverage. Then the average conductivity of each zone was calculated. Subsequently, the unshaken beverage was carefully subdivided into 4 equal layers, termed layer 1 (top) and layer 4 (bottom). Physicochemical properties were performed on the separated layers, including total protein (TP), viscosity, and particle size. Data were analyzed as repeated measures design using Proc MIXED procedure. As expected, the sample on d 0 was found to be homogenous in terms of composition and overall conductivity (0.0998 ± 7.5E-05 uS/cm) for all zones. However, the overall conductivity increased significantly ($P < 0.05$) from d 14 until d 88 in all the zones, followed by a sudden decrease on d 109 for all zones, then a significant increase until d 225. The TP content of layer 4 had increased from 7.86 ± 0.09 to 9.82 ± 0.69 by d 225 and can be attributed to the sedimentation of Κ-casein-depleted caseins. Consequently, the overall conductivity also increased. Furthermore, the change in conductivity correlated positively with the change in TP content at different layers. Therefore, ERT can be a potential tool to detect physiochemical changes and suggests its ability to detect the sedimentation of HPB in the early stages.

Key Words: conductivity, beverage, sedimentation

1014M Effect of polyphenols on functional properties of milk protein concentrate. A. Sharma*, R. Joshi, and P. Salunke, *South Dakota State University, Brookings, SD.*

Plant cells naturally contain polyphenolic compounds, which have the potential to boost human health. Foods rich in polyphenols are often consumed with milk such as coffee. The interaction between milk proteins and polyphenols may alter the functional and structural characteristics of milk proteins. However, these interactions are deeply affected by the nature of polyphenols and the type of milk protein involved in the reaction. In this study, the effect of ferulic acid (FA), cinnamic acid (CN), and caffeic acid (CA) at varying concentrations on the functional properties of milk protein concentrate (MPC) were investigated. The 5% and 10% protein solutions were prepared by diluting the concentrated MPC with distilled water at 45°C for 30 min. Separately, the FA, CN, and CA were added to the solution at the rate of 0.5% and 1% with continuous mixing for 15min. The samples were analyzed for pH, viscosity, heat and alcohol stability, color, emulsifying activity, and stability. Two-way ANOVA was performed in R and for mean comparison, Fisher's LSD test ($P < 0.05$) was applied. The pH of the 1% FA, CN, and CA was significantly lower as compared with the control indicating a reduction in pH as the concentration of polyphenols increased. The viscosity of the control at both protein levels was higher than the samples having polyphenols. Neither the polyphenol type nor concentration influenced the viscosity. The heat stability of 0.5% and 1% CA sample was significantly higher than the control whereas the heat stability of FA and CN samples were similar but significantly lower than the control. The control showed maximum alcohol stability but all polyphenol containing samples coagulated at 60% ethanol concentration. In color, the L* value of 1% CN was significantly higher than the control, however, a* value of 1% CA was significantly higher than 1% CN and control. Similarly, 1% CA had a significantly higher b* value than the control. In emulsifying activity and stability, there was no significant difference between the control and treated samples. In conclusion, the concentration and type of polyphenol can affect the functional properties of MPC.

Key Words: Milk protein concentrate, polyphenols, functional properties

ADSA-Graduate Student Competition: Production—Poster (MS)

1015M Effects of bovine somatotropin on development and feed efficiency of pre-pubertal Holstein × Gyr heifers. G. Berzoini Costa Leite*[1], A. L. Lacerda Sguizzato[2], G. Magalhães Santos[3], E. Ferreira Santos[2], S. E. Facione Guimarães[2], and M. I. Marcondes[1], *[1]Washington State University, Pullman, WA, [2]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, [3]Cenva Post Graduation, Viçosa, Minas Gerais, Brazil.*

Recombinant bovine somatotropin (rBST) is an inexpensive alternative strategy to enhance muscle deposition and growth of dairy heifers. It can alter nutrient partitioning, and its role in digestion may impact feed efficiency. This study aimed to assess the effects of rBST on feed efficiency and performance of pre-pubertal Holstein × Gyr heifers. Thirty-three animals with average body weight (BW) of 218 ± 49 kg and 14 ± 4 mo of age were blocked into 3 groups according to their initial BW (B1 n = 12: 273.6 ± 19.2 kg; B2 n = 11: 214.4 ± 18.3 kg; B3 n = 10: 161 ± 19.7 kg) and submitted to 3 experimental periods of 28 d (Table 1). Two treatments were randomly assigned to the animals within the block: T1: n = 17, placebo injections; or T2: n = 16, 500 mg rBST every 14 d; total of 7 shots/animal. The diet was formulated to obtain an average daily gain (ADG) of 1 kg/d. The 65:35 corn silage: concentrate diet was fed twice daily, and 5% as-fed leftovers were allowed. Heifers were weighed every 28 d before feeding. The rBST shots did not change dry matter intake (T1: 7.873; T2: 7.711 kg/d), nutrient digestibility, metabolizable protein intake (T1: 835; T2: 830 g/kg), metabolizable energy intake (T1:20.975; T2: 18.738 Mcal/kg), and their ratio (T1: 40.63; T2: 41.866 g/Mcal). There was no difference for final BW (305.19 and 314.58 for T1 and T2), ADG (1.12 and 1.15 kg/d for T1 and T2), or ADG/kg DMI (T1: 14.51; T2: 15.16). We concluded that the administration of rBST in crossbreed Holstein × Gyr pre-pubertal heifers does not improve feed efficiency, body weight, and growth.

Key Words: crossbreed, digestibility, recombinant bovine somatotropin

Table 1 (Abstr. 1015M). Feed intake and digestibility of Holstein × Gyr heifers submitted to placebo or recombinant bovine somatotropin rBST treatment

	Treatment			
Item	No rBST	rBST	SEM	*P*-value
Feed intake				
DM, kg/d	7.873	7.711	0.709	0.783
CP, kg/d	1.160	1.140	0.100	0.802
NDFap, kg/d	2.420	2.253	0.172	0.308
MEI, Macal/kg	20.975	18.738	1.509	0.186
MPI/g/kg	865.550	830.090	82.181	0.926
MP:ME, g/Mcal	40.630	41.866	1.321	0.513
Digestibility				
DM, g/kg	649.230	639.950	8.629	0.453
CP, g/kg	675.150	666.230	8.685	0.473
NDFap, g/kg	445.460	451.270	17.817	0.788

1016M Killed *Staphylococcus aureus* intramammary challenge induces subclinical mastitis and clear changes in milk composition but not milk yield. C. S. Gammariello*, M. Oliveira, G. M. Canny, K. M. Enger, and B. D. Enger, *Ohio State University, Wooster, OH.*

Subclinical mastitis accounts for the greatest financial loss to producers, primarily due to reduced milk production. Despite the significant cost, the mechanisms of how subclinical mastitis decreases milk production remain largely unknown. The objective of this study was to assess the acute response during subclinical mastitis on mammary gland productivity by examining changes in milk yield and composition. Mid-lactation primiparous Holstein cows (n = 4), averaging 115 d in milk, were acclimated to tie stalls were milked 3×/d using a quarter milker 3 d preceding the beginning of the experiment on Day 0. On Day 0, one udder half of each cow was randomly selected, and fore and rear quarters were each infused with 2 billion cfu of formalin-fixed *Staphylococcus* (*Staph.*) *aureus*; the contralateral quarters were infused with saline. Cows were milked 2 more days post infusion. Udder half milk weights were recorded and udder half milk samples were measured by DHIA laboratory. Milk somatic cell score (SCS), composition, and yield were analyzed using PROC GLIMMIX in SAS; fixed effects were treatment, time, and their interaction, and cow was a random effect and repeated measure across time. No quarters exhibited signs of clinical mastitis. *Staphylococcus aureus* udder half SCS were equivalent to saline udder halves at challenge ($P = 0.71$) but increased and remained greater than saline halves at all 5 subsequent milkings ($P \leq 0.02$). Milk yield ($P = 0.29$), fat percentage ($P = 0.62$), and urea nitrogen ($P = 0.96$) did not differ between udder half treatments, nor did energy corrected milk ($P = 0.39$). Lactose ($P = 0.87$) and protein ($P = 0.72$) concentrations were similar between udder half treatments at challenge, but *Staph. aureus* challenge significantly reduced lactose ($P \leq 0.03$) and increased protein percentages ($P \leq 0.01$) by 16 h post, which were maintained throughout the study. In this udder half model, changes in milk composition were observed during the initial stages of subclinical mastitis but milk yield was not notably affected. Future studies should aim to understand why certain milk components are altered by subclinical mastitis to explain the consequent shift in mammary gland productivity.

Key Words: inflammation, infection, milk synthesis

1017M Plasma oxylipid profile of postpartum dairy cows categorized into different systemic inflammatory grades in the first week after parturition. J. M. Grantz*[1], A. Mukhopadhyay[1], A. H. Jannasch[2], C. Ferreira[2], P. R. Menta[3], V. S. Machado[3], and R. C. Neves[1], *[1]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, [2]Metabolite Profiling Facility, Bindley Bioscience Center, Purdue University, West Lafayette, IN, [3]Department of Veterinary Sciences, Texas Tech University, Lubbock, TX.*

The objective of this study was to evaluate the plasma oxylipid profile of cows classified in one of 4 systemic inflammation categories based on plasma haptoglobin (Hp) concentrations assessed on days in milk (DIM) 1, 2, 3, 4, 5, and 7, in addition to the presence or absence of metritis within 14 DIM. Groups were classified as follows: 1) cows with a peak Hp concentration ≤3 DIM (early Hp peak; EarlyHp) and diagnosed with metritis; 2) cows with a peak Hp concentration 3 < DIM ≤7 (late Hp peak; LateHp) and diagnosed with metritis; 3) cows suffering from persistently elevated Hp concentrations assessed on DIM 4 and 7 while remaining apparently healthy during the first 21 DIM (PersistentHp); and, 4) cows not suffering from persistently elevated Hp concentrations while remaining apparently healthy during the first 21 DIM (LowHp). Six cows from each category were randomly selected from a plasma bank of a cohort study including 394 multiparous cows. Plasma samples on DIM 1 and 2, 3 and 4, and 5 and 7 were proportionally pooled (per cow basis) for lipidomics analysis. Statistical analyses were performed using SAS v9.4 and means were adjusted for multiple comparisons using the Tukey-Kramer's test. Comparisons for EarlyHp and LateHp were only performed on pooled samples from DIM 1 and 2 (before disease diagnosis). LowHp cows had increased plasma concentrations of (±)9(10)-diHOME compared with EarlyHp and LateHp cows ($P \leq 0.03$). LowHp cows tended to have increased plasma concentrations of 9(S)-HOTrE and (±)11(12)-EpETE compared with EarlyHp and LateHp ($P \leq 0.10$). Next, we sought to investigate whether cows classified as PersistentHp versus LowHp had time-dependent differences in oxylipid profiles. LowHp cows had increased concentrations of 19(R)-HETE compared with PersistentHp cows in a time dependent-manner ($P = 0.02$; only in pooled samples from DIM 5 and 7). Our results identified 4 lipids of interest that warrant further investigation to elucidate their function in dysregulated inflammatory processes of the postpartum dairy cow.

Key Words: oxylipid, systemic inflammation, metritis

1018M Evaluating the influence of heritable, metabolic, and production parameters on cyclicity resumption in a dairy with a robotic milking system.

S. Johnson*, T. Marins, S. Tao, and J. Bohlen, *University of Georgia, Athens, GA.*

The postpartum period is a challenging time in the dairy cow's life with production demands often overshadowing the need to resume cyclicity. Therefore, this study aimed to explore the metabolic markers, non-esterified fatty acids (NEFA) and glucose (GLU), alongside the heritable markers of insulin (IN), insulin resistance (INR), and anti-Müllerian hormone (AMH) and their influence on resumption of cyclicity postpartum in a robotic milking system (RMS). Holstein cows (n = 95) were enrolled at 14 ± 3 d in milk (DIM). Blood samples were taken weekly to evaluate metabolic profiles of NEFA, GLU, and IN until 52 ± 3 DIM. A surrogate index of RQUICKI was used to estimate INR. Animals were fitted with a DeLaval activity meter and monitored via DelPro Farm Manager. Both ++ and +++ reproductive attentions were used to identify an estrous event with estrous intensity (EI) recorded. Animals were recorded as normal (N) resumption of cyclicity if an estrous event was identified on or before 45 DIM or delayed (D) if after 45 DIM. Data were analyzed using the MIXED procedure of SAS with resumption of cyclicity as treatment and Spearman's correlation coefficients. The NEFA did not differ between treatment groups ($P > 0.05$) but decreased in all animals over time with a treatment*DIM interaction ($P < 0.05$). The IN increased over time in all animals ($P < 0.05$) but did not differ between treatment groups ($P > 0.05$). Milk production was higher in D animals over time compared with N ($P < 0.05$), which corresponded to lower circulating glucose ($P < 0.05$). Additionally, higher milk yield corresponded with increased RMS visits ($P < 0.05$). RQUICKI did not change over time for either treatment ($P > 0.05$). Both EI and DIM at cyclicity resumption were unrelated to all metabolic parameters ($P < 0.05$). The EI was not different in animals that had N (191% ± 6.6) versus D (179.1% ± 5.97) resumption ($P > 0.05$). the AMH was not different between N (223.4+28.62 pg/mL) and D animals (217.6+25.22 pg/mL) ($P > 0.05$) and AMH tended to increase EI at first estrus ($P = 0.052$). Milk production and its association with glucose and RMS visits are key drivers to cyclicity resumption in a RMS dairy.

Key Words: RMS, estrous, insulin

1019M Effects of differing durations of low feed intake on gastrointestinal tract function and recovery in cattle.

K. Lambert* and G. Penner, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

The objective was to determine the effect of 5 and 10 d of feed restriction (FR) on ruminal pH, short-chain fatty acid (SCFA) absorption, and gastrointestinal tract (GIT) permeability for cattle and their subsequent recovery. Six ruminally cannulated beef heifers were used in a replicated 3 × 3 Latin square design. Each period consisted of a 5-d baseline (BASE), 10-d treatment administration (TA), and 4 consecutive 7-d recovery phases (REC1 to 4). Treatments included ad libitum feed provision (CON) or 30% of the baseline DMI for 5 (FR5) or 10 d (FR10). Dry matter intake (DMI) and water intake (WI) were measured daily while ruminal pH was measured every 5 min. The temporarily isolated and washed reticulo-rumen technique (WRR) was used to evaluate SCFA absorption on the last day of TA and in REC3. Permeability of the total GIT and post-ruminal regions was assessed in BASE and TA based on a 48-h urinary appearance of Cr-EDTA and Co-EDTA. Data were analyzed with PROC MIXED (SAS 9.4) using treatment, phase, and their interaction as fixed effects and heifer and period × treatment as random effects. Dry matter intake and WI did not differ among phases for CON and among treatments during BASE; however, FR5 and FR10 had lesser DMI and WI during TA ($P < 0.01$). Heifers returned to BASE DMI by REC1 for FR5 and REC2 for FR10. While mean ruminal pH did not differ by phase for CON or among treatments during BASE, pH increased for FR5 and FR10 during TA and decreased below BASE values during REC1 ($P < 0.01$). The duration and area that pH was <5.8 were greatest for FR10 during REC1 ($P < 0.01$). Fecal pH was greater for FR treatments during TA than CON ($P < 0.01$). Treatment and phase effects were not detected for absorption of SCFA during the WRR. There were no differences in urinary Cr excretion while FR5 and FR10 had greater excretion of urinary Co than CON during TA ($P = 0.02$) but not during BASE. This study indicates that increasing the duration of FR increases the risk for ruminal acidosis when heifers return to ad libitum feeding and that intestinal permeability increases in response to low feed intake.

Key Words: barrier function, cattle, gastrointestinal tract

1020M Direct effects of heat stress on mitochondrial structure and energy metabolism in lactating dairy cows.

A. S. Marquez Acevedo*, R. J. Collier, and A. L. Skibiel, *University of Idaho, Moscow, ID.*

Heat stress negatively affects energy production through reduced mitochondrial integrity and function in vitro. However, effects on in vivo mitochondrial parameters are unknown. We hypothesized that heat stress directly affects energy metabolism through altered mito-nuclear signaling pathways and a compromised mitochondrial structure. Mid-lactation (94 ± 6 d in milk) multiparous Holstein cows (n = 23) were blocked by previous 305-d milk yield (MY) and parity and assigned to a heat-stressed (HS) or pair-fed thermoneutral (PFTN) group. Cows were housed in individual tie-stalls and acclimated for 3 d before experimental periods (P1 and P2). During P1 (7 d), all cows were thermoneutral and fed ad libitum. During P2 (10 d), HS cows were fitted with heat blankets and fed ad libitum whereas PFTN cows were maintained under thermoneutral conditions and pair-fed to match the reduction in feed intake of HS cows. Rectal temperature (RT), respiration rate (RR), and MY were recorded twice daily during P1 and P2. Blood samples were collected on D1, 5, 6 of P1 and D1, 5, 9 of P2 for cortisol quantification. On d 10 of P2, liver and mammary biopsies were taken from all cows. The ATP, ADP, and the expression of genes involved in energy metabolism (e.g., glucocorticoid and adrenergic receptors) were quantified by qRT-PCR. Mitochondria were imaged through transmission electron microscopy and mitochondrial number, area, and aspect ratio quantified. Data were analyzed using *t*-tests or mixed models with fixed effects of period, treatment, and their interaction and cow ID as a random effect. The HS cows had an average of 39.0°C rectal temperature during P2. THI of the barn was 58 ± 5 across the study. Both RT and RR were higher for HS compared with PFTN during P2 ($P < 0.01$) but not P1. The MY was similar between groups in P1, but was lower for HS group compared with PFTN in P2 ($P = 0.004$). There tended to be fewer mammary mitochondria in HS cows relative to PFTN ($P = 0.08$); however, mitochondrial area and aspect ratio were similar between groups. The ATP, ADP, and cortisol concentrations were similar between groups. Our results suggest our heat stress challenge had limited effects on energy metabolism and mitochondrial morphology.

Key Words: adenosine triphosphate, bioenergetics, histology

1021M Postpartum acetylsalicylic acid administration and calcium supplementation: Effects on clinical health events and milk production. P. Zarei*, E. Jimenez, J. Spring, M. Dailey, M. Martinez, E. Hovingh, and A. Barragan, *Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA.*

The objective of this study was to assess the effects of the combination of acetylsalicylic acid administration and calcium supplementation after calving on clinical health events, culling rate, and daily milk yield in the first 60 DIM in multiparous (≥2 lactation) Holstein dairy cows. Within 12 h after calving, multiparous cows were randomly allocated to one of 4 study groups: 1) ASA (n = 109) = cows received 2 oral administrations with acetylsalicylic acid 24 h apart (125 g/cow/d; 4 480-grain aspirin boluses); 2) ASACAL (n = 114) = cows received 2 oral administrations with acetylsalicylic acid (125 g/cow/d; 4 480-grain aspirin boluses) and calcium (43 g/cow/d; 2 calcium boluses) 24 h apart, 3) CAL (n = 117) = cows received 2 oral administrations with calcium (43 g/cow/d; 2 calcium boluses) 24 h apart, and 4) UNT (n = 109) = cows remained untreated. Clinical disease event, culling rate, and daily milk yield data were collected from on-farm computer records. The data were analyzed using MIXED and GLIMMIX procedures of SAS. There was no difference on the incidence of clinical health events between treatment groups. Cows treated with CAL tended ($P = 0.09$) to have a higher culling rate compared with UNT cows (ASA = 9.67 ± 3.08%; ASACAL = 8.16 ± 2.77%; CAL = 12.74 ± 3.32%; UNT = 2.10 ± 1.47%). Cows treated with ASA tended ($P = 0.07$) to produce 2.55 kg/d more milk in the first 60 DIM compared with UNT cows (ASA = 48.80 ± 0.76 kg/d; ASACAL = 46.87 ± 0.76 kg/d; CAL = 47.18 ± 0.74 kg/d; UNT = 46.25 ± 0.75 kg/d). These findings suggest that a combination of acetylsalicylic acid administration and calcium supplementation after calving may not have positive effects on cow health or production, and that postpartum acetylsalicylic acid administration may be beneficial for milk production in multiparous Holstein dairy cows.

Key Words: postpartum period, acetylsalicylic acid and calcium, milk production

ADSA-Graduate Student Competition: Production—Poster (PhD)

1022M Performance, sorting behavior, and nondisease probability of Holstein calves fed different physical forms of starter. I. R. R. Castro1,2, G. B. C. Leite[2], J. C. C. Chagas[3], G. A. Fields[2], A. E. Bartelheimer[2], A. L. Harder[2], D. V. Landin[2], I. F. Carrari*[2], and M. I. Marcondes[2], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Washington State University, Pullman, WA,* [3]*Swedish University of Agricultural Sciences, Umeå, Sweden.*

The physical form of starter feed (PFSF) plays a role in the development of calves as ruminants. Thus, to study the effects of the PFSF on feed intake, growth performance, and the health of dairy calves, we used 24 female Holstein calves (5 d of age; 40.4kg ± 3.9 of BW ± SD) were enrolled in a 67d randomized block design (n = 12 calves per treatment). Calves were individually housed and assigned to one of the 2 treatments: textured feed starter (TFS; mix of pellet plus whole-kernel corn), and pelleted feed starter (PFS). Calves were offered milk replacer in a step-down feeding scheme and had free access to drinking water and the starter (intakes measured daily). Weight and withers height were recorded weekly. The sorting index was determined by a ro-tap shaker. Each calf was examined daily for any clinical signs of diseases. After, the nondisease probability was calculated by the Kaplan-Meier method considering the significant health parameters. Treatments and periods were used as fixed effects. Block and interaction between treatment and period were used as random effects. There was no effect of the PFSF on starter ($P = 0.056$) and water ($P = 0.059$) intakes. The PFSF did not affect body weight ($P = 0.109$), average daily gain ($P = 0.664$), and withers height ($P = 0.257$). The nondisease probability showed that TFS animals decreased health occurrences about general attitude ($P = 0.010$), eye discharge ($P = 0.004$), total respiratory score ($P = 0.019$) and fecal score ($P = 0.040$). Calves fed TSF sorted toward small particles (0.4 and 0.3-mm sieves; Table 1), and PSF sorted toward big particles. Overall, whole-kernel corn as starter texturizer may not improve the intake and performance of young dairy calves compared with PFS diets, however we observed a health improvement in the TSF group.

Key Words: body development, health score, particle size

1023M Endotoxemia induces lipolysis and alters adipose tissue function in dairy cows. M. Chirivi*[1], L. Worden[2], J. Parales-Giron[2], A. L. Lock[2], and G. A. Contreras[1], [1]*Department of Large Animal Clinical Sciences Michigan State University, East Lansing, MI,* [2]*Department of Animal Science, Michigan State University, East Lansing, MI.*

The periparturient period is characterized by intense lipolysis in adipose tissues (AT) and inflammation that together increase the risk of disease. Endotoxemia is often associated with periparturient diseases. In vitro, endotoxins increase lipolysis in adipocytes, however the systemic effect of endotoxemia on AT lipolytic activity and function is unknown. Multiparous Holstein dairy cows [204 (SD = 21.7) DIM] were infused IV with LPS from *E. coli* O55:B5 (n = 4)] at 1 µg/kg BW or 100 mL of saline infusion control (CON n = 4). Plasma samples collected at −24, 0, 2, 6, 12, 24, 48, 72, 96, and 120 h relative to infusion were used to quantify nonesterified fatty acids (NEFA; mmol/L), β-hydroxybutyrate (BHB; mmol/L), calcium (Ca$^+$; mg/dL), LBP (mg/mL), and haptoglobin (Hp; mg/mL). Subcutaneous AT (SCAT) biopsies were collected (right flank) 24 h after infusion. The SCAT were incubated in the presence of the lipolytic agent isoproterenol (ISO = 1 µ*M*, BASAL = 0 µ*M*) for 3 h. Lipolysis was assessed by glycerol release (nmol glycerol/ mg of AT). A linear mixed model was used to evaluate mean differences. Compared with CON, LPS cows increased NEFA at 24 h with a peak at 48 h (1.44 vs 0.78 ± 0.30; $P < 0.05$). LBP, and Hp were elevated in LPS cows at 12 and 24 h with LPB peaking at 24 h (15.19 vs 1.9 ± 0.60) and Hp at 96 h (1.86 vs 0.66 ± 0.18; $P < 0.001$). LPS reduced BHB by 2h (0.16 vs 0.62 ± 0.04), and calcium by 6h (6.92 vs 9.75 ± 0.48) returning to pre-infusion levels at 24 h and 48 h, respectively ($P < 0.001$). LPS lowered BASAL lipolysis in AT (0.32 vs 2.34 ± 0.60; $P < 0.05$). ISO-induced lipolysis did not differ between LPS and CON. These data suggest that endotoxemia activates lipolysis and systemic inflammation. The reduced BASAL lipolysis may be associated with the depletion of triglyceride storage within the AT after lipolysis. Future studies will explore the effects of endotoxemia on AT inflammation and macrophage trafficking and its association with metabolic disease in dairy cows.

Table 1 (Abstr. 1022M). Sorting index (%) influenced by feeding textured feed starter (TSF) or pelleted feed starter (PFS)

Particle size, mm	Treatment			*P*-value		
	TSF	PSF	SEM	Treatment (T)	Week (W)	T × W
4.0	98.2	97.1	26.7	45.6	47.3	46.4
2.0	110	108.5	4.1	83.1	47.1	68.7
1.18	100.1	91.1	49.6	70.1	60.3	46.1
0.71	116.7	112.9	20.1	89.7	0.8	43.6
0.425	68.8	118.3	13.3	1.1	92.7	40.2
0.3	75.3	122.8	15.9	6	48.2	38.3
Pan	124.5	144.7	66.7	51.1	2.8	56.4

Key Words: endotoxemia, lipolysis, adipose tissue

1024M Feeding rumen-protected methionine and calcium salts enriched in omega-3 fatty acids modify plasma and liver phosphatidylcholine and phosphatidylethanolamine concentrations of periparturient dairy cows. T. L. France*[1], K. Juarez-Leon[1], A. Javaid[1], P. Deme[2], N. J. Haughey[2], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Johns Hopkins University, Baltimore, MD.*

Rumen-protected (RP)-Met feeding increases milk production in dairy cows. The objective was to investigate the effects of feeding RP-Met and calcium salts (CS) of fatty acids (FA) enriched without or with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; i.e., n3FA) in periparturient cows. In a randomized complete block study design, 75 multiparous Holstein cows were assigned to 1 of 4 treatments (n = 18–19/diet): 1) Met deficient (-Met) with CS not enriched in n3FA (-n3FA; 0%n3FA; EnerGII; Virtus Nutrition, USA), 2) Met adequate (+Met; Smartamine M; Adisseo Inc., France) with -n3FA, 3) -Met with CS enriched in n3FA (+n3FA; 4%n3FA; EnerG-3; Virtus Nutrition), or 4) +Met with +n3FA from wk −3 to + 4 wk relative to parturition. Cows were fed corn silage-based total mixed rations, pre- and postpartum, which were formulated to provide Met at ≤ 0.96 or ≥ 1.13 g per Mcal metabolizable energy for -Met and +Met, respectively. The CS were fed at 1.5% ration dry matter pre- and postpartum. Plasma and liver collected on d +21 were analyzed for phosphatidylcholine (PC), phosphatidylethanolamine (PE), and lysophosphatidylcholine (LPC) by LC-MS. Data were analyzed using PROC MIXED of SASv9.4. Planned contrasts included: 1) effect of Met (-Met vs. +Met), 2) effect of n3FA (-n3FA vs. +n3FA), and 3) effect of co-supplementation (+Met/+n3FA vs. +Met/-n3FA and -Met/+n3FA). Liver LPC-20:5 and −22:6 concentrations were greater in +n3FA, relative to -n3FA ($P < 0.01$). We observed greater plasma LPC-22:6 in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P < 0.01$; 0.04 vs. 0.02 arbitrary units (AU)). Liver PC-38:6 concentrations were greatest in +n3FA, relative to -n3FA ($P < 0.01$; 10.5 vs. 8.63 AU) and were negatively correlated with liver triglyceride levels ($r = -0.25$; $P = 0.04$). Liver PE-38:6 concentrations were greater in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P < 0.01$; 2.94 vs. 2.27 AU). Lower liver PC-38:6:PE-38:6 was observed in +Met/+n3FA, relative to +Met/-n3FA and -Met/+n3FA ($P < 0.01$; 3.63 vs. 5.01). Feeding adequate Met and n3FA altered plasma and liver PC and PE profiles, suggesting improved liver function in early lactation.

Key Words: methionine, omega-3, transition

1025M Utilization of algae biomass as a partial replacement for soybean meal in the diet of dairy cows in vitro. R. R. Lobo*[1], M. U. Siregar[1], S. S. da Silva[1], A. R. Monteiro[2], G. Salas-Solis[1], A. C. S. Vicente[1], J. Vinyard[1], M. L. Johnson[1], S. Ma[1], E. Sarmikasoglou[1], and A. P. Faciola[1], *[1]University of Florida, Gainesville, FL, [2]University of Sao Paulo, Piracicaba, SP, Brazil.*

The objective of this study was to evaluate the effects of partially replacing soybean meal (SBM) with algae sources on in vitro ruminal fermentation. Using 6 fermenters in a 3 × 3 replicated Latin-square with 3 periods of 10 d each, we tested 3 treatments: a control diet (CRT) with SBM at 17.8% of the diet; and 50% SBM replacement with either Chlorella (CHL) or Spirulina (SPI). The basal diet was formulated to meet the requirements of a 680 kg Holstein dairy cow producing 45 kg/d of milk with 3.5% fat and 3% protein. All diets had a similar nutritional composition (16.0% CP; 34.9% NDF; 31.0% starch, DM basis) and fermenters were fed 106 g/d split into 2 feedings. After 7 d of adaptation, samples were collected for 3 d of each period for analyses of ruminal fermentation at 0, 1, 2, 4, 6, and 8 h post-morning feeding. Statistical analysis was performed with MIXED procedure of SAS and the treatment effect was depicted using orthogonal contrasts (CRT *vs* algae; and CHL *vs* SPI), and significance was declared when $P \leq 0.05$. When compared with CRT, algae had a greater NDF degradability (44 vs 53%) and propionate molar proportion (24 vs 27%), lower ammonia concentration (10 vs 8 mg/dL) and flow (20 vs 17 g/d) and lower butyrate molar proportion (18 vs 16%). When comparing the 2 algae sources, CHL had a greater dry matter degradability (68 vs 64%) and molar proportion of acetate (49 vs 46%), and lower molar proportion of iso-acids (7.4 vs 5.6%). In addition, when compared with SPI, CHL had a lower crude protein degradability (56 vs 60%) and ammonia flow (0.13 vs 0.21 g/d), which increased dietary RUP (31 vs 35%) and improved the efficiency of nitrogen utilization (46 to 50%) and nitrogen capture (36 vs 39%) by the ruminal microbial population. In conclusion, when 50% of SBM is replaced by algae, in a high-producing dairy cows' diet, ruminal fermentation is positively modulated, improving fiber degradability and reducing the flow of ammonia, which could be an indication of better nitrogen utilization. In addition, Chlorella stimulated a greater nitrogen efficiency, by improving RUP and bacterial nitrogen flow when compared with Spirulina.

Key Words: chlorella, spirulina, N metabolism

1026M Activation of immune signaling pathways by microRNAs extracted from bovine colostrum. R. Santos*[1], A. Brown[1], Y. Ahn[2], U. Bickel[2], and F. Rosa[1], *[1]Texas Tech University, School of Veterinary Medicine, Amarillo, TX, [2]Texas Tech University, Health Sciences Center, School of Pharmacy, Amarillo, TX.*

MicroRNAs (miRNAs) are non-coding RNA sequences that attach to targeted mRNA resulting in cellular and protein regulation. MicroRNAs in milk can serve as biomarkers of inflammation and might help neonatal immune system regulation. This study aimed to elucidate the activation of immune signaling pathways by miRNAs extracted from bovine colostrum. The miRNA was isolated from bovine colostrum (n = 10 samples) using commercial kits and quantified using real-time qPCR assays. After exposing RAW-Blue Cells, a mouse macrophage cell line, to *E. coli* lipopolysaccharide (LPS), cells were incubated with a pool of miRNAs. The transcription factor NF-kB activity was measured for 24 h post-challenge using spectrophotometry absorbance. Statistical analysis was performed using the PROC MIXED procedure of SAS with treatment and time (repeated measurements) as the fixed effects and well within treatment as the random effect. Significance was declared at $P < 0.05$. Our results demonstrated that the miRNA dose (1µg and 0.1µg) triggered the activation of the transcription factor of inflammation NF-kB in a dose-dependent manner ($P = 0.01$). Several miRNAs including let-7a-5p, miR-101–3p, miR-29C-3p, miR-21–5p, miR-142, and miR-340–3p were identified in the bovine colostrum samples used for this in vitro assay. Such miRNAs are involved in immune signaling regulation and cell apoptosis. Thus, our findings indicate that miRNAs extracted from bovine colostrum can potentially enhance the immune system (i.e., regulate immune pathways) of neonatal calves if transferred via colostrum.

Key Words: microRNAs, colostrum, bovine

1027M Prevalence and timing of bovine leukemia virus infection in dairy youngstock. M. Sokacz*[1], K. R. B. Sporer[2], C. Droscha[2], P. Bartlett[3], B. Norby[3], and T. M. Taxis[1], *[1]Michigan State University Department of Animal Science, East Lansing, MI, [2]CentralStar Cooperative, Lansing, MI, [3]Michigan State University College of Veterinary Medicine Large Animal Clinical Sciences, East Lansing, MI.*

The objective of this study is to investigate when animals become first infected with Bovine leukemia virus (BLV). Bovine leukemia virus is a blood-borne pathogen responsible for the development of bovine leukosis. Transmission of BLV is possible by both vertical and horizontal transmission. Horizontal transmission has been found to be associated with various management practices, such as the reuse of needles and obstetric sleeves between animals. The most recognized effects of BLV infection are lowered milk production, decreased cow longevity, and decreased animal welfare. Due to these impacts inflicting the most detriment on lactating cows, BLV research has primarily focused on the milking herd. To accomplish our objective of identifiying when animals first become infected, we conducted a study to longitudinally follow calves to determine BLV infection status in commercial dairy herds. Currently, assessment is occurring by testing animals for BLV as neonate, prebreeding heifer, bred heifer, first, and second lactation animals. The infection status of BLV is determined from blood samples collected at each time point and assayed via qPCR for BLV provirus. Neonates were enrolled (n = 254) on a weekly basis from 5 Michigan commercial dairy farms over the course of 9 mo. The median age at which neonates were first enrolled in the longitudinal study was 5 d. Two years following the neonate enrollment period, preliminary data revealed an upward trend in youngstock BLV prevalence, most notably within the first year of life. The average BLV neonate prevalence across the enrolled farms was 1.2% ± 1.3. As those neonates aged to become prebreeding heifers, the BLV prevalence increased to 13.4% ± 3.9 and subsequently to 17.8% ± 6.2 as bred heifers. To add perspective to the youngstock prevalence, the average milking herd BLV prevalence among the 5 farms is 32.0% ± 12.0. Initial results show that BLV infection status rapidly increased within the first year of life. With events such as weaning, vaccination, and housing changes, the first year of youngstock life likely presents BLV transmission risks not previously studied.

Key Words: animal health, disease

1028M Use of long short-term memory models with integrated cow-level data for early prediction of clinical ketosis in dairy cows.

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The use of precision dairy technologies is rapidly expanding in dairy science with an overwhelming amount of cow-level data being generated daily to be used by producers and consultants. However, data integration from different automated systems is still challenging and not used to its potential in real-time decision-making. Long short-term memory (LSTM) models are deep learning models that are capable of learning temporal patterns in complex data and making sequence predictions. The objective of this study was to evaluate the use of LSTM models for their ability to predict clinical ketosis within 21 d in milk (DIM) using daily milk production variables and rumination time obtained from a commercial dairy farm in IN that utilizes a voluntary milking system. Additionally, we used Explainable Artificial Intelligence methods to understand variable impact on model predictions. Data on daily milk yield, milk fat-to-protein ratio (FPR), number of robot visits, rumination time, and disease events during 0 to 21 DIM were retrospectively obtained from February 2020 to July 2022. A total of 3,603 cow-lactations were included in the analysis. Prevalence of clinical ketosis was 5.1%. Diagnosis was done using the Lely health report and confirmed by the herd veterinarian. Deviations from the median value of each variable by parity and DIM were used instead of raw values. Six LSTM models were developed with varying prediction lengths (d −3, −2, or −1 before ketosis diagnosis). Model performance was assessed using repeated stratified 10-fold cross-validation for 10 repeats. The Shapley additive explanations framework was used for model explanation. Model sensitivities were 79%, 68–70%, and 69%, and model specificities were 81–82%, 66–70%, and 63% when predicting ketosis at 1, 2, or 3 d before diagnosis, respectively. Model explanation revealed that lower milk yield, lower number of robot visits, lower rumination time, and higher milk FPR were more likely to result in a positive prediction. Our results demonstrate the potential of on-farm milk and rumination data to be integrated in early prediction of clinical ketosis.

Key Words: ketosis prediction, deep learning

1029M State of the Tennessee value-added dairy industry.

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Few value-added dairy (VAD), dairies that process their milk into a salable dairy product, enterprise economics existed in 2020. This project aimed to describe the current state of Tennessee's (TN) VAD. A 50-question in-person survey was presented to dairy producers and processors. The survey included 5 sections: 1) respondent information, 2) farm information, 3) processing information, 4) processing equipment, and 5) business economics. A mixture of short-answer, multiple choice, and Likert-Scale questions were used. Data were reported for 16 cattle (VAD-C) and 3 small ruminant (VAD-S) in 16 TN counties. Data were separated into currently in business (current; C) and considering opening (prospective; P) VAD-C (n = 9 and n = 7, respectively) and VAD-S (n = 2 and n = 1, respectively). Data were described using the MEANS and FREQ procedures of SAS 9.4. Current and P VAD-C respondents were 38 ± 11 and 38 ± 13 yr old, respectively, while VAD-S C and P processors were 66 ± 13 and 60 yr old. Seven VAD-C processed their milk, 8 were considering processing their milk, 4 processed purchased milk, and 2 were considering processing purchased milk. All VAD-S were either processing or considering processing their own milk. Farming activities were C VAD-C's primary income source (36 ± 44%), while P VAD-C relied on off-farm income (62 ± 39%). Similar to P VAD-C, most income for VAD-S was obtained from off-farm sources (≥99%). In TN, 6% of all cow dairies were VAD, primarily located in East TN, while all 3 VAD-S were located in Middle TN. The largest quantity of VAD-C producers produced ice cream, but fluid milk had the highest production volume (1,669,990 L). Creamline milk and aged cheese were the most often considered for production by VAD-C, while all 3 VAD-S were either considering or currently making cultured products and soft cheese. Current and P VAD-C and VAD-S producers were in good financial standing with a low debt-to-asset ratio (<40%). Study outcomes were used to aid farmers considering entering or expanding a value-added enterprise through marketing, production, or economic suggestions.

Key Words: value-added, local, benchmarking

ADSA-USD Competition: USD Original Research Poster Presentations

1030M Effect of drought stress on in situ ruminal starch digestibility of corn grain. J. Becker*[1], H. Galyon[1], J. Bell[2], Q. Xue[2], T. Marek[2], and G. Ferreira[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Texas A&M AgriLife Research and Extension Center, Amarillo, TX.*

The hypothesis of this study is that drought stress does not affect in situ ruminal starch digestibility. Therefore, the objective was to determine the effect of drought on in situ ruminal starch digestibility of corn grain. Corn grain samples from 4 commercial hybrids were obtained from a previous study performed in Amarillo, TX in 2020. All corn hybrids were subjected to 2 irrigation regimens: 100% evapotranspiration (100 ET) and 50% Evapotranspiration (50 ET), which received 526 and 229 mm of total water, respectively. All grain samples were ground to pass through a 1-mm screen of a cutter mill, and 3.6 g of ground samples was placed into a 10 X 20 Dacron porous bags. After sealing, bags were incubated in the rumen of 2 rumen-cannulated Holstein milking cows for 0, 2, 4, 6, 9, 12, 24, and 48 h. After incubation all bags were rinsed in a washing machine (3 cycles of 3 min) with tap water. All starch residues were reground to pass through a 0.5-mm screen of a cyclone mill and analyzed for starch concentration. Kinetic parameters of in situ starch digestibility (ISSD) were determined using the NLIN procedure of SAS according to the model ISSD = A + B x (1-e(-kdxT)) where A is the starch solubilized at T = 0, B is potentially digested starch estimated as 100 – C, C is the undegraded starch at T = 48, and T is the incubation time. The time at which 50% of the starch is degraded (T50) was also determined. The MIXED procedure of SAS was used to contrast the irrigation treatments with a model that included the fixed effect of treatment, the random effect of hybrid, and the random residual error. Neither fraction A (14.8%; $P < 0.23$), fraction B (83.2%; $P < 0.16$), fraction C (2.1%; $P < 0.16$), kd (7.39%/h; $P < 0.31$), or T50 (7.72 h: $P < 0.19$) were affected by irrigation. Based on the conditions of this study, irrigation did not affect in situ ruminal starch digestibility.

Key Words: starch digestibility, corn, drought

1031M Impact on cheese yield of nonstandardized milk with seasonal variation, a 2-year retrospective. E. Cole*, S. L. Beckman, and P. Salunke, *South Dakota State University, Brookings, SD.*

Historically, seasonal variation in milk components has been seen to directly impact cheese yield. Consequentially, many cheese plants standardize milk before it goes into a cheese vat, increasing the total processing time. Over time, practices on the farm have been aimed at increasing milk quality with nutrition, genetics, and new technologies. Research needs to be done on the seasonal impacts in milk on cheese yield as the dairy industry has evolved with modern methods. Therefore, the objective of this research is to see the correlation between, modern, seasonal milk components on cheese yield without component standardization before entering the cheese vat. Milk was obtained from a university dairy farm and then transported to the same university's dairy plant. Component analysis for protein and fat, using Fourier transform infrared spectroscopy, was conducted on raw milk. The milk was then used to make cheddar cheese without milk component standardization. Comprehensive data analysis was done on the cheddar cheese produced from January 2021 to October 2022. A total of 67 Cheddar cheese blocks were evaluated for fat, moisture, salt, and cheese yield. The data were divided into the respective month of milk used. The correlation between monthly mean protein percentage and monthly mean cheese yield was determined in R. Results show that the average percent yield per month differed significantly ($P = 0.016$) and ranged from 9.46% to 10.62%. The highest average cheese yields were observed in January (10.62%) and December (10.43%). The lowest average yields were in July (9.46%) and September (9.52%). There is a significant positive correlation $r = 0.76$ ($P = 0.004$) between the average protein % in milk and average cheese yield, as the protein was highest in January, February, and December. Likewise, the average fat % was highest in the winter season and lowest in the summer ranging from 3.30% to 3.50%. This 2-year evaluation shows that, even with improved techniques, genetics, and knowledge of cheese-yield data due to historical research, the milk before standardization continues to vary, making cheese yield dependent on seasons.

Key Words: cheese yield, protein, seasons

1032M Development of whey protein-lignin based film materials for food packaging applications. Y. Deng*, S. Kolodjski, Y. Kim, and G. Lewis, *University of Wisconsin-River Falls, River Falls, WI.*

Whey protein (WP) and lignin are by-products of cheese production and the pulp and paper industry, respectively. This project aims to combine WP, glycerol, and lignin to develop biodegradable films with improved water barriers and mechanical strengths for food packaging applications. Reconstituted WP solutions (6% wt/vol) were held in a water bath with glycerol and lignin (if applicable) at different temperatures (60–95°C) for 30 min to induce different levels of WP denaturation (20°C films were made without heat treatment as the control). The SDS-PAGE showed that the film-forming solution prepared at 90°C for 30 min resulted in 88.7% WP denaturation, while it was only 12.3% for WP prepared at 60°C for 30 min. Further results revealed that WP denaturation increased available thiol groups, which were likely responsible for forming the polymeric structure of WP films. With altered polymeric structures, different levels of WP denaturation altered film properties such as film thickness, water vapor permeability (WVP), water solubility, mechanical strength, and light barrier properties. Both WVP and water solubility were inversely correlated with the percent denaturation of WP. The WVP of 60°C and 90°C WP films were 2.20 ± 0.34 mg·m-1·hr-1·kPa-1 and 1.83 ± 0.41 mg·m-1·hr-1·kPa-1, respectively (errors represent 95% CI of triplicate measurements). WVP of 20°C film could not be measured as the film started to rupture within 3–4 h of the WVP measurement, indicating its inability to block moisture due to its extremely hygroscopic nature. The addition of lignosulfonate (at 5% wt/wt of WP) improved water barrier properties by 10%, while the inclusion of alkaline lignin increased the mechanical strength of WP films. WP films with alkaline lignin also had greater opacity than those with WP only or WP+lignosulfonate films. Overall, the results highlight the importance of WP denaturation in film properties and the potential benefits of different types of lignin for improving film properties.

Key Words: whey protein, lignin, food packaging

1033M Impact of feeding branched-chain volatile fatty acids during the dry period on colostrum composition and neonatal calf muscle metabolic activity. B. L. Gast*, L. M. Beckett, E. Tobolski, L. Jones, K. Gouveia, J. P. Boerman, and T. M. Casey, *Purdue University, West Lafayette, IN.*

Feeding branched-chain volatile fatty acids (BCVFA) improved lactation performance, but little is known of the effects of BCVFA feeding on colostrum components and impacts on the neonatal calf. We hypothesized BCVFA feeding during the dry period may increase fat and protein in colostrum, and induce higher muscle metabolic activity in calves. Forty dry Holstein cows were enrolled at −42 d before expected calving and allocated to 1 of 2 treatments: control (CON; soy hulls) or BCVFA consisting of isobutyrate (30 g DM), isovalerate (15 g DM), 2-methylbutyrate (15 g DM) top dressed on dry cow TMR. Forty calves were immediately enrolled at birth and fed their dam's colostrum at <4h and 12h postnatal (PN). Calf colostrum intake was standardized to 15% of birth weight with 10% being fed at the first feeding and 5% fed at the second feeding. At 24 h PN, blood was collected, a biopsy of the semitendinosus muscle was performed, and metabolic activity was measured using an Alamar blue assay, which indirectly measures NAD+ reduction to NADH, as fluorescence per 15 min. Colostrum was analyzed for fat and protein concentration using creamatocrit and Bicinchoninic acid (BCA) assays. Immunocrit was measured to evaluate passive transfer. A mixed model was used to examine the effects of treatment, time and the interaction of treatment and time on the muscle metabolic activity. Muscle metabolic activity was greater ($P = 0.01$) for BCVFA (4070370 ± 455124 fluorescence · g of tissue^{-1} · 15 min^{-1}) treatment than CON (2505555 ± 444156 fluorescence · g of tissue^{-1} · 15 min^{-1}). BCVFA feeding had no effect on calf birth weight, immunocrit, or colostrum fat or protein concentration ($P > 0.10$). Taken together, these data indicate that supplementing cows with BCVFA during the dry period increased muscle metabolic activity of calves at 24 h after birth. The mechanism of BCVFA supplementation on calf development needs to be further explored, but it did not affect percent fat or protein of colostrum, and it is possible calves are experiencing BCVFA impacts in utero.

Key Words: calf muscle, branched-chain volatile fatty acid, colostrum composition

1034M Molecular and gene expression changes in liver tissue from mid-lactation dairy cows supplemented with methionine during a subclinical mastitis challenge.

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The objectives were to assess molecular changes in the liver of cows supplemented with methionine during solely a subclinical mastitis challenge (SMC). Thirty-two multiparous Holstein cows (145 ± 51 DIM) were enrolled in a randomized complete block design and assigned to either a basal diet (CON; n = 16) or a basal diet supplemented with rumen-protected methionine (SM; n = 16, Smartamine M 0.09% DM). The dietary treatment was administered at −21 d relative to a SMC, and data were collected from 0 to 3 d. At 0 d relative to SMC, the mammary gland rear right quarter was infused with 500,000 cfu of *Streptococcus uberis* (O140J). Blood samples were collected during SMC to assess inflammation, oxidative stress, and liver function biomarkers. Liver biopsies were taken −10 and 1 d relative to SMC. Genes related to methionine and glutathione metabolism, inflammatory response, and oxidative stress were analyzed via qPCR and Western blot. Data were analyzed using the PROC MIXED procedure of SAS with TRT and TRT*TIME interaction as an effect. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. There was no TRT*TIME interaction for any of the evaluated genes. A trend ($P = 0.07$) for greater milk yield (+0.9 kg) was observed in SM cows than CON. Methionine metabolism genes *MAT2A* and *PEMT* were upregulated ($P = 0.02$) in SM cows compared with CON. The gene *BHMT* was downregulated ($P = 0.03$) in SM cows compared with CON. Compared with CON, a trend ($P = 0.09$) was observed for upregulation of *MAT1A* in SM cows. The immune cell signaling gene *MYD88* was downregulated ($P = 0.01$) in SM cows compared with CON. A trend ($P = 0.06$) in haptoglobin (*HP*), a positive acute-phase response, was observed, where *HP* was downregulated in SM cows compared with CON. A transcription factor involved in antioxidant regulation, *NRF2,* was upregulated ($P = 0.01$) in SM compared with CON. A trend ($P = 0.09$) was observed in glutathione synthetase (*GSS*), which directs the final step in glutathione biosynthesis, where *GSS* was upregulated in SM cows compared with CON. The lower reduction in milk yield in SM cows during the SMC could be associated with upregulation of antioxidant-related genes and downregulation of inflammatory-related genes.

Key Words: methionine, inflammation, oxidative stress

1035M Evaluating the influence of glucose and nonesterified fatty acids during the transition period on characteristics of cyclicity resumption postpartum.

N. Hendrix*, S. Johnson, T. Marins, S. Tao, and J. Bohlen, *University of Georgia, Athens, GA.*

Reproductive efficiency is dictated by several factors including genetics, environment, nutrition and management. Consequently, nutrient partitioning and its effects specifically on the early postpartum period and reproductive success are of specific interest. Therefore, this study aimed to investigate changes in circulating nonesterified fatty acids (NEFA) and glucose during the periparturient period on the days in milk (DIM) at and intensity of the first postpartum estrous event. Holstein (n = 22) and Jersey (n = 13) cows were enrolled at approximately −14 d before calving (d 0) at the University of Georgia Teaching Dairy in Athens, GA. Blood samples, body condition scores, and milk weights were taken at d −14, d −7, d 0, d 7, and d 14. Serum samples were subsequently analyzed for NEFA (μEq/L) and glucose (mg/dL) concentration. Animals were fitted with the DeLaval activity meter and monitored from day of calving until the first postpartum estrous event using Delpro$^{\text{TM}}$ Farm Manager. Using the algorithm contained within this system, a ++ or +++ alert and/or 120% estrous intensity (EI) was used to confirm an estrous event with the EI recorded for each event. Normal resumption of cyclicity was considered when an estrous event occurred at ≤45 DIM with anything after 45 DIM considered delayed. Data were analyzed using the MIXED procedure of SAS and Spearman's correlation coefficients. Analysis of the data collected revealed NEFA circulation to be significantly lower in the pre versus postpartum period ($P < 0.01$). Holsteins had higher circulating NEFA concentrations at days −14, −7, 0, and 7 compared with Jerseys ($P < 0.01$). Glucose was significantly higher in the pre versus postpartum period and continued to decline from 0 to 7 and 7 to 14 DIM ($P < 0.01$) in all animals. Overall, DIM at first estrus was lower in Jerseys ($P = 0.04$) but unaffected by glucose and NEFA ($P > 0.10$). EI was unaffected by glucose, NEFA, and DIM at cyclicity resumption but tended to be higher in Jerseys ($P = 0.10$). Though metabolic tendencies were as expected, the differences in breeds related to the transition period and cyclicity resumption warrants further investigation.

Key Words: NEFA, glucose, cyclicity

1036M Understanding postpartum factors effecting fertility rates in dairy cattle across body condition score.

K. Hill*, E. Eckelkamp, K. McLean, and S. Moorey, *University of Tennessee, Knoxville, TN.*

The oocyte environment is crucial to successful maturation and development of a viable embryo. This environment includes the surrounding follicle and follicular fluid. Substances from circulating blood can cross into follicular fluid through the blood follicular barrier. This barrier is both charge and size dependent, meaning that sera and follicular fluids may correlate but will not match. Body condition of both women and cattle can severely affect the follicular fluid environment during oocyte maturation by impacting metabolomic profiles, proteins, or other molecules. Reactive oxygen species (ROS) leads to both negative and positive effects on ovulation, oocyte maturation, and fertility. In a previous metabolomics study, thin cows had signs of negative energy balance and tricarboxylic acid (TCA) cycle intermediates in sera. Increased TCA cycle intermediates point to increased mitochondrial activity, a major ROS source. The long-term goal of this research was to identify factors that played a substantial role in fertility in comparison to a concurrent study in Angus cattle pre and postpartum. Sera and β-hydroxybuterate (BHBA) samples were collected from 20 second-lactation dairy cattle at 7, 21, and 60 ± 3 d in milk. Sera were analyzed for ROS content and metabolomic profiling. Blood BHBA was determined using BHBCheck. Metabolomic profiles, ROS, and BHBA concentrations were analyzed against body condition score and reproductive success metrics. Relationships between serum BHB, cow BCS, or days postpartum and serum ROS were determined through a multiple linear model using R software. Analysis of variance (Type III Sum of Squares) was utilized to determine significance for categorical variables within the model. Significance was determined at $P < 0.05$. β-hydroxybuterate and ROS were positively correlated. Elevated ROS was detected in extreme body condition score cattle when compared with ideal. Increased ROS was associated with increased days open and breedings per cow. Our results will serve as continual data for understanding metabolic profiles of differing body compositions and health factors that could affect oocyte maturation.

Key Words: oocyte, reactive oxygen species, negative energy balance

1037M Optimization of casein micelle nanoparticle formation using high-pressure homogenization.

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Casein proteins are the most abundant proteins in milk. They orient themselves into micellar structures to create hydrophobic, or water repelling, cores. When this protein structure is disrupted and then reaggregated, the proteins can form nanoparticles. Different substances can be encapsulated inside these nanoparticles, which improves their stability and bioavailability. One technique known to induce nanoparticle formation is high pressure homogenization (HPH). In this research, skim milk (SM, at 20% vol/vol) was processed using HPH (0–300 MPa) with ethanol (EtOH) at different concentrations (0–60%) and temperatures (5–65°C) to find the optimal nanoparticle formation conditions. Absorbance (at 400 nm) was used to monitor casein micelle dissociation. All procedures were completed in a randomized order with triplicate samples, and Minitab software was used to conduct a 1-way ANOVA with Tukey's test applied for mean comparisons and significance of treatment at $P < 0.05$. The control sample, with no HPH, at 65°C had a significant ($P = 0.001$) decrease in sample absorbance from 0.156 ± 0.01 with 0% EtOH (i.e., 20% SM, 80% distilled water) to 0.052 ± 0.03 with 60% EtOH (i.e., 20% SM, 20% distilled water, and 60% EtOH). This reflects the dissociation of casein micelles with a high EtOH concentration at elevated temperatures. With HPH of 300 MPa and a temperature of 65°C, the average absorbance of 20% SM solutions significantly decreased from 0.126 ± 0.019 at 0% EtOH to 0.036 ± 0.032 at 60% EtOH ($P = 0.026$). At 5°C with or without HPH, the ethanol did not dissociate the casein proteins and showed possible aggregation. The results of these experiments reflect more successful casein micelle dissociation, which is the initial step in nanoparticle formation, with the application of HPH, higher EtOH concentrations, and elevated temperatures.

Key Words: casein proteins, high-pressure homogenization, nanoparticles

1038M Evaluation of effectiveness between 2 caustic paste brands and volumes when disbudding dairy calves.

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Two main brands of caustic paste are routinely used in the United States to disbud dairy calves; Dr. Naylor's and Dr. Larson's are both commercially available and routinely purchased by commercial dairy farms. The objectives of this study were to evaluate paste brand and volume on horn growth, wound size, and healing rate. Sixty-two female Friesian Holstein calves were enrolled in the study at 1 – 3 d of age. Calves were housed individually and fed 3 L milk replacer 2 times per day for the first 4 d and then 3 L milk replacer 3 times per day until weaning began at 49 d when milk feeding was reduced to one time daily for a week until 56 d. At enrollment, calves were randomly assigned in a 2×2 factorial plus a sham to one of the following treatments: Dr. Naylor's at 0.2 mL of caustic paste (N2) or 0.4 mL of caustic paste (N4) or Dr. Larson's at 0.2 mL of caustic paste (L2) or 0.4 mL of caustic paste (L4) or Sham where petroleum jelly was applied (S). Before application of caustic paste or petroleum jelly, calves received a cornual lidocaine block and oral Meloxicam (1 mg/kg body weight) 15 min before treatment. Wound diameter (mm) was measured with digital calipers weekly until 8 wk of age; horn regrowth was checked weekly until 8 wk of age. Repeated mixed linear regression models were used for analysis. Least squares means of average wound size for wk 1–8 between caustic paste brands were not different ($P = 0.2$) where L measured 22.09 ± 0.69 mm and N measured 23.25 ± 0.61 mm. Least squares means of average wound size was affected by paste volume ($P < 0.001$) where 0.2 mL measured 20.96 ± 0.62 mm and 0.4 mL measured 24.39 ± 0.68 mm. At 8 wk of age, horn regrowth was recorded for one calf in N2, L4, and N4 treatments. Wound size was significantly affected by week ($P < 0.0001$) but there were no significant interactions between brands and volumes ($P < 0.07$). Overall, this study suggests that farmers may purchase different brands of caustic paste and expect similar results in wound size however an increasing volume of caustic paste applied will increase wound size.

Key Words: Dairy calves, caustic paste brand, caustic paste volume

1039M Changes in oxylipid concentrations in dairy calves in response to wildfire-$PM_{2.5}$ exposure.

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Wildfires produce fine particulate matter ($PM_{2.5}$) that cause health issues in humans, such as pulmonary and cardiovascular disease, thought to be attributed to inflammation. Although there are many studies focused on human health, there is very little known about the impacts of wildfire-$PM_{2.5}$ on livestock. Our group previously found wildfire-$PM_{2.5}$ increased selective markers of inflammation and calf mortality. Oxylipids are pro- and anti-inflammatory polyunsaturated fatty acids that play a role in the development of cardiopulmonary pathologies in humans. Our objective was to determine how wildfire-$PM_{2.5}$ affects the concentration of oxylipids as a measure of inflammation. Blood samples were collected from 13 Holstein heifer calves (monitored from birth to weaning) before, during, and after a wildfire smoke event during the summer of 2022.

The $PM_{2.5}$ concentrations, air temperature, and relative humidity were monitored hourly through a local monitoring station. Daily average $PM_{2.5}$ ranged from 1.5 μg/m^3 before to 113.5 μg/m^3 during the wildfire smoke event. Oxylipids were extracted from blood plasma and concentrations were determined using liquid chromatography-mass spectrometry (LC-MS). Data were analyzed using mixed models in SAS, with $PM_{2.5}$ and temperature-humidity index (THI) as fixed effects and the oxylipids as the dependent variable. Significance was declared at $P < 0.05$. When the $PM_{2.5}$ increased from a wildfire event, concentrations of 9-HODE ($P = 0.005$), 3-HODE ($P = 0.007$), 13-OxoODE ($P = 0.007$), 9,10-DiHOME ($P = <0.0001$), 12,13-DiHOME ($P = 0.01$), 11-HETE ($P = 0.03$) also increased. Further, 9,10-EpOME ($P = 0.02$) increased as THI increased whereas 12,13-EpOME, 17,18-DeHETE, 11,12-EET, 11,12-DHET, and 8,9-DHET did not respond to $PM_{2.5}$ or THI. These results indicate that wildfire smoke can modify pro- and anti-inflammatory oxylipids present in the body that have implications for the calves' health.

Key Words: inflammation, wildfire fine particulate matter, Holstein

Animal Behavior and Well-Being 1

1040M The climatic cost of impaired welfare in dairy sheep farming: A scenario study. L. Lanzoni*[1], K. Waxenberg[2], R. Ramsey[2], R. M. Rees[2], J. Bell[2], E. D. Costa[3], S. Throude[4], G. Vignola[1], and A. S. Atzori[5], *[1]Department of Veterinary Medicine, University of Teramo, Teramo, Italy, [2]Scotland's Rural College, Edinburgh, United Kingdom, [3]Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy, [4]Department of Farming Techniques and Environment, Institut de l'Élevage, Lyon, France, [5]Department of Agriculture, University of Sassari, Sassari, Italy.*

Small ruminant farming, which accounts for 6.5% of these livestock-derived greenhouse gas (GHG) emissions, has a high potential to reduce its environmental impact through improved farming practices. Sustainable mitigation policies should achieve win-win mitigation strategies in line with the "One Welfare" approach, simultaneously improving animal, human and environmental welfare. The present work aims to quantify the GHG abatement potential of various health and welfare improvements of dairy sheep farms in the Mediterranean basin. A dairy sheep farm representative of the Mediterranean semi-intensive farming system was selected as a baseline scenario. From this baseline, 6 impaired welfare scenarios, covering the most common impaired welfare conditions (i.e., mastitis, lameness, gastrointestinal nematodes infestation – GIN, high stocking density, heat stress and water deprivation) were modeled using published data. Each of the scenarios was characterized by different input values, used to assess the carbon footprint with Agrecalc©. The variation in emission intensity (EI) between the baseline and the scenarios for producing 1 kg of fat and protein-corrected milk (FPCM) was then assessed. All the impaired welfare scenarios presented an increase in EI compared with the baseline (2.38 kg CO2eq/kg FPCM). The high stocking density scenario, with animals housed in < 1.5 m^2/head, was associated with the highest increase in EI from the baseline (+10.0%, 2.84 kg CO2eq/kg FPCM). The mastitis (2.55 kg CO2eq/kg FPCM), GIN (2.54 kg CO2eq/kg FPCM), and lameness (2.43 kg CO2eq/kg FPCM) scenarios caused rises in EI of +6.8%, +6.5% and +2.1%, respectively. The water deprivation (+1.6%) and thermal discomfort (+1.0%) scenarios showed lower increases in EI. The present results suggest that adopting good practices to enhance animal welfare, driving the conditions from impaired to baseline, is likely to proportionally mitigate the farm's environmental impact. Enhancing welfare on dairy sheep farms is undoubtedly a prerequisite for resilient and sustainable animal food systems, even before considering other mitigation actions.

Key Words: sustainability, one welfare, dairy sheep

1041M Dietary supplementation of vitamin D_3 and Ca partially recover compromised lying behavior and its circadian rhythm in lactating cows under heat stress. K. Wang*[1], A. Ruiz-González[2,3], S. E. Räisänen[1], V. Ouellet[3], A. Boucher[3], D. E. Rico[2], and M. Niu[1], *[1]Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland, [2]Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Deschambault, QC, Canada, [3]Department of Animal Science, Université Laval, Québec, QC, Canada.*

The objective was to characterize the time budget and circadian rhythm of lying behavior in dairy cows during heat stress (HS) and to assess the effect of dietary supplementation of vitamin D_3 and Ca (+D_3/Ca). Twelve multiparous Holstein cows were used in a split-plot design with the level of dietary vitamin E and Se as main plots (LESe: 11 IU/kg and 0.55 ppm; HESe: 223 IU/kg and 1.8 ppm). Within each plot, cows were randomly assigned to 1) HS (D_3: 800 IU/kg; Ca: 0.67%), 2) HS+D_3/Ca (D_3: 3,764 IU/kg; Ca: 0.97%), or 3) thermoneutral pair-fed to HS (TNPF) in a Latin Square design with 14-d periods and 7-d washouts. Lying time was measured with HOBO® Loggers in 15-min intervals. Difference in daily (DLT) and hourly (HLT) lying time between treatments were analyzed using a mixed model. Cows in HS had reduced ($P < 0.05$) DLT compared with TNPF in both LESe (487 vs. 677 min/d) and HESe (546 vs. 780 min/d), whereas HS+D_3/Ca increased ($P < 0.05$) DLT by 64 min/d relative to HS in LESe. A treatment by time interaction ($P < 0.05$) was observed for HLT: lying time was reduced in HS relative to TNPF during early morning (0000–0600 h) and late evening (1800–2400 h). The diurnal patterns of lying behavior were characterized by fitting a cosine function of time in linear mixed model. Daily rhythmicity of lying was detected ($P < 0.05$) for cows in TNPF and HS+D_3/Ca, but not in HS ($P = 0.06$). Cows in TNPF had the highest mesor (the average level of diurnal fluctuations; 34.2 min/h) and amplitude (the distance between the peak and mesor; 17.9 min/h). The acrophase (time of the peak) occurred after midnight in all treatments. In conclusion, reduced daily lying time and disrupted circadian rhythms in dairy cows under HS can be partially restored by +D_3/Ca. Lastly, restricted feeding might alter cows' natural behavior, as shown by increased ($P < 0.05$) lying time in TNPF during the first 4 d of each period as DMI was gradually restricted. Pair-feeding might be a good model to investigate production and physiology under HS, but it may introduce biases when used for animal behavior research.

Key Words: heat stress, lying behavior, circadian rhythm

1042M Dairy cull cow condition and its effect on selling price in Québec, Canada. M. Puerto-Parada*[1], S. Buczinski[1], J. Dubuc[1], L. Blouin[2], and M. Villettaz-Robichaud[1], *[1]Université de Montréal, Saint-Hyacinthe, Québec, Canada, [2]Producteurs de bovins du Québec, Longueuil, Québec, Canada.*

Specific culling decisions vary depending on economics and specific situations affecting the animal and the herd. Although the sale of cull animals represents only a small percentage of a dairy farm's income, improving the welfare and quality of these animals could raise their sale price. Our objective was to assess the general physical condition of cull dairy cows sold at auction markets in Québec and to evaluate the relationship between their condition and sale price. A cross-sectional observational study was conducted in 3 auction markets. Cull dairy cows sold at auction were evaluated during their sale, in the auction ring using standardized data collection sheets. Registered data for each cow included auction and month of sale, breed, price, body condition score (BCS), locomotion, and udder characteristics. Descriptive statistics were performed to gather culled cows' characteristics. Univariable linear regression was used to evaluate the association between cow's characteristics and the final price. Multivariable linear regression including each characteristic, auction and month of the sale were then performed. A total of 5,614 dairy cows were observed between February and December 2022. Price ($/lb of live weight) obtained for each animal varied between 0 and $2.4 CAD (Median = 0.81, Mean = 0.80). Among all dairy cows observed, 90% were Holstein, 3% were Jersey, and 7% were of another dairy breed. Very skinny (BCS ≤ 2) cows' price was $0.23/lb lower than cows considered to have a BCS > 2 ($P < 0.01$). Lame cows' price was $0.09/lb lower than non-lame cows ($P < 0.01$). Cows with full udder were sold $0.06/lb less than cows with an empty udder ($P < 0.01$). Cows with a red or swollen udder were sold $0.07/lb lower than cows without abnormalities ($P < 0.01$). This study provides a better understanding of the condition of cull dairy cows sold at auctions in Québec. The characteristics having the greatest impacts on selling price could be used to identify targeted improvement strategies to be avoided by producers.

Key Words: animal welfare, cull dairy cow, auction market

1043M Investigating the efficacy of *Faecalibacterium prausnitzii*: A putative psychobiotic on health, growth, and behavior of dairy calves during the preweaning period.

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Early-life microbial interventions in neonatal dairy calves offers promise to improve animal health and well-being via modulating the microbiome-gut-brain-axis (MGBA). Although the use of probiotic in dairy calves has been investigated to some extent, their effect on MGBA and ultimately animal well-being has not been investigated till date. The purpose of this study is to evaluate the effects of administering *Faecalibacterium prausnitzii* (a butyrate producing bacteria) on the growth, health and behavioral responses of neonatal dairy calves. Twenty-four female Holstein dairy calves were enrolled immediately after birth and randomly assigned to 4 groups: control (CC); acidified control (AC); control/*Faecalibacterium* (CF); and acidified/*Faecalibacterium* (AF). The experiment lasted for 8 weeks where CF and AF calves were dosed at 2nd and 16th day after birth with 60 mL (1.34 × 107 cfu/mL) of activated *F. prausnitzii* whereas the other 2 groups had received blank media as placebo. All calves were measured for live weight, fecal scores performed and sampled for feces, blood throughout the experimental period and analyzed for systemic responses. Findings revealed that dosing neonatal dairy calves with *F. prausnitzii* resulted in an increased weight gain ($P < 0.05$), lowered incidence of diarrhea ($P < 0.05$) and also reduced cortisol ($P < 0.05$) but tended to increase serotonin ($P < 0.12$). There were numerical increases in anti-inflammatory (IL-6) but decreased pro-inflammatory (IL-10) responses in dosed calves compared with placebo calves. Calves were subjected to open field and novel object tests which revealed significant differences in behavioral responses between dosed and placebo calves. Overall, the oral administration of *F. prausnitzii* improves the calf's health, growth, and behavioral responses during the neonatal period. It can be concluded that *F. prausnitzii* may serve as a psychobiotic via modulating MGBA.

Key Words: *Faecalibacterium prausnitzii*, psychobiotic, preweaning dairy calves

1044M A global review of dairy quality assurance: What do we know and where are we headed?

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Dairy cattle health and welfare continue to be growing concerns for dairy producing nations around the world, which has led to the development of many quality assurance (QA) programs. Our objective was to conduct a global review of QA programs for dairy cattle welfare to better understand their goals, structure, and impacts. An environmental scan was conducted to identify prominent dairy QA programs that were used for industry and customer assurance in North America, Europe, the UK, Australia, and New Zealand. An evaluation framework was developed to systematically review each program's structure, standards, evaluation process, enforcement, and adoption. A total of 35 programs were identified for inclusion in this review. The evaluation was done using all publicly available information for each program. Interviews were conducted with representatives from 7 global QA programs to gather information about program structure, implementation, and communication strategies. While many programs had similar goals, the auditing approaches (e.g., second party vs. third party), program structures (e.g., voluntary vs. mandatory) and data collection methods (e.g., self-reported vs. on-farm evaluation) varied considerably. Most programs were voluntary or required for market access (80%), with few programs being mandated by government. Most (57%) programs required some form of on-farm evaluation; however, the depth and rigor varied considerably. Producer buy-in to program participation was noted as a challenge by most program representatives. Lessons learned from developing animal care programs included that clear and direct communication is key. Educating stakeholders on program goals, structure, and purpose was thought to be a foundational step in building interest and support. Animal welfare areas of future focus included pain management, surplus calves, antibiotic use, cow-calf separation, and permanent tethering. Areas of program importance included improved animal welfare assessments, sampling, and data collection, a desire to produce science and outcomes-based standards, and program cost vs value.

Key Words: welfare, quality assurance, environmental scan

1808W Effects of udder edema on parlor behavior in first and second-lactation Holstein dairy cows.

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Udder edema (UE) is a disorder that occurs around the transition period. However, there are gaps in our knowledge about its effects on parlor behavior. The aim of this study was to examine the prevalence and effects UE has on behavior in the milking parlor during udder preparation and active milking from 2 to 9 DIM in first (FL) and second lactation (SL) Holstein dairy cattle. Each cow (n = 375) was observed once and monitored from the point of first contact during udder preparation through the first 5 min of active milking. Behavior measurements include step, kick, and kicking off the milking unit (KOU). Cows were determined to have UE when the rear udder medial suspensory ligament lost definition and was softened due to the presence of interstitial fluid. Linear regression models of each milking session phase were analyzed and the interaction between lactation and edema presence was examined. In this study, 95.6% FL cows and 81.3% SL cows presented with UE. First lactation cows with UE had a higher step rate (3.97 steps/session) when in contact during udder preparation and attachment (2.80 steps/session), and kick rate during milking (2.68 kicks/milking session) when compared with SL cows with UE (2.37 steps/session, 1.25 steps/session, 1.24 kicks/milking session). The FL cows with UE had a lower step rate during milking (6.04 steps/milking session) compared with FL cows without UE (7.20 steps/milking session). The FL cows with UE had a higher average KOU count ($\mu = 0.220$ kick-offs) than SL cows with UE ($\mu = 0.029$ kick-offs) and FL cows without UE ($\mu = 0.091$ kick-offs). When comparing FL and SL cows without UE, FL cows had higher kick rates during attachment and during active milking, whereas SL cows had lower step rates during udder preparation, unit attachment, and active milking. The results indicate that UE is prevalent among dairy cattle, and the disorder has effects on behaviors presented in the milking parlor. Reducing UE has the potential to decrease step and kick behavior that pose a risk to dairy caretakers in the milking parlor and improve welfare of transition cows.

Key Words: udder edema, behavior, parlor

1046M Hoof disorders in Korean dairy cattle and the correlation of farm conditions to their prevalence.

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This study investigated the prevalence of hoof disorder (HD) in intensive dairy cattle farms in Korea and correlated the farm conditions to the HD prevalence. From 15 dairy cattle farms, a total of 877 cattle were inspected for HDs comprising claw horn lesions (CHLs) and non-lesion HDs. The 4 farm conditions assessed which may serve as risk factors were level of bedding, floor wetness, floor sharpness, and trimming invasiveness. Each condition was rated on a 1–3 scale (1 = poor, 2 = fair, 3 = good), with a total score range of 4–6 for poor condition, 7–9 for fair condition, and 10–12 for good condition. Prevalence was evaluated at the animal, foot, and farm levels using frequency distributions. Farm clustering and correlation analysis were performed using principal component analysis (PCA) and Pearson correlation, respectively. A total of 310 HD cases in 276 (31.47%) cattle with at least one case of HD was observed. Most of these were due to non-lesion HDs (24.63%), followed by infectious and noninfectious CHLs, affecting 6.16% and 4.56% of cattle, respectively. Bovine digital dermatitis (BDD) was the most prevalent among CHLs at animal and farm levels. Non-lesion HDs are highly prevalent on the front feet, mostly due to excessive hoof growth, while CHLs were more prevalent on the rear feet. The prevalence of HDs was higher in farms with poor conditions and was less prevalent in farms with good conditions. Farms with poor conditions and high HD prevalence were found to cluster together based on PCA analysis, suggesting similarities in their management practices. Lastly, correlation analysis showed that good farm condition has a significant negative correlation with the high prevalence of HDs. This suggests implementing favorable management practices to address potential risk factors for hoof disorders and to promote better hoof health for the overall welfare of animals. HDs have been neglected in Korea for over 2 decades and this study has shown that CHLs have increased sharply in the country compared with previous studies.

Key Words: Hoof disorders, farm condition, claw horn lesions

1047M Effects of heat stress and abomasally infused fish oil on lactating cow behavior.

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Omega-3 fatty acids in fish oil can reduce inflammation and hyperthermia, which could modulate animal behavior during heat stress. Twelve Holstein cows (38.5 ± 9.8 kg milk/d; 85 ± 33 DIM) were randomly assigned to 1) Heat stress + corn oil (HS; 55% 18:2 n-6; Max. THI = 82), 2) Heat stress + fish oil (HSFO; 8.3% EPA, 19% DHA; Max. THI = 82) and 3) thermoneutral pair feeding + corn oil (Max. THI = 64; TNPF) in a replicated incomplete Latin Square design with 2 10-d periods. Oils were abomasally-infused at 160 g/d. All cows were fitted with an automated device to record lying time (LT) and bouts (LB) every minute. Physiological indicators of HS such as respiratory rate (RR), rectal (RT) and skin temperature (ST) were measured 3x/d. Pearson correlations were used to determine associations between physiological and behavioral indicators of HS. Difference in LT and LB between treatments were analyzed in a mixed model. The circadian rhythms of lying behavior were then characterized by fitting a cosine function of time into linear mixed model. There were negative correlations between all physiological and behavioral indicators of HS (r: −0.29 to −0.36; all $P < 0.05$) with the strongest correlation observed between LT and RT. Daily number of LB did not differ between treatments, but relative to TNPF cows, HS cows spent on average 2h/d ($P = 0.01$) less time lying, whereas LT of HSFO cows tended to be higher compared with HS cows by 67 min/d. A treatment by time interaction ($P < 0.05$) was observed for lying behavior where LT was reduced for both HS treatments compared with TNPF during early morning (0000–0600 h) and late evening (1800–2400 h). Daily rhythmicity of lying was detected ($P < 0.05$) for all treatments. Cows in the TNPF treatment had the highest average level of diurnal fluctuations (31.9 min/h) and amplitude (11.5 min/h). The peak amplitude of lying occurred after midnight for all treatments, but earlier for TNPF cows. Together, these outcomes indicate that cows under HS adapt their lying behavior to dissipate more heat, while fish oil infusion can modulate these adaptations.

Key Words: hyperthermia, behavior, circadian rhythm

1048M Associations between personality traits of dairy cows and their heifer offspring.

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Personality traits of dairy cattle affect behavior and production, but it is not well known how heritable these traits may be. The objective of this study was to determine the correlation between personality traits of dairy cows and their heifer offspring. Twenty-three Holstein dairy cows had their personality traits assessed at 24 d before calving and 24 d after calving, and their offspring were assessed at 7 mo of age. Personality traits of all animals were assessed through observation of behaviors in response to a novel environment, object, and human. Principal components analyses (PCA) identified the traits of active, exploratory and bold in the pre-partum test conducted on dams (76% cumulative variance), and the traits of active and exploratory in the postpartum test (79% cumulative variance). Linear regression models were built using the REG procedure of SAS to assess the consistency of cow scores across calving, as well as possible associations between factor scores of the heifers and their dams and between each of the heifer PCA factors. Cow scores within the active and exploratory traits were consistent before and after calving ($P < 0.01$, $R^2 = 0.35$ and $P = 0.02$, $R^2 = 0.10$, respectively). From personality assessment in heifers, PCA resulted in 3 traits from the novel object test (bold, exploratory-active, and social; 81% cumulative variance), and 2 traits from the novel human test (exploratory-active and social; 74% cumulative variance). Cows who were more exploratory pre-partum were associated with having heifers that were less bold in the novel object test ($P = 0.01$, $R^2 = 0.26$). Cows who were more active pre-partum tended to be associated with heifers who were more exploratory-active in the novel object test ($P = 0.06$, $R^2 = 0.16$), while cows who were more active postpartum tended to be associated with heifers that were more bold in the novel object test ($P = 0.07$, $R^2 = 0.15$). The data from this study indicates that there are some associations between the personality traits of cows and heifer offspring, which with further investigation may allow prediction of heifer personality and better inform young stock management.

Key Words: personality, behavior, heritability

1049M Individual feeding consistency across stocking densities and feed efficiency in lactating cows.

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Our objective was to evaluate individual feeding pattern consistency at different stocking densities and associations with feed efficiency. Lactating Holstein cows (32 primiparous, 32 multiparous; 146 ± 35 mid-trial DIM, mean ± SD) were randomly assigned to share 8 Roughage Intake Control bins with cows of the same parity and similar BW (16 cows/block; 2 cows:1 bin). Feeding patterns were evaluated at 3 stocking densities (1:1, 2:1, 4:1 cows/bin) during 1 h tests within each block (2 tests/stocking density; 6 tests/cow) following 2 h of feed deprivation. Cows in each block were randomly assigned to subgroups of 8 for the 1:1 tests. Latency to the first bin visit, number of visits, total DMI, eating rate, and total eating time were calculated for each cow and averaged between replicates. Analysis was performed with 63 cows. To evaluate consistency between stocking densities, individual stability scores (ISS) were calculated for each feeding variable using normalized z-scores to compare pairwise combinations (A. 1:1 vs 2:1, B. 1:1 vs 4:1, C. 2:1 vs 4:1). Spearman rank correlations were used to compare ISS between A vs B and A vs C. Residual feed intake (RFI) was calculated by regressing DMI on secreted milk energy, median DIM, metabolic BW, and ΔBW, each nested within parity, over 45 d. Pearson correlations between RFI and ISS were used to assess relationships between consistency and feed efficiency. Cows with higher ISS (more consistent) for 1:1 vs 2:1 were also more consistent for 1:1 vs 4:1 (R range = 0.33 to 0.51; $P \leq 0.01$) for all feeding variables except latency to the first bin visit (R = 0.21, $P = 0.10$). Similar consistency was found for number of visits between 1:1 vs 2:1 and 2:1 vs 4:1 (R = 0.30, $P = 0.02$), but not for other variables (R range = 0.08 to 0.30; $P \geq 0.15$). Feeding variable ISS and RFI were not correlated for any stocking density comparison (R range = −0.08 to 0.19, $P \geq 0.14$). Cows remained consistent between a non-competitive environment and the more competitive environments. However, consistency in these brief testing scenarios was not correlated with RFI over the longer-term study.

Key Words: behavioral consistency, residual feed intake

1050M Evaluation of stocking density on feeding patterns in lactating cows.

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Our objective was to evaluate the impact of stocking density on feeding patterns. Lactating Holstein cows (32 primiparous, 32 multiparous; 146 ± 35 mid-trial DIM, mean ± SD) were housed in a freestall pen and randomly assigned to blocks of 16 cows sharing 8 Roughage Intake Control (RIC) bins (2:1 cows/bin standard density) with cows of the same parity and similar BW. Feeding patterns were evaluated at 3 stocking densities (1:1, 2:1, 4:1 cows/bin) during 1 h tests for each block (2 tests/stocking density; 6 tests total/cow). Within each block, cows were randomly assigned to subgroups of 8 cows for the 1:1 tests. Cows were feed deprived for 2 h before each test. For each test, latency to the first bin visit, number of visits, total DMI, eating rate, and total eating time were calculated from RIC data for each cow and averaged between test replicates. Analysis was performed with 63 cows. Latency was analyzed with a generalized linear mixed model using a gamma distribution. The remaining variables were analyzed with linear mixed models; number of visits and eating rate were $\log_n$ transformed, with back-transformed means and 95% confidence intervals reported. All models included a fixed effect of stocking density and a random effect of cow within block. As stocking density increased, so did latency to the first bin visit [2.8 min (2.1, 3.7) at 1:1 vs. 5.4 (4.0, 7.2) at 2:1 vs. 14.9 (11.0, 20.2) at 4:1; $P < 0.001$] and eating rate [0.16 kg/min (0.14, 0.17) at 1:1 vs. 0.18 (0.16, 0.19) at 2:1 vs. 0.21 (0.20, 0.23) at 4:1; $P < 0.001$], whereas DMI (6.3 ± 0.2 kg at 1:1 vs. 5.1 ± 0.2 at 2:1 vs. 3.3 ± 0.2 at 4:1; $P < 0.001$) and total eating time decreased (40.3 ± 1.2 min at 1:1 vs. 28.6 ± 1.2 at 2:1 vs. 16.2 ± 1.2 at 4:1; $P < 0.001$). Cows visited the bunk most often at the intermediate stocking density [5.8 (5.2, 6.4) at 1:1 vs. 6.9 (6.2, 7.7) at 2:1 vs. 4.3 (3.9, 4.8) at 4:1; $P < 0.001$]. Video analysis of competitive interactions and proximity to occupied feeding bins is in progress. As stocking density increased, cows appeared to modulate their bunk visits and eating rates to adjust for greater competition and less opportunity to gain feed bunk access.

Key Words: competition, feeding behavior, social dynamic

1051M Role of methodology and operationalization for inferring dominance hierarchy from observed behavior of dairy cows.

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A variety of methods are available for inferring a dominance order within a group of cows from observations of their interactions. In addition to selecting a method, an investigator must formulate a dominant/subordinate behavior operationalization (DSBO; i.e., a working definition of agonism for purposes of replicable empirical measurement). This work aims to estimate how much results differ among methods and among DSBO. We observed 492 interactions among 69 cows at the feed bunk (headlocks) in the 2 h following 8 milkings over 3 d. We noted whether the dominant behavior was displayed by the cow who initiated the interaction (offensive dominance; OD) or by the one who did not initiate (defensive dominance; DD) and whether there was physical contact. We experimented with 4 DSBO: 1) applying no restrictions (i.e., using n = 492 interactions), 2) using only OD cases (n = 385), 3) double counting

Table 1 (Abstr. 1051M). Kendall correlations of dominance among methods or among dominant/subordinate behavior operationalizations (DSBO)

Type of correlation	Median	Minimum	Maximum
Among methods, within DSBO 1	0.77	0.67	0.91
Among methods, within DSBO 2	0.79	0.67	0.96
Among methods, within DSBO 3	0.75	0.68	0.90
Among methods, within DSBO 4	0.79	0.68	0.90
Among DSBO, within Galindo-Broom index	0.72	0.59	0.92
Among DSBO, within Lamprecht index	0.71	0.59	0.97
Among DSBO, within Kondo-Hurnik index	0.74	0.63	0.97
Among DSBO, within Mendl index	0.70	0.58	1.00
Among DSBO, within David's score	0.75	0.63	0.93
Among DSBO, within Elo ratings	0.70	0.57	0.87

contact cases (n = 833), and 4) including contact cases only (n = 341). We experimented with 6 methods: Galindo-Broom index, Lamprecht index, Kondo-Hurnik index, Mendl index, David's scores, and random-order Elo ratings. We computed Kendall correlations (using R v. 4.2.2) among the resulting dominance orders in 2 groups: 1) correlations among methods within each of the DSBO and 2) correlations among DSBO within each of the methods (Table 1). Correlations among methods and among DSBO fall into a similar range (approximately 0.55–1.0), indicating that an inferred dominance order can vary as much due to choice of DSBO as choice of method.

Key Words: dominance, behavior, methods

1052M Effects of weaning and tyndallized *Lactobacillus helveticus* supplementation on dairy calf behavioral and physiological indicators of affective state. B. K. McNeil[1], D. L. Renaud[2], M. A. Steele[1], L. R. Cangiano[1,3], M. F. Olmeda[1], and T. J. DeVries*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [3]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objectives of this study were to determine if weaning dairy calves would induce indicators of a negative affective state, and if tyndallized *Lactobacillus helveticus* (TLH) supplementation would reduce them. A total of 23 male Holstein calves were enrolled in the study on d 1 of life. Calves underwent an abrupt stepdown weaning from 9 L/d on d 34 to 0.4 L/d on d 42, fed at 150 g of milk replacer (MR) powder/L. Calves were assigned to 1 of 2 treatments: 1) control (CON; n = 11) and 2) 5 g/d of TLH at 10^9 cfu/g split over and mixed into 2 daily MR feedings from d 3–42 (TLH; n = 12). Lying behavior was tracked from d 21–41. On d 33, 37, and 41, infrared eye images were taken to determine maximum eye temperature (MET), saliva samples were collected for cortisol concentration, and play assessments were done (each assessment included 30 s to add bedding to the pen and a 3 min observation period following). On d 34, 38, and 42, blood samples were collected for serotonin concentration. On d 43, a subset of calves (n = 5/treatment) were euthanized to collect gut and brain samples for serotonin concentration. Data were analyzed using mixed-effect linear regression models with repeated measures where appropriate. Weaning resulted in 4.3 fewer lying bouts/d, 11.7 min longer lying bout length ($P \leq 0.01$), 8.9 s shorter play duration per assessment, and 1.1 fewer play counts per assessment ($P \leq 0.01$). No changes in lying time (1105.2 ± 2.4min/d; $P = 0.13$), MET (37.0 ± 0.1°C; $P = 0.8$), saliva cortisol (0.13 ± 0.01 µg/dL; $P = 0.8$), nor blood serotonin (3985.6 ± 108.7 ng/mL; $P = 0.6$) were detected with initiation of weaning. TLH supplementation was associated with 24.2 min/d less lying time throughout ($P \leq 0.09$), and 9.9 s greater reduction in play duration from preweaning ($P = 0.04$) and 0.05 µg/dL higher salivary cortisol ($P = 0.01$) and 0.5°C higher MET ($P = 0.08$) during weaning. No treatment differences in lying bout count (22.0 ± 0.2 bouts/d), length (52.7 ± 0.6 min/bout; $P > 0.4$), or blood serotonin ($P > 0.3$) were detected throughout, nor colon (153.1 ± 14.1 n*M*/mg), ileum (148.3 ± 5.5 n*M*/mg), prefrontal cortex (113.3 ± 7.6 n*M*/mg), and brain stem (107.4 ± 5.5 n*M*/mg) serotonin concentrations ($P > 0.3$). Overall, weaning resulted in behavioral changes, while the results of TLH supplementation are inconclusive.

Key Words: paraprobiotic, gut-brain axis, welfare

Animal Health 1

1053M Variables associated with the magnitude and time of the nadir body condition score during the early lactation of Holstein cows. C. Hernandez-Gotelli*[1], D. Manriquez[1], J. Azocar[3], A. De Vries[2], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*DeLaval Inc., Madison, WI.*

The objective was to test the association between health status and milk yield and the magnitude and time of nadir BCS (nBCS) during early lactation of Holstein cows. This retrospective observational study used data collected from 12,042 lactations in 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy operation in Colorado, USA. Scores generated by an automated BCS camera system at calving (BCS1) and nadir (nBCS; lowest daily BCS from calving to 100 DIM) were selected and subsequently categorized into quartiles (Q1 = lower BCS). Potential explanatory variables included health status and average daily milk yield during the first 30 DIM (M30; categorized into quartiles). Health-related events occurring before nBCS were categorized into reproductive (REP; dystocia, retained fetal membranes, metritis, and pyometra), metabolic (MTB; clinical hypocalcemia, subclinical ketosis, and left displaced abomasum) or other disorders (OTH; lameness, clinical mastitis, digestive problem, injury, and respiratory disease). Lactations were classified as affected by REP, MTB, OTH, multiple categories (MLT), or unaffected (HLT). Data were examined using logistic regression and ANOVA. Multivariable models considered parity category, calving season, BCS1, and M30 as covariables. Healthy cows had greater nBCS (2.97) and reached nBCS earlier (40.9 ± 0.33 DIM) than cows that develop one (2.91; 49.5 ± 0.41 DIM) or multiple (2.85; 53.8 ± 0.49 DIM; $P < 0.0001$) health events in any of the proposed categories before nadir. Similarly, nBCS and DIM at nadir differed by system affected, with OTH and MLT cows evidencing the lowest nBCS (OTH = 2.90 and MLT = 2.86) and the longest time to nBCS (OTH = 54.2 ± 0.58 DIM and MLT = 53.4 ± 0.58 DIM). Cows in the lowest M30 quartile had the greatest nBCS values (2.97), while cows in the highest M30 quartile had the lowest nBCS (2.90). Time to nBCS was also different by M30 category, with low-producing cows consistently reaching nBCS earlier (Q1 = 41.7 DIM) than cows with greater milk yield (Q2 = 45.0 DIM, Q3 = 48.1 DIM, and Q4 = 51.6 DIM; $P < 0.0001$). Disease and high milk yield were associated with lower nBCS and increased DIM at nadir.

Key Words: nadir body condition, health, milk

1054M Impact of the reduction of metabolizable proteins in the ration during the transition period on immune and metabolic status of dairy cows. G. Tapp[1,2], F. Beaudoin[1], D. Ouellet[1], F. Malouin[1], P. Lacasse[1], and C. Ster*[1], [1]*AAFC- Sherbrooke R&D Centre, Sherbrooke, QC, Canada,* [2]*Biologie, Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada.*

Previous studies have shown that milk production is responsive to the level metabolizable proteins (MP) in early lactation. The objective of this study was to improve the energy balance and health of cows (n = 23) by temporarily reducing milk production via a reduction of MP in the ration. Control cows received a diet covering 100% of MP requirements (100%; n = 12) during the whole project (from 28 d before to 28 d after calving). Test cows received a diet covering 80% of MP requirements (80%; n = 11) during the treatment period (from 15 d before to 14 d after calving) and 100% before and after the treatment period. Blood was collected weekly to assess cows' metabolic and immune status. After calving, milk production was recorded daily and milk was collected weekly to evaluate its composition. Data were analyzed for each period (before, during and after the treatment) as a complete randomized design with the MIXED procedure of SAS. The milk production of the 80% group tended to be lower than the 100% group (30.8 vs 33.2 kg/d; $P = 0.09$) during the treatment period, but not after ($P = 0.68$). There was no difference for dry matter intake, energy-corrected milk, somatic cell count, and milk fat and protein content. The 80% group tended to have more plasma β-hydroxybutyrate during treatment period (694.6 vs 574.0 µmol/L, $P = 0.09$). The 80% group had less blood glucose (~0.3 mmol/L) on D7 and D10 ($P \leq 0.01$). Blood bilirubin, GGT, AST and GLDH were not affected during the treatment period, but GLDH activity was greater (35.2 vs 14.7 U/L, $P = 0.05$) in the 80% group during the post-treatment period. No differences were noted for free fatty acids nor the markers of the mobilization of muscle reserves (AST and CK). The granulocytes of the 80% group tended to show a greater oxidative burst during treatment period ($P = 0.09$). The treatment did not alter the lymphocytes proliferation nor granulocytes phagocytosis. In conclusion, a 20% reduction in MP intake did not affect cow health, but did not reduce milk production enough to diminish metabolic and immune disturbances during the transition period.

Key Words: energy balance, immunodepression

1055M Effect of supplementing one or 2 calcium boluses at calving on serum pH and minerals, performance, rumination, and activity of multiparous dairy cows. D. Duhatschek[1], B. Newcomer[2], G. M. Schuenemann[3], B. T. Menichetti[4], S. Paudyal*[1], V. N. Gouvêa[1], and J. M. Piñeiro[1], [1]*Department of Animal Science, Texas A&M University, College Station, TX,* [2]*Large Animal Clinical Sciences Department, Texas A&M University, College Station, TX,* [3]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [4]*Inter-Ag Nutrition Services, South Solon, OH.*

The objectives were to compare the supplementation of a single dose with one or 2 calcium boluses at parturition on serum pH and minerals, activity, rumination, milk yield, health events, and culling up to 60 DIM. A total of 1,022 multiparous dairy cows from a dairy farm in Texas were enrolled. Cows were fed acidogenic diets prepartum with an average (±SD) urine pH of 5.5 ± 0.3 within one week before expected parturition. At parturition, cows were randomly supplemented with one (1CB; n = 501) or 2 (2CB; n = 521) calcium boluses each with at least 50 g of Ca, 2.9 g of Mg and 60 IU of Vitamin D3 (RumiLife® CAL24, Genex). Blood samples were collected at parturition and between 4 and 28 h after treatment administration from a subgroup of cows (1CB = 246; 2CB = 251). Samples were centrifuged within 4 h and analyzed within 24 h to assess serum pH and minerals (Stat Profile Prime®, Nova Biomedical; Table 1). Milk yield, sold, death and health events up to 60 DIM were obtained from the farm management software. Activity and rumination data from −2 to 5 d relative to parturition were measured using activity monitor collars (Heat Time® Pro+, Allflex). Data were analyzed using MIXED or GLIMMIX procedures of SAS. Predictor variables in all models included treatment and lactation, and for blood parameters, the time between treatment and blood sampling was included. Compared with 1CB, 2CB decreased serum pH ($P = 0.01$), increased K ($P = 0.03$), and tended ($P \geq 0.05 < 0.10$) to increase serum iCa and Cl (Table 1). No differences between treatments were observed for rumination, activity, milk yield, culling, or health events

Table 1 (Abstr. 1055M). Effects of supplementing one (1CB) or 2 (2CB) calcium boluses to multiparous dairy cows at calving on serum pH and minerals 4 to 28 h after parturition

Item	1CB	2CB	SEM	*P*-value
pH	7.51	7.50	0.004	0.01
minerals (mmol/L)				
iCa	1.064	1.076	0.006	0.07
iMg	0.765	0.757	0.007	0.28
Cl	110.6	111.0	0.29	0.097
Na	140.7	140.5	0.24	0.27
K	4.85	4.96	0.053	0.03

$(P \geq 0.10)$. Compared with supplementing 1CB to multiparous cows fully acidified prepartum, supplementing 2CB does not seem to improve their performance.

Key Words: calcium bolus, serum minerals, performance

1056M Identification of potential blood biomarkers for early detection of periparturient diseases: A systematic review and meta-analysis.

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The transition period is associated with a high incidence of several peripartum diseases. Identification of blood biomarkers for early detection in peripartum period could decrease the incidence and severity of these diseases, whereby positively affecting dairy farm profitability. Thus, the objective of this study was to examine whether blood nonesterified fatty acid (NEFA), β-hydroxybutyric acid (BHB) and glucose could be used to predict health status of Holstein cows and what could be the best time for prediction in peripartum period. Initially, 787 studies from 2012 to 2022 were recovered from databases (Web of Science, PubMed and Scopus) through search terms like Holstein Friesian, peripartum period, blood metabolites, nonesterified fatty acids, β-hydroxybutyrate, and glucose. Different diseases included were ketosis, hypocalcemia, metritis, and mastitis. Then, many studies were excluded from analyses based on the breed (other than Holstein Friesian), sex (other than cows), stage of lactation (beyond 60 d prepartum or postpartum), disease that are studied only at prepartum or postpartum period, metabolites values shown in ratios or differentials and metabolite values without errors terms. The exclusion terms reduced the number of studies to 43, which were included in the final analyses. A random effect model was applied in the comprehensive meta-analysis software (Version-4) to determine the effect size in terms of means and standardized mean difference (SMD). In this respect, the healthy and disease consensus mean values were computed for peripartum period (Table1). The overall pooled effects (SMD) of NEFA, BHB and glucose between healthy and disease animals were significantly different ($P < 0.05$), reflecting their potential to be used for prediction purposes. However, the significances varied at different stages of the peripartum period (Table 1). In short, analyses of these studies provided consensus values for healthy and disease Holstein cows at different stages of the peripartum that could be used as reference for future studies. Further, the SMD analyses showed that NEFA and BHB seems to be better predictors for health status than glucose and 4 weeks before and after calving appeared to be the important time period that could potentially be used for predictive purposes.

Key Words: β-hydroxybutyric acid, nonesterified fatty acid

1057M *Bacillus* direct-fed microbial impacts intestinal butyrate-producing microbial populations during feed restriction in mid-lactation Holstein cows.

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Butyrate-producing bacteria in the ruminant gastrointestinal tract benefit the hindgut mucosa by converting fiber into butyrate, which serves as primary energy source for enterocytes and as local anti-inflammatory. This study evaluated the effects of a *Bacillus subtilis* and *Bacillus pumilus*-based direct-fed microbial (DFM; Strateris ECL; United Animal Health, Sheridan, IN) on beneficial butyrate-producing bacterial populations and key hindgut pathogens during feed restriction (FR) in mid-lactation Holstein cows. Thirty-six cows were randomly assigned to 1 of 3 dietary treatments (TRT): 1) control (CON; 7.5g/d rice hulls; n = 12), 2) DFM10 (10 g/d *Bacillus* DFM, 4.9×10^9 cfu/d; n = 12) or 3) DFM15 (15 g/d *Bacillus* DFM, 7.4×10^9 cfu/d; n = 12). After a 32-d pre-feeding phase with CON, DFM10, or DFM15, cows continued to receive their respective TRT during the 3 5-d experimental periods (P): a baseline (P1) with ad libitum feed intake, a FR period (P2) at 40% of P1 feed intake, and an ad libitum recovery period (P3). Rectal swab samples were collected from all cows on P1d3, P2d4, and P3d5 for DNA isolation and qPCR. Butyryl-CoA CoA transferase gene (*But*, from

Table 1 (Abstr. 1056M). Consensus mean values of blood nonesterified fatty acid (NEFA; µmol/L), BHB (mmol/L), and glucose (mg/dL) for healthy and disease cows and significance of standardized mean difference (SMD) at peripartum period

		Prepartum		Postpartum	
Condition	Metabolite	Week 8	Week 4	Week 4	Week 8
Healthy Mean (CI)	NEFA	158 (105–211)	317 (168–466)	396 (294–497)	466 (291–641)
	BHB	0.32 (0.29–0.35)	0.71 (0.57–0.85)	0.66 (0.58–0.75)	0.71 (0.56–0.86)
	Glucose	Studies not found	57 (53–62)	59 (55–64)	60 (56–63)
Disease Mean (CI)	NEFA	109 (73–146)	617 (490–744)	1,011 (751–1,271)	311 (268–354)
	BHB	0.41 (0.31–0.51)	0.51 (0.43–0.59)	1.5 (1.26–1.74)	0.62 (0.55–0.69)
	Glucose	Studies not found	52 (49–55)	54 (44-64)	61 (58–63)
Significance of SMD (*P*) (healthy vs. disease)	NEFA	0.166	0.019	<0.001	<0.001
	BHB	0.145	0.037	<0.001	0.129
	Glucose	Studies not found	0.044	0.095	0.404

butyrate-producing anaerobic bacteria), pan-bacterial 16S gene, and a panel of hindgut microbial virulence genes were evaluated. Absolute gene quantities were normalized to 5.0×10^9 pan-bacterial 16S copies per reaction to control for microbial and host DNA concentration. The qPCR data were analyzed with repeated measures ANOVA by P and TRT. *But* was higher ($P = 0.0096$, +0.17 $\log_{10}$ copies) in DFM10 cows relative to DFM15 and to CON ($P = 0.052$, +0.13 $\log_{10}$), and did not differ by P. Microbial virulence genes differed or trended by P (*C. perfringens* α toxin *cpa*, $P = 0.010$; *E. coli* zinc transporter *ZnT*, $P = 0.055$; intimin *eaeA*, $P = 0.012$; enteroaggregative stable toxin *EAST1*, $P = 0.056$), decreasing with FR in P2 or P3 relative to P1 regardless of TRT. Though *Bacillus* TRT did not affect pathogen burdenin healthy cows in this trial, DFM10 increased butyrate-producing bacteria, which promote intestinal health through immune modulation and energy availability.

Key Words: *Bacillus,* probiotic, butyrate

1058M Epidemiology of high liver triglyceride prediction: Associations with postpartum performance metrics.

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The study objective was to evaluate postpartum consequences (CON) for high liver triglyceride cases (HTG; triglyceride >22% tissue dry matter) as predicted by previously validated and published blood biomarker prediction models (BP). Multiparous Holstein cows (n = 529) were enrolled on 3 farms (X, Y, and Z). Coccygeal blood was collected at 7 ± 1 and 14 ± 1 d in milk (DIM). Blood plasma analyte concentrations were used in 3 BP models to predict HTG (case vs no-case) based on 7 (BP7) or 14 DIM (2 analyte panels: BP14A and BP14B) data. Farm records (DairyComp 305) provided the CON data. The data were analyzed by generalized estimating equations (GEE; SAS 9.4) with CON as the response and fixed effects of BP, lactation group (LG), farm, and all interactions. Interactions were backward eliminated based on GEE model BIC. In-text comparison descriptions are HTG cases relative to no-cases. First test-day (5 to 40 DIM) energy corrected milk (ECM) yield for HTG was greater at farm X but lower at farm Z with BP7 ($P < 0.01$, BP × farm); also, first test-day ECM had marginal evidence to be greater for HTG within 4+ parity cows with BP14A ($P = 0.06$, BP × LG). Second test-day (30 to 75 DIM) ECM yield had marginal evidence to be greater for HTG cows with BP14A ($P = 0.10$); while there was evidence of a 3-way interaction (BP × LG × farm) with BP7 ($P = 0.09$) and BP14B ($P = 0.05$). Retained placenta and metritis had greater incidence for HTG cows when predicted by BP7 ($P \leq 0.02$). Hyperketonemia (blood β-hydroxybutyrate ≥1.2 m*M*) had greater incidence in HTG cows for all BP ($P \leq 0.04$). Using BP14A and BP14B, HTG cows had greater incidence of displaced abomasum ($P \leq 0.05$). Retention rate (alive and not sold) from 0 to 60 DIM for HTG was greater on farm X and lower on farm Z with BP14A ($P = 0.02$, BP × farm); marginal evidence of a 3-way effect was detected with BP14B ($P = 0.07$, BP × LG × farm). First service conception rate for HTG was greater at farm X and lower at farm Z with BP14B ($P = 0.05$, BP × farm). Based on the associations with economically relevant CON, the BP evaluated may be useful tools for cow- and herd-level assessment of metabolic health.

Key Words: early lactation, lipidosis, longevity

1059M Abomasal infusion of branched-chain amino acids or branched-chain keto-acids alter neutrophil immunometabolic gene expression in early lactation dairy cows.

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Inflammation greatly impacts health and performance of dairy cows during early lactation. The objectives of this study were to determine the effect of branched-chain amino acids (BCAA) and branched-chain ketoacids (BCKA) on neutrophil phagocytic and oxidative burst function as well as mRNA expression of genes regulating neutrophil adhesion, chemotaxis and migration, Toll-like receptor pathway, and antioxidant metabolism in the first 3 weeks postpartum. Thirty-six multiparous Holstein cows were used in a randomized block design experiment. Cows were abomasally infused for 21 d after parturition with solutions of saline (CON, n = 12); BCAA (n = 12) including 67 g valine, 50 g leucine, and 34 g isoleucine; and BCKA (n = 12) including 77 g ketovaline, 57 g ketoleucine, and 39 g ketoisoleucine. All cows received the same diet. Neutrophils were isolated from blood collected on d 10 and d 20 of lactation. Treatment effects were determined using PROC GLIMMIX in SAS. No treatment differences ($P > 0.48$) were observed for phagocytosis or oxidative burst activity upon pathogen challenge. Cows receiving BCAA had significantly higher expression of antioxidant enzyme *GPX1* compared with both CON and BCKA ($P \leq 0.01$), suggesting enhanced reduction of pro-oxidant with CON. Expression of lysosomal protein *MPO* was lower in BCAA cows compared with CON ($P = 0.03$), which likely was associated with reactive oxygen production in neutrophils. Expression of pro-inflammatory cytokine receptor kinase *IRAK1* is higher in BCKA cows compared with CON or BCAA cows ($P \leq 0.05$) as well as expression of anti-inflammatory cytokine receptor *IL10RA* in BCKA cows compared with CON ($P = 0.03$). Overall, results suggest that neutrophil transcriptome respond differently to BCAA or BCKA supplementation during early lactation. Further work is required to understand underlying the mechanisms to the changes observed in this study.

Key Words: branched-chain amino acids, branched-chain ketoacids, early lactation

1060M Gut microbiome is linked to functions of peripheral immune cells in transition cows during excessive lipolysis.

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This study aimed to investigate the potential links between the gut microbiome and postpartum immunosuppression in transition dairy cows with excessive lipolysis. The blood and fecal samples were collected at 7 d postpartum. Cows with plasma nonesterified fatty acid (NEFA) levels >750 μmol/L were defined as high lipolysis (HNF, n = 9), whereas, those with NEFA levels <600 μmol/L were defined as low lipolysis (LNF, n = 9). The peripheral immune cells were collected to perform single-cell RNA sequencing (scRNA-seq), the fecal samples were conducted the analysis of 16S amplicon sequencing, metagenomics, and targeted metabolomics, the plasma were collected to measure the blood parameters and targeted metabolomics. The wilcx test was used to identify the differentially abundant taxa. The Student's *t*-test were used to compare the blood paramaters. Use of scRNA-seq identified 10 different immune cell types, and enrichment analysis revealed a downregulation of immune functions in HNF compared with LNF ($P < 0.001$). Beta diversity analysis revealed a clustering of fecal bacterial communities based on lipolysis grouping ($P = 0.001$). Totally, 9 differentially bacterial genera and 21 species were identified between 2 groups. Functional analysis showed that secondary bile acids (SBA) biosynthesis was significantly higher in HNF cows than LNF cows ($P = 0.047$), and the total SBAs with higher tendency ($P = 0.10$) in HNF.

Furthermore, the plasma glycolithocholic acid (GLCA, $P = 0.02$) and taurolithocholic acid (TLCA, $P = 0.03$) content was lower in HNF than LNF. Additionally, the results showed that monocyte (MON) exhibited a uniquely higher bile acid receptor expressing score. The Takeda G protein-coupled bile acid receptor 1 (GPBAR1) expressing was lower ($P = 0.007$) in $CD14^+$monocyte ($CD14^+$MON) in HNF cows. Enrichment analysis revealed that immune functions were significantly downregulated in $CD14^+$MON ($P < 0.001$) isolated from HNF cow and significant correlated with BA metabolism ($P < 0.001$). In summary, our results showed that the gut microbial alteration and reduced plasma GLCA and TLCA could contribute to the immunosuppression of $CD14^+$MON during excessive lipolysis by decreasing the expression of GPBAR1 in transition dairy cows, which provide a novel insight for the healthy feeding of transition dairy cows.

Key Words: bile acids, gut microbiome, immunosuppression

1061M Does knowledge of blood calcium at 2 DIM impact decisions of calcium supplementation? H. A. McCray*, C. R. Seely, and J. A. A. McArt, *Cornell University College of Veterinary Medicine, Ithaca, NY.*

Delaying oral Ca supplementation may benefit cows with low blood Ca at 4 DIM, a time when subclinical hypocalcemia (SCH) is associated with negative health and production outcomes. Our objective was to determine if delayed oral Ca supplementation improves Ca status at 4 DIM and milk production in cows with reduced blood Ca at 2 DIM. Data were collected from a previously conducted randomized controlled trial on multiparous cows (n = 518) from 4 farms in NY. Cows were randomly assigned to 1 of 2 treatment groups at calving: 1) control (CON; no Ca supplementation, n = 259); 2) bolus (BOL; 43g oral Ca administered at 48 and 72 h post-calving, n = 259). We used generalized linear mixed models to identify serum total Ca (tCa) concentrations at 2 DIM that maximized the difference in milk yield to diagnose SCH. Cows were classified as normocalcemic (NC; parity 2 tCa >1.9 mmol/L; parity 3 tCa >1.87 mmol/L; n = 457) or SCH (parity 2 tCa ≤1.9 mmol/L; parity 3 tCa ≤1.87 mmol/L; n = 61). Cows were further classified into 1 of 4 SCH-treatment groups (SCHTRT) based on 2 DIM SCH status and random treatment allocation: 1) NC-CON (n = 165), 2) SCH-CON (n = 29), 3) NC-BOL (n = 165), or 4) SCH-BOL (n = 27). Generalized linear mixed models were used to analyze the difference in tCa at 2 and 4 DIM and milk yield for the first 10 wk of lactation between SCHTRT groups with separate analyses performed for parities 2 and 3. For both parities, SCH-CON and SCH-BOL cows had lower tCa at 2 DIM than NC-CON and NC-BOL cows ($P < 0.001$). At 4 DIM, tCa concentrations were similar for all SCHTRT groups in parities 2 ($P = 0.7$) and 3 ($P = 0.6$). Mean milk yield differed between SCHTRT groups for both parities. For parity 2, SCH-CON and SCH-BOL cows produced more milk than NC-CON and NC-BOL cows ($P = 0.002$). In parity 3, SCH-BOL cows produced 56.3 (95% CI = 53.1, 59.3) kg, SCH-CON 51.7 (48.6, 54.7) kg, NC-BOL 50.6 (49.0, 52.2) kg, and NC-CON 48.7 (46.9, 50.5) kg of milk ($P = 0.001$). Our results suggest that while delayed Ca bolus administration does not improve blood Ca status when compared with controls, it does support increased milk production in specific groups of cows.

Key Words: subclinical hypocalcemia, oral bolus

1062M Effect of supplementing rumen-protected arginine on immune and inflammation status of transition dairy cows. A. Fraz*[1], F. T. Saputra[1], T. M. Adeoti[1], B. Souza Simões[1], U. Arshad[1], A. Husnain[1], M. C. Perdomo[1], Y. Sugimoto[2], J. E. P. Santos[1], and C. D. Nelson[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Ajinomoto Co, Inc., Tokyo, Japan.*

The objectives were to evaluate the effect of supplying 30 g/d metabolizable Arg supplemented to diets as rumen protected Arg (RPA, Ajinomoto Co., Inc., Japan) on the immune and inflammation status of transition dairy cows. Holstein cows were blocked by parity (n = 42 nulliparous, n = 60 parous) and energy-corrected milk yield at 250 d of gestation and randomly assigned to RPA, providing 30 g/d of metabolizable arginine or control (CON), providing 30 g/d of metabolizable protein from soybean meal. Blood was sampled from coccygeal vessel at d 250 and 260 of gestation and d 3, 7, and 14 postpartum to assess leukocyte profiles and neutrophil phagocytic and oxidative burst functions in total blood. Immune cell markers (CD172, CD14, CD21, CD4, CD8, $\gamma\delta$ TCR, CD62L, and CD11b) and neutrophil phagocytic and oxidative burst capacities were analyzed by flow cytometry. Data were analyzed with mixed models for effect of treatment (CON vs. RPA), parity, day and all possible interactions. Significance was declared at $P < 0.05$ and tendencies were declared at $P < 0.1$. Feeding RPA increased ($P = 0.05$) monocytes as a percentage of leukocytes (CON = 7.7 vs. RPA = 9.0 ± 0.6%) and tended ($P = 0.06$) to increase the percentage of monocytes that expressed CD14 compared with CON (CON = 77.1 vs. RPA = 80.3 ± 1.5%) during the postpartum period. An interaction between treatment and parity ($P = 0.04$) was observed for CD62L expression on CD14 positive monocytes because feeding RPA increased CD62L on monocytes of nulliparous cows but decreased CD62L on monocytes of parous cows. The percentages and concentrations of granulocytes, B cells, CD4 T cells, CD8 T cells and $\gamma\delta$ T cells in blood did not differ between treatments. Likewise, oxidative burst and phagocytic capacities of neutrophils did not differ between treatments. Feeding RPA decreased serum haptoglobin concentrations at d 0 (CON = 801 vs. RPA = 559 ± 71 μg/mL) and d 3 (CON = 1,110 vs. RPA = 979 ± 71 μg/mL) relative to calving compared with CON. Supplementing RPA to dairy cows in transition period increased the proportion of classical monocytes in blood but decreased haptoglobin, an indicator of less inflammation, compared with CON.

Key Words: arginine, immunity, inflammation

1063M Comparison of metabolic and immune biomarkers between primiparous and multiparous dairy cows during the peripartum period. A. Corset*[1,2], A. Boudon[1], S. Philau[1], O. Dhumez[1], A. Remot[3], P. Germon[3], and M. Boutinaud[1], [1]*INRAE-Institut Agro Rennes Angers, UMR 1348 PEGASE, Saint-Gilles, France,* [2]*Biodevas Laboratoires, Savigné-l'Évêque, France,* [3]*INRAE, Université de Tours, UMR 1282 ISP, Nouzilly, France.*

The peripartum period can decrease immune capacity leading to a higher risk of infectious diseases. The objective of this study was to investigate the influence of parity on metabolic biomarkers (NEFA, glucose, β-hydroxybutyrate, urea) and immunity (cytokines) before calving and at the beginning of lactation. Fifteen Holstein cows were classified into 4 groups according to calving date with 7 primiparous heifers and 8 multiparous cows. Blood samples were collected 3 weeks before calving, and 4, 8, 12 weeks postpartum and milk was samples at the same times only after calving. Cows were fed ad libitum a total mixed diet based on maize silage and milked twice a day. Milk minerals were measured by ICP-OES. In the plasma, metabolic markers were analyzed by spectrophotometry. Plasma pro- and anti-inflammatory cytokines were analyzed after an ex vivo challenge of whole-blood cells with or without heat-killed *Escherichia coli* using a bead-based multiplex assay. Data were analyzed with a mixed model including parity, week, and their interaction as fixed effects, and cow as a random effect. Milk production was higher in multiparous than in primiparous cows (x vs y kg/d on average over 12 weeks). During the first weeks of lactation, NEFA and BOH (parity x week interaction effect), and urea (parity effect) were higher in multiparous cows, whereas glucose was lower. Milk cell count was lower in multiparous than in primiparous cows in the 2nd week after calving but was always lower than 100,000 cells/mL. Parity did not affect the integrity of the mammary epithelium (milk Na^+/K^+ ratio). The key inflammatory cytokines CXCL10, CCL2, IL1Rα and IFNγ were lower in the plasma of multiparous cows. At the 3rd week before and the 8th week after calving, the immunity of multiparous cows could be less effective with lower levels of IL1α and TNFα, compared with primiparous cows. The parity effects on immunity contrasted with what is observed for the metabolic markers and milk production. The effect of parity must be considered for the research studies of immunity in dairy cows during the peripartum period.

Key Words: parity, cytokine, dairy cows

1064M The relationship among serum haptoglobin concentration, fecal pH, and milk production in dairy cows immediately after calving. R. M. Duperron, L. E. Engelking, and M. Oba*, *University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to evaluate relationships among serum haptoglobin concentration, fecal pH, and milk production. We hypothesized that serum haptoglobin concentration would be negatively correlated to fecal pH and milk production postpartum. Thirty Holstein cows (14 primiparous and 16 multiparous) were fed a common prepartum diet (15.8% crude protein (CP), 31.7% forage neutral detergent fiber (FNDF), and 19.4% starch) and a common postpartum diet (16.3% CP, 25.7% FNDF, and 25.5% starch). Dry matter intake and milk yield were recorded daily. Blood was sampled on d 1, 3, and 5 postpartum, and analyzed for serum concentration of haptoglobin, and plasma concentrations of fatty acids, β-hydroxybutyrate, and glucose. Fecal pH was measured on d 1, 3, and 5 postpartum. Correlation among the variables and their differences among days relative to calving were analyzed using Multivariate Methods and *t*-test, respectively, with JMP 16.1.0 (SAS Institute Inc.). There were no relationships among serum haptoglobin concentration, fecal pH, and milk production. However, serum haptoglobin concentration was negatively correlated to dry matter intake on 2 d before calving ($r = -0.50$, $P < 0.01$), and positively correlated to plasma fatty acid concentration ($r = 0.33$, $P < 0.05$). Dry matter intake increased linearly after calving and was highest on d 5 postpartum (18.3 kg/d; SEM = 0.64 kg/d; $P < 0.05$), and fecal pH decreased linearly after calving and was lowest on d 5 postpartum (7.78 vs. 7.50 vs. 7.39, respectively for d 1, 3, and 5; SEM = 0.035; $P < 0.05$). However, concentrations of serum haptoglobin (0.43, 0.96, and 0.77 mg/mL, respectively for d 1, 3, and 5; SEM = 0.15 mg/mL; $P < 0.05$) and plasma β-hydroxybutyrate (1.3, 1.5, and 1.3 mmol/L, respectively for d 1, 3, and 5; SEM = 0.08 mmol/L; $P < 0.05$) were highest on d 3 postpartum. These results suggest that fecal pH may not be associated with systemic postpartum inflammation indicated by serum haptoglobin concentration, but that prepartum feed intake can be a predictive measurement of postpartum inflammatory response.

Key Words: inflammation, fecal pH, milk production

1065M Decreased in lactose percentage in milk due to quarter health disorder and negative energy balance of dairy cows. A. Hamon*[1], S. Dufour[2], D. Kurban[2], C. Hurtaud[1], S. Lemosquet[1], R. Gervais[3], and J. Guinard-Flament[1], *[1]PEGASE, INRAE, Institut Agro, Rennes, France, [2]Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada, [3]Université Laval, QC, Canada.*

Several studies described variations in lactose percentage (LP) in dairy cows during udder inflammation or negative energy balance (NEB). However, their joint effects on LP were never described together. This was the aim of a longitudinal observational study performed on 5 Quebec dairy farms using automatic milking systems. Quarter milk samples were collected every other week from 5 to 300 DIM. Quarter health status was described by combining inflammation status (SCC- or SCC+: < or ≥100 000 cells/mL) and microbial status (Path- or Path+: presence or not of pathogens on a milk culture). Cows with NEB in early lactation (DIM <70) were identified using milk BHB content: 0.15 m*M* = BHB-; 0.15–0.19 m*M* = BHB+; > 0.19 m*M* = BHB++. A total of 14,583 quarter foremilk samples were collected from 380 lactating cows. The quarter level LP was analyzed using a mixed linear regression model with the following fixed effects: quarter health status, parity, delay between last milking and sampling, quarter milk yield (in kg/d), DIM, and herd. A random quarter intercept with a repeated measures correlation structure and a cow random intercept were also specified. The LP of SCC+ quarter milk was lower (−0.17 ± 0.014%; $P < 0.01$) for both primiparous and multiparous. LP was also lower in the presence of *Streptococcus dysgalactiae* and *Staphyloccocus aureus* regardless of the quarter inflammation status (SCC- or SCC+): −0.13 ± 0.029% for primiparous and −0.11 ± 0.027% for multiparous cows ($P < 0.01$). BHB+ and BHB++ cows in early lactation had lower LP (−0.04 ± 0.012% and −0.12 ± 0.021% respectively) compared with BHB- cows ($P < 0.01$). A larger decrease of LP was observed in SCC+ quarters from BHB+ and BHB++ cows, respectively −0.19 ± 0.021% and −0.27 ± 0.020% and that, in both parity groups ($P < 0.01$). Moreover, the cumulative effect of the quarter health status and NEB on milk LP was greater with higher NEB. Our findings suggest the necessity to jointly take in consideration both quarter health status and NEB when using LP as a health indicator.

Key Words: lactose, udder quarter health, negative energy balance

1066M Single-nuclei transcriptome reveals depot-specific changes in adipose tissue of dairy cows with subclinical ketosis. T. C. Michelotti[1], A. P. Tegeler[1], J. F. Fiallo[1], L. Flores[1], A. De-la-Cruz[1], O. J. Benitez[1,2], D. Dutton[2], V. Machado[1], and C. Strieder-Barboza*[1,2], *[1]Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, [2]School of Veterinary Medicine, Texas Tech University, Amarillo, TX.*

We evaluated the cellular transcriptional heterogeneity of visceral (VAT) and subcutaneous (SAT) adipose tissue in dairy cows with subclinical ketosis (SCK). Six multiparous Holstein cows (DIM 7 ± 2) were enrolled in a case control study based on SCK diagnosis (blood BHB in mmol/L): SCK (n = 3, BHB ≤1.4 and ≤2.6) and control, non-ketotic (NK, n = 3, BHB ≤ 0.8). Omentum VAT and flank SAT were collected via laparotomy, and nuclei purified. Single-nuclei RNA sequencing was performed using 10x Genomics and Cell Ranger pipelines, then analyzed in Seurat v3.1.4. Differently expressed genes were defined by Log2FoldChange > 0.5 and Adj. *P*-value < 0.05. Expression of signature genes identified 5 main cell types across SAT and VAT: e.g., *PDGFRA* for adipose stem and progenitor cells (ASPC); *ADIPOQ* and *LEP* for mature adipocytes (AD); *VWF* and *PECAM1* for endothelial cells (EC); and *MRC1, MSR1*, and *CD52* for immune cells (IM). Statistical analysis was performed using SAS v.9.4. with a 95% CI. Both groups had increased lipolysis, evidenced by high serum NEFA concentrations, which were greater in SCK vs. NK (1.2 vs. 0.9 ± 0.07 mEq/L). The most abundant cell type across SAT and VAT was AD, with ± 40% of total nuclei. VAT had a lower proportion of ASPC and IM, and greater abundance of EC than SAT. Cell type frequency did not differ between SCK and NK. There was a depot-dependent contrasting pattern of *HSPA1A* expression, an anti-inflammatory heat shock protein: AD, ASPC and EC had greater *HSPA1A* expression in SCK SAT vs. NK SAT, while *HSPA1A* expression was decreased in AD, ASPC and EC of SCK VAT vs. NK VAT. Regardless of depot, the expression of extracellular matrix genes (e.g., *SPP1* and *COL3A1*) was decreased in EC, ASPC and AD in SCK compared with NK. Gene expression of immune response markers (e.g., *CD63, LYZ*, and *CFD*) was downregulated in IM of SCK SAT vs. NK SAT, however these differences were not observed for VAT. Our results evidence unique differences in the transcriptional profile of SAT and VAT's cell types during subclinical ketosis, suggesting depot-specific contributions to the pathogenesis of ketosis in dairy cows.

Key Words: single-cell transcriptomics, postpartum, inflammation

1067M Prepartum acetylsalicylic acid in high-priority cow groups: Effects on metabolic status, systemic inflammation, and daily milk yield.

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The objective of this study was to assess the effects of prepartum administration of acetylsalicylic acid on metabolic status, systemic inflammation, and daily milk yield in high-priority cow groups (i.e., over conditioned cows [BCS ≥3.75 pts.], primiparous cows, and cow with calving disorders [i.e., stillbirth, dystocia, and/or twins]). At 14 d before the expected calving date, cows (n = 404) and heifers (n = 160) were blocked by body condition score (optimal = 3–3.5; high ≥3.75) and parity (nulliparous; parous), and randomly allocated to one of 2 treatment groups: 1) ASA (n = 286): receive one oral administration of acetylsalicylic acid (4 boluses; 480 grain/bolus; 125 g/d); or 2) PLC (n = 278): receive one oral treatment with gelatin capsules filled with water. Body condition score (BCS) was assessed, and blood samples were collected (i.e., β-hydroxybutyrate [BHB] and haptoglobin [HP] concentration assessment), weekly starting 1 week before treatment until 3 weeks after calving. Daily milk yields were collected for the first 50 DIM from on farm computer records. The data were analyzed using MIXED procedure of SAS as a randomized complete block design. Multiparous cows treated with ASA had lower BCS at calving, and 7 ± 3, 14 ± 3 and 21 ± 3 DIM compared with multiparous cows treated with PLC. There was no difference on BHB concentrations in the first 21 ± 3 DIM between treatment groups. Cows and heifers treated with ASA had lower HP concentrations at 7 ± 3 DIM compared with cows and heifers treated with PLC (ASA = 58.78 µg/mL,95% CI = 34.94–88.77; PLC = 128.50 µg/mL, 95% CI = 93.56–168.98; $P = 0.03$). There was an interaction between treatment and day, where cows and heifers treated with ASA produced more milk (x = 1.66 kg/d) in certain days (n = 7) during the first 2 weeks after calving compared with PLC cows and heifers. These findings suggest that treatment with prepartum acetylsalicylic acid may have positive effects on systemic inflammation and daily milk yield, while may negatively affect body condition in multiparous cows.

Key Words: prepartum acetylsalicylic acid, systemic inflammation, daily milk yield

1068M Epidemiology of high liver triglyceride prediction: Prediction model agreement and case prevalence.

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The study objective was to use previously validated and published blood biomarker prediction models (BP) to evaluate high liver triglyceride (HTG; triglyceride >22% tissue dry matter) prevalence on dairy farms. On 3 farms (X, Y, and Z), multiparous Holstein cows (n = 529) were enrolled at 22 ± 5 d prepartum. Body condition score (BCS) was evaluated at enrollment and 14 ± 1 d in milk (DIM). Coccygeal blood was collected at 7 ± 1 and 14 ± 1 DIM. Blood plasma analyte concentrations were used in 3 BP models to predict HTG (case vs no-case) based on data from 7 (BP7) or 14 DIM (2 analyte panels: BP14A and BP14B) data. Analytes included glucose (BP14A, BP14B), fatty acids (all BP), β-hydroxybutyrate (BHB; BP14A, BP14B), cholesterol (BP7, BP14A), aspartate transaminase (BP14A, BP14B), alanine transaminase (BP14A, BP14B), and magnesium (BP14B). Hyperketonemia (HYK) was diagnosed when blood BHB ≥1.2 m*M*. Data were analyzed (SAS 9.4) using generalized estimating equations (GEE) with BP prediction, HYK status, or BCS loss as the response; the GEE models' fixed effects were farm, calving month, and farm × month. Bonferroni's method was used for pairwise mean comparisons. Agreement between BP were tested by Kendall rank correlation. The overall BP case prevalence was 52%, 61%, and 54% with BP7, BP14A, and BP14B, respectively. Agreement between BP was found ($P < 0.09$) with $\tau = 0.29$, 0.08, and 0.54 for BP7 vs BP14A, BP7 vs BP14B, and BP14A vs BP14B, respectively. Farms did not differ in any BP case prevalence ($P > 0.34$). With BP7, monthly case prevalence differed ($P < 0.01$); September had lower prevalence (31 ± 4%) than June (61 ± 6%), July (70 ± 5%), and August (58 ± 4%). Monthly case prevalence was not different ($P > 0.18$) with other BP. Peripartum BCS loss differed farm × month ($P = 0.02$); farm X had less BCS loss in September than all other farm × month ($P \leq 0.10$). The overall prevalence of HYK, a HTG comorbidity, was 30% and did not differ between farms ($P = 0.40$). All BP had agreement with HYK ($P < 0.01$, $\tau = 0.12$ to 0.24). These HTG predictions may be a scalable opportunity to discover HTG associations with economically relevant outcomes.

Key Words: transition period, lipidosis, ketosis

1069M Mitochondrial uncoupling protein 1 expression is increased postpartum in adipose tissue of Holstein dairy cows.

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Mitochondrial uncoupling protein 1 (UCP1) is a thermogenic peptide that contributes to non-shivering thermogenesis in brown and beige adipocytes. In fetal calves, UCP1 expression starts during the last trimester of gestation, decreasing rapidly 2–3 weeks after birth. In monogastrics, UCP1 expression can be reinstated in white adipocytes by acute adrenergic stimulation. However, the expression of UCP1 in bovine adipose tissue (AT) is not well studied. The objective of our study was to characterize UCP1 in AT of periparturient dairy cows. Subcutaneous AT (SCAT) samples were collected from multiparous Holstein cows (n = 12) at 11 ± 3.6 d before calving (PreP), and 6 ± 1.0 d (PP1) and 13 ± 1.4 d (PP2) after parturition. The averages (mean ± SD) for BCS, MEq, and parity were 3.53 ± 0.22, 32182 ± 3752 kg, and 2.67 ± 0.65, respectively. All animals received a common diet during the close-up (30 d pre-calving to parturition) and fresh (1–15 DIM) periods to meet NRC (2001) requirements. The SCAT were fixed in 4% paraformaldehyde, blocked in paraffin, and then sectioned (4 µm). Sections were stained with an antibody against UCP1 (1:800) followed by rabbit on rodent HRP-polymer. Imaging was performed using an

Olympus SLIDEVIEW VS200 Research Slide Scanner. The UCP1 expression was quantified using ImageJ software (particle count/adipocyte count). Statistical analyses were performed using a mixed-effect model including the random effect of cow and fixed effect of time where time was included as a repeated measure. We observed a time effect on UCP1 protein expression reflected by higher HRP signal at PP1 and PP2 (2.09 and 2.86 ± 0.48) compared with PreP (0.72 ± 0.48; $P < 0.05$). Our results provide novel evidence for the browning of white AT in dairy cows after calving. Whether this reflects an increase in thermogenesis or an upregulation of the mitochondrial bioenergetic capacity is unknown. Further genomic and proteomic analysis are warranted to better understand UCP1-mediated energy expenditure in bovine AT especially during times of intense lipolysis and metabolic stress such as during the periparturient period.

Key Words: mitochondrial uncoupling protein 1, brown fat, postpartum period

1070M Prepartum anti-inflammatory therapies in high-priority cow groups: Effects on cow health and reproductive performance.

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The objective of this study was to assess the effects of prepartum administration of anti-inflammatory therapies on the incidence of diseases and reproductive performance in high-priority cow groups (i.e., over conditioned cows [BCS ≥3.75 pts.], primiparous cows, and cow with calving disorders [i.e., stillbirth, dystocia, twins]). At 14 d before the expected calving date, cows (n = 170) and heifers (n = 63) were blocked by body condition score (optimal = 3–3.5; high ≥3.75) and parity (nulliparous; parous), and randomly allocated to one of 3 treatment groups: 1) ASA (n = 80): receive one oral administration of acetylsalicylic acid (4 boluses; 480 grain/bolus; 125 g/d); 2) MEL (n = 76): receive one oral administration with meloxicam (1mg/kg of BW), or 3) PLC (n = 77): receive one oral treatment with gelatin capsules filled with water. Serum β-hydroxybutyrate concentrations and clinical metritis were assessed weekly for the first 21 DIM. Clinical disease events in the first 60 DIM and reproductive performance by 300 DIM were collected from on-farm computer records. The data were analyzed using MIXED, GLIMMIX and LIFETEST procedures of SAS as a randomized complete block design. Primiparous cows treated with MEL and ASA tended to have lower incidence of stillbirth compared with primiparous cows treated with PLC. Over conditioned cows treated with ASA had lower clinical health events incidence compared with PLC and MEL cows (ASA = 21.66 ± 5.37%; MEL = 43.33 ± 6.49%; PLC = 43.1 ± 6.60%; $P = 0.02$). Primiparous cows treated with ASA tended to have lower incidence of clinical metritis compared with MEL and PLC cows. Interestingly, multiparous cows treated with ASA required less days to become pregnant compared with MEL and PLC cows (ASA = 121.48 ± 6.96%; MEL = 145.49 ± 9.26%; PLC = 147.01 ± 9.80%; $P = 0.01$). These findings suggest that treatment with pre-partum anti-inflammatory therapies may have positive effects on postpartum cow health and reproductive performance in high priority cow groups. Further studies with larger data sets are needed to confirm association observed in this trial.

Key Words: prepartum anti-inflammatory therapies, cow health, reproductive performance

1071M The effect of oral or subcutaneous calcium at calving on ionized calcium and milk yield in Holstein cows fed anionic diets.

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Hypocalcemia is a common metabolic disease in dairy cattle. Although anionic diets during the prepartum period have been fed to prevent clinical hypocalcemia, subclinical hypocalcemia is still prevalent in mature dairy cows. Thus, Ca supplementation at calving has become a common strategy to improve performance of postpartum (pp) cows. The aim was to assess the effect of oral or subcutaneous Ca at calving on the concentrations of ionized calcium [iCa] and milk yield during the early pp of multiparous Holstein cows. The study was conducted in 2022 at the University of Missouri. An anionic diet was fed for 28 d before expected parturition (DCAD −120 mEq/kg; ([Na]+[K] − [Cl]+[S]) based on a commercial product. Multiparous cows with normal parturition were randomly assigned to 3 groups: CON no treatment (2L = 5; 3L = 3; ≥4L = 6); SQ (2L = 5; 3L = 3; ≥4L = 7), subcutaneous injection, 500 mL of calcium gluconate 23% once within 6 h pp; BOL (2L = 6; 3L = 3; ≥4L = 8), 2 oral boluses of $CaCl_2$/calmin 50 g (RumiLife CAL24 Bolus, Genex) once within 6 h pp. Blood samples were taken at calving before treatment and at d 1, 2, 3, 4, and 7 pp. Milk yield was recorded daily for 15 d pp. iCa (mmol/L) (Cal-Meter, Horiba, Japan) in plasma, and BHB (mmol/L) in blood (BHB Check Plus, PortaCheck) were measured. Results were analyzed by a mixed model for repeated measures. For [iCa] there was a triple interaction (treatment × day × parity; $P = 0.07$). Within cows of ≥4L BOL had lower [iCa] than SQ at d 2 pp (1.0 vs 1.2 mmol/L, respectively), but higher [iCa] than SQ at d 7 pp (1.14 vs 0.98 mmol/L, respectively). For milk yield in cows of 2 L and 3 L, SQ had higher milk yield from d 8 to 15 than BOL (34.5 vs 31.9 kg/d, respectively) ($P \leq 0.05$); however, in 4L there was a higher milk yield from d 9 to 14 in BOL than CON (35.4 vs 28.0 kg, respectively) ($P < 0.10$). It is concluded that SQ and BOL Ca improved performance of pp Holstein cows, but oral Ca improved consistently milk yield in mature cows when compared with controls and improved calcemia at 7 d pp when compared with SQ group. These results suggest that oral calcium is an efficient strategy to prevent hypocalcemia in mature Holstein cows fed prepartum anionic diets.

Key Words: ionized calcium, milk yield, Holstein

1072M Association of fetid vaginal discharge or vaginal discharge appearance with cytological endometritis in Holstein cows.

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The evaluation of vaginal discharge is a common practice for the diagnostic of metritis; however, the association of vaginal discharge evaluation and cytological endometritis was not explored. Furthermore, whether the discharge's appearance and odor characteristics differ in the association with cytological endometritis is unknown. Thus, we aimed to determine the association of fetid vaginal discharge (FVD) or vaginal discharge appearance (MCS) with endometrial cytology (CYTO) at 15 or at 30DIM. We conducted a pooled statistical analysis of 5 studies, including individual data from 280 multiparous Holstein cows. Evaluations of vaginal discharge were performed at 4, 7, 10, 13, and 15DIM (±1 d). The vaginal discharge was scored according to smell (FVD; 0 = no odor, 1 = fetid odor), and appearance (MCS; 0 = clear mucus or mucus containing <50% white mucopurulent material; 1 = discharge containing ≥50% purulent material or sanguineous discharger). Cytology of the endometrium was performed using a cytology brush (Andwin Scientific). Cutoff values for the polymorphonuclear cells (PMN) percentage were determined by taking the median value of the data set for PMN percentage at 15 DIM (24%) and at 30 DIM (7%). Statistical analyses were performed using generalized linear mixed models in SAS, considering cow as the experimental unit and experiment as a random effect. Having FVD at 4 DIM was associated with greater odds of having cytological endometritis at 15DIM (OR = 3.84, 95% CI = 1.19–12.9, $P = 0.03$), while having MCS at 4 DIM was also associated with greater odds of having CYTO at 15 DIM (OR = 2.22, 95% CI = 1.11–4.44, $P = 0.04$). In addition, having FVD at 13 DIM was associated with greater odds of having CYTO at 15 DIM (OR = 3.42, 95% CI = 1.66–7.08, $P = 0.01$) and at 30 DIM (OR = 2.01, 95% CI = 1.03–3.90, $P = 0.04$). There was no association of MCS and CYTO at 30DIM ($P > 0.21$). In conclusion, these results suggest that the evaluation of vaginal discharge odor may be an adequate and practical indicator of the inflammatory status of the uterine environment at 15 and 30 DIM.

Key Words: polymorphonuclear cells, vaginal discharge, uterus

1073M Associations of heat stress during the transition period with herd-level markers of energy metabolism in early-lactation cows.

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The objective of this study was to identify associations between temperature-humidity index (THI) during the transition period and markers of energy metabolism in early-lactation cows on commercial dairy farms in Ontario, Canada. From April to October 2022, 40 farms (190 ± 140 milking cows, mean ± SD) were visited 6 times; once every 4 wk. On each visit, blood samples were collected from up to 12 fresh cows (n = 1,530) within 14 DIM (7.2 ± 4.3 DIM; range = 0–14) to measure β-hydroxybutyrate (BHB), glucose, and nonesterified fatty acids (NEFA). Herd-level averages of these markers were calculated for each farm and visit. Ambient temperature and relative humidity were recorded every 10 min with loggers placed in both the close-up and fresh cow pens of each farm. Daily average THI (THIa; 65.2 ± 3.1, range = 57.7–71.1) and daily average maximum THI (THIm; 70.5 ± 2.8, range = 63.8–75.3) were calculated for each farm for 21-d periods before visits 2–6. To do this, we combined the THI data from 1) 14 d in the close-up pen (d −21 to −8 before each visit), and 2) 7 d in the fresh cow pen (d −7 to −1 before each visit). Separate mixed-effect linear regression models, using visit as a repeated measure, were used to identify the associations of both THI measures with herd-level averages of BHB (0.75 ± 0.30 mmol/L), glucose (2.94 ± 0.44 mmol/L), and NEFA (0.51 ± 0.18 mmol/L). THIa ($P = 0.01$) and THIm ($P = 0.02$) were negatively associated with herd-average blood glucose concentrations; each 1-unit increase in these measures was associated with a reduction of 0.03 mmol/L in blood glucose. No associations were detected between the tested THI measures and BHB and NEFA concentrations. In conclusion, measures of THI indicating modest heat stress during the transition period were associated with herd-level blood glucose concentrations in early-lactation cows in commercial dairy farms in Ontario.

Key Words: heat stress, transition cows, energy metabolism

1074M Postpartum acetylsalicylic acid administration and calcium supplementation: Effects on cow metabolic status and uterine health.

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The objective of this study was to assess the effects of the combination of acetylsalicylic acid administration and calcium supplementation after calving on body condition score (BCS), β-hydroxybutyrate (BHB) concentration, and incidence of subclinical ketosis and clinical metritis in multiparous (≥2 lactation) Holstein dairy cows. Within 12 h after calving, multiparous cows were randomly allocated to one of 4 treatment groups: 1) ASA (n = 109) = cows received 2 oral administrations with acetylsalicylic acid 24 h apart (125 g/cow/d; 4 480-grain aspirin boluses); 2) ASACAL (n = 114) = cows received 2 oral administrations with acetylsalicylic acid (125 g/cow/d; 4 480-grain aspirin boluses) and calcium (43 g/cow/d; 2 calcium boluses) 24 h apart, 3) CAL (n = 117) = cows received 2 oral administrations with calcium (43 g/cow/d; 2 calcium boluses) 24 h apart, and 4) UNT (n = 109) = cows remained untreated. Body condition score was assessed, and blood samples were collected (i.e., BHB concentration assessment) at 7 ± 3, 14 ± 3 and 21 ± 3 DIM. Cows that presented a BHB concentration of > 1.2 mmol/L were classified as having subclinical ketosis. Clinical metritis was defined as the presence of watery red-brownish fetid vaginal discharge and was assessed weekly using a Metricheck® device for the first 21 DIM. The data were analyzed using MIXED and GLIMMIX procedures of SAS. There was no difference on BCS between treatment groups (ASA = 3.5 ± 0.06 pts.; ASACAL = 3.25 ± 0.06 pts.; CAL = 3.5 ± 0.06 pts.; UNT = 3.25 ± 0.06 pts.; $P = 0.67$). Cows treated with ASACAL tended ($P = 0.05$) to have lower BHB concentrations in the first 21 DIM compared with UNT cows (ASA = 1.04 mmol/dL, 95% CI = 0.93–1.16; ASACAL = 0.95 mmol/dL; 95% CI = 0.86–1.05; CAL = 0.99, 95% CI = 0.89–1.11; UNT = 1.10 mmol/dL, 95% CI = 0.99–1.22). Cows treated with CAL tended ($P = 0.05$) to have lower incidence of subclinical ketosis in the first 21 DIM compared with UNT cows (ASA = 21.39 ± 8.84%; ASACAL = 21.6 ± 8.95%; CAL = 9.73 ± 4.92%; UNT = 26.67 ± 9.99%). There was no difference in the incidence of clinical metritis between treatment groups. These findings suggest that a combination of acetylsalicylic acid administration and calcium supplementation after calving might have positive effects on metabolic status in multiparous dairy cows.

Key Words: postpartum period, acetylsalicylic acid and calcium, metabolic status

1075M Epidemiology of high liver triglyceride prediction: Peripartum risk factors.

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The study objective was to evaluate peripartum risk factors (RF) for high liver triglyceride cases (HTG; triglyceride >22% tissue dry matter) as predicted by validated blood biomarker prediction models (BP). On 3 farms, multiparous Holstein cows (n = 529) were enrolled at 22 ± 5 d prepartum; body condition score (BCS) was evaluated then and at 14 ± 1 d in milk (DIM). Coccygeal blood was collected at 7 ± 1 and 14 ± 1 DIM. Blood plasma was analyzed for metabolite and protein biomarker concentrations that were input into 3 BP models predicting HTG (case vs no-case) based on 7 (BP7) or 14 DIM (2 analyte panels: BP14A and BP14B) data. Potential RF data were extracted from farm records (DairyComp 305). Data were analyzed by generalized estimating equations (GEE; SAS 9.4, GENMOD) with BP prediction as the response and fixed effects of RF, lactation group (LG), farm, and all interactions. Interactions were backward eliminated based on GEE model BIC. Greater HTG risk was associated with older LG for all BP ($P < 0.03$). Based on BP7, dry period length was positively associated with HTG risk ($P = 0.01$) but depended on LG when using BP14A and BP14B ($P \leq 0.03$, RF × LG). Longer calving intervals had greater HTG risk with all BP ($P \leq 0.05$). Greater HTG risk was associated with longer previous lactation DIM with BP14A ($P = 0.05$) and BP14B ($P = 0.09$). With BP7, greater HTG risk was associated with greater calving difficulty ($P < 0.01$). A 3-way interaction was detected ($P < 0.01$) for a calf sex effect on HTG risk with BP14A. Gestation length and prepartum BCS were not associated ($P > 0.10$) with HTG risk. Peripartum BCS loss was associated with greater HTG risk with BP14A ($P < 0.01$), but depended on farm ($P \leq 0.04$, RF × farm) when using BP7 and BP14B. Greater previous lactation 305 d mature equivalent milk production was negatively associated with HTG risk with BP7 ($P = 0.06$), and a 3-way interaction was detected ($P < 0.01$, RF × LG × farm) with BP14A. Subclinical mastitis cases at dry off (somatic cell score ≥4) increased HTG risk with BP14A ($P = 0.02$). Identification of RF by using BP may reveal management interventions that improve cow health and longevity.

Key Words: transition period, lipidosis, metabolic health

1076M Association between body condition score and ultrasound abdominal fat in Jersey cows.

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Abdominal fat is more metabolically active than subcutaneous fat. Thus, cows with more abdominal fat are at greater risk of developing more diseases such as fatty liver, displacement of the abomasum, and ketosis. Body condition score (BCS) is a subjective tool to assess subcutaneous fat deposition, as a result, it may only moderately predict specific areas of the abdominal cavity for fat deposition. The aim was to evaluate the amount of fat accumulated in different areas of the abdominal cavity in Jersey cows and its association with their BCS. From a Texas commercial farm, 21 non-pregnant nonlactating Jersey cows homogenous in weight and size from 5 to 7 parturition were selected at random. Cows were placed in a headlock line and body scored (scale 1 to 5 with a 1/4 unit increment) by the same evaluator. Ultrasonographic evaluation was performed using a Sonosite machine in duplicate. Ultrasonography was carried out on key anatomical points of the body to predict total abdominal fat (AT), retroperitoneal fat (RT), omental fat (OT), and mesenteric fat (MT; Table 1). A regression analysis for each abdominal fat depot and the BCS was run using SAS (2017). Models from the lowest to the largest r^2 are reported. The models were: $MT = 1.4819x - 1.7207$ ($r^2 = 0.023$); $RT = 2.8483x - 5.5353$ ($r^2 = 0.1047$); $OT = 5.0395x - 7.3852$ ($r^2 = 0.323$); $AT = 16.145x - 37.282$ ($r^2 = 0.369$). Correlation between BCS and abdominal fat depots were lower for mesenteric and retroperitoneal fat (≤10%), but higher for omental and total abdominal fat (32 and 37%, respectively). Then, cows were divided based on the median value of BCS distribution to high (≥3.5) and low (≤3.25). Those with high BCS had significantly larger amounts of fat deposited in abdominal cavity than cows with low BCS. It is concluded that BCS has a low to moderate association with abdominal fat depots, which supports the notion that fat accumulation patterns and metabolic turnover between abdominal and subcutaneous fat differ from each other.

Key Words: abdominal fat, Jersey, body condition score

Table 1 (Abstr. 1076M). Predicted kilograms of fat by ultrasound in Jersey cows with high and low BCS

BCS (1–5)	Abdominal fat (kg)	Retroperitoneal fat (kg)	Omental fat (kg)	Mesenteric fat (kg)
≤3.25 (n=8)	8.90 ± 1.86	2.41 ± 0.63	7.72 ± 0.62	1.86 ± 0.48
≥3.50 (n=13)	21.5 ± 1.96	4.86 ± 0.72	10.6 ± 0.70	3.57 ± 0.49
P-value	<0.001	0.03	0.01	0.04

1077M Whole blood cytokine response and milking performance during the transition period of healthy and diseased multiparous Holstein dairy cows on an automatic milking system.

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To compare the whole blood cytokine response and productive performance of healthy and diseased cows during the transition period, 74 multiparous cows (parity = 2.4) on a guided-flow automatic milking system (AMS) were monitored for health disorders (retained placenta, milk fever, ketosis, metritis, clinical mastitis, displaced abomasum) during the first 21 DIM. Cows were divided into 2 groups: Healthy cows (HC, n = 22, no diagnosed disease); and diseased cows (DC, n = 52, diagnosed with ≥1 diseases). Milk yield, pellet intake at AMS, and number of AMS visit were recorded daily. Blood was drawn from all cows on −10 ± 5 d before expected calving to collect serum. Additional blood samples were collected at the same time from a subset of cows (HC: n = 19; DC: n = 41) and cultured ex vivo without (CON) or with lipopolysaccharide (LPS, 1 μg/mL) for 24 h. Serum and culture supernatant were analyzed for tumor necrosis factor (TNF)-α, interleukin (IL)13, IL1α, and interferon (IFN)-γ. Data were analyzed using PROC MIXED of SAS. Fixed effects included group, time and their interaction with cow as a random variable. During the first 21 DIM, HC produced more milk (44.6 vs. 36.8 kg/d, $P < 0.01$) and consumed more pellets (3.14 vs. 2.96 kg/d, $P = 0.03$), but had similar AMS visit per day (3.1 vs. 2.9, $P = 0.15$) than DC. Relative to DC, HC tended to have greater ($P = 0.08$) serum concentrations (pg/mL) of TNF-α (612 vs. 886), IL13 (2188 vs. 3172) and IL1α (92 vs. 134) but had similar ($P = 0.20$) IFN-γ concentration. When stimulated by LPS, culture supernatant of HC had greater IFN-γ (332 vs. 210 pg/mL, $P = 0.04$) and tended to have greater IL13 (1846 vs. 1217 pg/mL, $P = 0.07$) concentrations than DC. Both HC and DC cows had similar cytokine concentrations in supernatant collected from CON culture. In conclusion, occurrence of health disorders reduced milk yield and pellet consumption but did not affect the number of AMS visits in early lactation. Greater whole blood cytokine responses of HC before calving may suggest greater immune responses to prevent disease occurrence.

Key Words: cytokines, disease, automatic milking

1078M Investigating risk factors for subclinical ketosis in robotic milking systems.

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In robotic milking systems, cows are free to milk more often and typically produce higher quantities of milk, leading to increases in energy required to support this level of production. Subclinical ketosis (SCK) is a prevalent illness caused by severe energy deficiency in dairy cattle. Therefore, the objectives of this study were to describe SCK in robotic milking herds, and to explore factors associated with SCK. A total of 430 cows across 2 commercial robotic milking herds in the Fraser Valley of British Columbia were enrolled in this study 1 week before dry off and followed until 60 d in milk (DIM) of the next lactation. Blood samples were collected from the time of dry off, weekly through the prepartum period, the day of calving until 4 DIM, then every other day until 14 DIM, and a final sample at 21 DIM. Blood was analyzed cow-side using a hand-held device (FreeStyle Precision Neo, Abbott Diabetes Care, Saint Laurent, QC, Canada), for β-hydroxy butyrate (BHB) and glucose and sent off for analysis for nonesterified fatty acids (NEFA). Data were analyzed using the GLIMMIX procedure with estimates of odds ratios in SAS software (SAS institute Inc., Cary, NC). Subclinical ketosis was defined as BHB ≥1.2 mmol/L and was diagnosed 426 times among 142 cows in both farms. Of the cows diagnosed with SCK, 63% had 2 or more SKC events. The prevalence of SCK varied across DIM, with the highest prevalence occurring at 8 DIM (21%). Additionally, cows with a body condition score of 3.5 or greater in the dry period were 1.4 times (95% confidence interval (CI) = 1.05–1.88) more likely to experience SCK. Cows in lactation 3 and higher were 1.4× more likely to experience SCK, with highest incidence occurring in 7th lactation cows (95% CI = 1.09–1.79). Cows with longer dry periods (>62 d) were 3.0 times (95% CI = 2.2–4.1) more likely to experience SCK compared with cows with a dry period of 51 d or less. Therefore, cows with greater body condition prepartum, longer dry period lengths, and higher lactation numbers are more susceptible to SCK in robotically milked herds.

Key Words: robotic milking systems, subclinical ketosis

1079M Predicting dyscalcemia at 4 days in milk using activity and rumination data in multiparous Holstein cows.

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Dyscalcemia (DYS), reduced blood Ca at 4 DIM, is associated with reduced milk production and reproduction and increased risk for negative health events. Cowside testing of blood Ca to diagnose DYS is difficult and alternative methods to identify DYS are needed. Our objectives were to use activity and rumination data to predict DYS. Data were collected from multiparous Holstein cows (n = 182) from 2 herds in NY. Cows were affixed with ear or neck loggers to record daily activity (AT; arbitrary units (AU)/d, defined by manufacturer) and rumination (RT; min/d) times. Daily AT and RT were collected from 14 d before calving until 14 DIM. No cows received supplemental Ca or experienced clinical hypocalcemia. A blood sample was collected at 4 DIM and analyzed for total Ca (tCa); cows were classified as DYS if tCa ≤2.2 mmol/L (n = 57), or eucalcemic (EUC) if tCa >2.2 mmol/L (n = 125). We used linear mixed models to analyze differences in AT and RT between calcemic groups. Logistic models were used to predict the probability of DYS from AT and RT variables. Prepartum AT was similar between EUC and DYS cows (402.0 ± 10.4 AU/d and 395.1 ± 14.5 AU/d, respectively; $P = 0.5$). Postpartum, EUC cows had greater AT than DYS cows (436.1 ± 10.7 vs. 407.8 ± 14.7 AU/d, respectively; $P = 0.004$). Prepartum RT was similar between EUC and DYS cows (512.6 ± 9.8 min/d and 504.2 ± 14 min/d, respectively; $P = 0.3$). Postpartum, EUC cows had greater RT than DYS cows (512.3 ± 10.5 min/d vs 480.5 ± 15 min/d, respectively; $P < 0.001$). The prediction model including daily changes in RT between 0 and 4 DIM and raw AT for 1 to 4 DIM yielded the greatest predictive capacity (AUC = 80%, sensitivity = 45%, specificity = 94%, negative predictive value = 79%, positive predictive value = 76%, accuracy = 78%). Our findings suggest that differences exist in postpartum AT and RT between cows that experience DYS and those that remain EUC. Utilizing AT and RT data in the immediate postpartum period shows utility in identifying cows with DYS at 4 DIM which could be beneficial to aid in management decisions that may ameliorate the associated negative outcomes.

Key Words: dyscalcemia, activity time, rumination time

1080M Association between access to grazing and respiratory disease in Holstein cows in organic-certified herds.

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The objective of this study was to evaluate the association between grazing during dry-off, calving, and early lactation and the probability of respiratory disease events (RES) in the first 30 DIM in Holstein cows under organic-certified management. A retrospective study was performed, including 64,666 lactations from 8 organic dairies located in northern Colorado and west Texas. The data set included respiratory events in the first 30 DIM, along with dry-off, calving, and grazing information between January 2016 and December 2018. Cows had access to pasture for at least 120 d during spring and summer and were managed differently in the grazing and non-grazing seasons. However, supplementation was provided in all seasons and farms. Potential risk factors included parity (primiparous vs. multiparous), calving season (CS), access to grazing at dry-off (DG), at calving (CG), and within 21 DIM (G21). Data were analyzed using multivariable logistic regression and the models were adjusted considering the year of calving and operation as random effects. After edits, the total number of cows in the data set was 40,920. Moreover, 33.8% and 66.2% of the lactations corresponded to primiparous and multiparous cows, respectively. Overall, 3.1% of the lactations (primiparous = 2.8%; multiparous = 3.2%) had at least one RES case within 30 DIM. Parity, CS, DG, and G21 were significantly associated with RES (Table 1). Despite the low incidence of respiratory disease in the first 30 DIM, there may be management opportunities for the prevention of respiratory disorders during grazing.

Key Words: pasture, cow health, transition

Table 1 (Abstr. 1080M). Adjusted odds ratios of respiratory events within 30 DIM by variable of interest

Variable	Category[1]	OR	95% CI	*P*-value
Parity	Primiparous vs. multiparous	2.15	1.30–3.53	0.002
Calving season	Spring vs. winter	2.09	1.74–2.52	<0.001
	Summer vs. winter	1.27	1.05–1.53	0.01
	Fall vs. winter	0.93	0.78–1.09	0.3
Grazing at dry-off	Yes vs. No	1.81	1.14–2.85	0.01
Grazing at calving	Yes vs. No	1.00	0.85–1.16	0.76
Grazing within 21 DIM	Yes vs. No	1.23	1.03–1.48	0.02

Table 1 (Abstr. 1081M). Adjusted odds ratios of leaving the herd (combined live culling and death) during the first 30 DIM by variable of interest

Variable	Category	OR	95% CI	*P*-value
Parity	Primiparous vs. multiparous	2.73	1.70–4.38	<0.001
Calving season	Spring vs. winter	1.00	0.83–1.19	0.98
	Summer vs. winter	1.28	1.07–1.53	0.006
	Fall vs. winter	1.33	1.16–1.51	<0.001
Grazing at dry-off	Yes vs. No	1.85	1.16–3.03	0.009
Grazing at calving	Yes vs. No	1.32	1.13–1.53	<0.001
Grazing within 21 DIM	Yes vs. No	0.96	0.79–1.16	0.69

1081M Association between access to grazing and the risk of leaving the herd in organic-certified Holstein cows. A. Velasquez-Munoz*[1], D. Manríquez[2,3], and P. Pinedo[2], [1]*Universidad Catolica de Temuco, Temuco, Chile,* [2]*Department of Animal Sciences, Colorado State University, Fort Collins CO,* [3]*National School of Veterinary Medicine of Toulouse, Toulouse, France.*

The objective of this study was to assess the association between access to grazing during dry-off, calving, and early lactation and the odds of leaving the herd (LHD) due to culling or death in the first 30 DIM. A retrospective study was performed with data from 64,666 lactations in 8 dairy operations under organic certification, located in northern Colorado and west Texas. The data set included information on culling and death within the first 30 DIM, previous dry-off date, calving date, and grazing dates, between January 2016 and December 2018. Cows had access to pasture for at least 120 d during spring and summer and supplementation was provided in all seasons and farms. Data were analyzed using multivariable logistic regression. Potential risk factors of LHD included in the analyses were parity (primiparous vs. multiparous), calving season (CS), and access to grazing at dry-off (DG), at calving (CG), and within 21 DIM (G21). In addition, year of calving and operation were included as random effects. The total number of cows in the data set was 40,920. Moreover, 33.8% and 66.2% of the lactations corresponded to primiparous and multiparous, respectively. Overall, 3.4% of the cows (primiparous = 2.5%; multiparous = 3.8%) left the herd within 30 DIM. Parity, CS, DG, and CG were significantly associated with LDH (Table 1). Considering the results, access to grazing during dry-off and calving might be associated with an increased risk of leaving the herd in the early lactation.

Key Words: deaths, culling, organic

1082M Exploration of dairy cow holobiont revealed an important sharing of microbes between anatomic sites within individual hosts throughout lactation but sharing was limited in the herd. M. Mariadassou[1], X. Nouvel[2], F. Constant[3], D. Morgavi[4], L. Rault[5], S. Barbey[6], E. Helloin[7], O. Rué[8], S. Schbath[1], F. Launay[6], O. Sandra[9], Y. Le Loir[5], P. Germon[7], C. Citti[2], S. Even*[5], [1]*Université Paris-Saclay, INRAE, MaIAGE, Jouy-en-Josas, France,* [2]*IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France,* [3]*Ecole Nationale Vétérinaire d'Alfort, Université Paris-Saclay, UVSQ, INRAE BREED, Maisons-Alfort, France,* [4]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champanelle, France,* [5]*STLO, INRAE, Institut Agro, Rennes, France,* [6]*INRAE, UE326 Unité Expérimentale du Pin, Gouffern en Auge, France,* [7]*ISP, INRAE, Université de Tours, Nouzilly, France,* [8]*Université Paris-Saclay, INRAE, BioinfOmics, MIGALE bioinformatics facility, Jouy-en-Josas, France,* [9]*Université Paris-Saclay, UVSQ, INRAE, BREED, Jouy-en-Josas, France.*

Microbiota is now considered as a major determinant of holobiont phenotypes. The present study aimed at exploring microbiota associated to various body sites of dairy cows to 1) identify determinants of the microbiota composition and 2) evaluate the intra- and inter-animal microbial sharing. Microbiotas from the mouth, nose, vagina and milk of 45 primiparous lactating dairy cows were characterized 1 week pre-partum and 1, 3, and 7 mo postpartum by metataxonomics. Cows with different scores of susceptibility to mastitis were used, as defined by the French national genomic evaluation system (genotyping). Data were analyzed using the DADA2 pipeline. Statistical analyses on ∝- and β-diversity were performed using R and specialized packages (phyloseq, DESeq2). Microbiota differed between body sites and changed over the lactation as a result of physiological and environmental changes (from indoor with corn and grass silage to outdoor). An important sharing of microbes was revealed within animals between nearby anatomic sites (up to 32% of Amplicon Sequence Variants (ASVs) of the oral microbiota shared with the nasal microbiota) but also between distant ones (e.g., milk with nasal microbiota). In contrast, microbes shared between animals within the herd were limited (<7% of ASVs shared by more than 50% of the herd for a given site and time point) and they were mainly found in the mouth and nose. Although they shared a common environment and diet, each animal thus hosted a specific set of bacteria, suggesting a host control. Interestingly, the score of susceptibility to mastitis was slightly related to the milk microbiota composition, confirming a link between host genetics and microbiota. In conclusion, this work highlights an important intra-animal sharing of microbes between different body sites, whereas sharing of microbes was limited between animals within the herd, supporting a host regulation of body-associated microbiotas. A better understanding of the host determinants shaping microbiota will allow to modulate the host-microbiota interplay for health and production.

Key Words: dairy cow holobiont, dairy cow microbiota, mastitis susceptibility

Breeding and Genetics 1: Inbreeding, Crossbreeding, and Lifetime Performance

1083M Identification of US Jersey bulls for germplasm preservation. K. Srikanth*[1], M. A. Jaafar[1], M. Neupane[2], J. Metzger[3], H. Ben Zaabza[2,4], S. McKay[4], H. J. Huson[1], C. P. Van Tassell[2], and H. D. Blackburn[5], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Baltimore Ave, Beltsville, MD,* [3]*Trans Ova Genetics, Sioux Center, IA,* [4]*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT,* [5]*National Animal Germplasm Program, USDA, Fort Collins, CO.*

The National Animal Germplasm Program (NAGP) conserves livestock genetic resources in the US. Periodic comparison of the germplasm collection (GPC) with the in situ population and determining animal relatedness, inbreeding, and missing genetic representation are necessary to understand collection completeness and plan future collection development. In this study we compared genetic diversity in US Jersey cattle between GPC and in situ population. Genotype and pedigree information on 40,831 Jersey bulls were obtained from the CDCB, including the 776 Jersey bulls preserved in the GPC. 88% of the bulls in the data set were born after 2010, however among the GPC only 23% of the bulls were from this period. Principal component analysis (PCA) showed that the animals clustered by decade of birth. To identify animals representative of missing diversity for the GPC, we performed Ward's minimum variance clustering of the bulls in GPC with i) top 1000 sires of bulls, ii) top 1000 sires of cows and iii) bulls with the top 1% net merit (TOPNM). Results showed that the GPC is missing genetic variation found in the TOPNM bulls but not among the other 2 categories evaluated. Based upon absent alleles it was determined that 0.6% of the alleles in TOPNM and 1.71% of the alleles in the GPC were not present in the GPC and TOPNM, respectively. Clusters 1 through 3 included only bulls from GPC, while clusters 6 through 8, were predominantly TOPNM bulls. ADMIXTURE analysis revealed unique genetic signatures within each cluster. Cross population selection signature analysis based on XP-EHH between bulls in cluster 1–3 and 6–8 showed selection on BTA 5, 9 and 13. Runs of homozygosity (ROH) analysis showed high ROH frequencies on BTA 1, 2, 3, 5, 7 and 20 in TOPNM. Inbreeding estimates based on ROH length class (F_{ROH}) suggested that historical inbreeding and recent inbreeding accounts for most of the inbreeding in the GPC and TOPNM, respectively. Results suggest a broad array of Jersey genetic diversity, some (1.71%) no longer present in the in situ population, has been captured by GBC; however the identified collection gaps provide a basis for future collection development.

Key Words: Jersey, genetic diversity, germplasm

1084M Effects of type traits, inbreeding, and production on survival in US Jersey cattle. B. M. Nascimento*[1], C. W. Wolfe[2], K. A. Weigel[1], and F. Peñagaricano[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*American Jersey Cattle Association, Reynoldsburg, OH.*

Greater longevity is associated with lower replacement costs, higher average milk production, and fewer replacement heifers needed. Longevity data are obtained late in life, and for this reason stayability, defined as the probability of survival from birth until a certain age, can be used as an alternative measure. The objective of this study was to evaluate the effects of different type traits, inbreeding, and production level on the stayability of Jersey cows to various ages and assess trends over time. Data consisted of 460,172 to 204,658 stayability records, depending on length of the opportunity period, for survival from birth until 36, 48, 60, 72, or 84 mo of age. Threshold models were used to analyze the stayability traits, including different type traits, inbreeding coefficient, and within-herd production level as explanatory variables. Heritability estimates for stayability traits ranged from 0.05 (36 mo) to 0.22 (84 mo). As expected, the probability of survival decreased as age increased. Highly productive cows were more likely to survive than their poor-producing contemporaries regardless of age and the type trait evaluated. Our data indicate that farmers' selection decisions tend to punish poor production at early ages and reward high production at later stages. Inbreeding negatively impacted the probability of survival, especially when inbreeding coefficients exceeded 10%, and this impact was most noticeable at 48 mo of age or later. Some type traits, such as stature and foot angle, had little impact on the probability of survival. Other type traits, such as strength, dairy form, rump width, and rear legs, showed higher probability of survival at intermediate scores, while other type traits, such as fore udder attachment, rear udder height, udder depth, and final score showed higher probability of survival at higher scores. Finally, our results indicate that the probability of survival has decreased in the last decade, probably due to a greater number of heifers available and, therefore, higher culling rates.

Key Words: probability of survival, stayability, threshold model

1085M Multibreed variance components and genetic parameters for crossbred dairy animals. G. Vargas*, N. Vukasinovic, D. Gonzalez-Peña, T. Passafaro, Z. Ahmed, A. Kulkarni, C. Przybyla, and D. Nkrumah, *Zoetis Genetics, Kalamazoo, MI.*

Reliable multi-trait estimates of genetic variance and heritabilities are a powerful tool in any genetic improvement program to facilitate total merit selection objectives. The objective of this study was to estimate multibreed covariance components and genetic parameters for 27 traits divided in 7 basic groups using data from Holstein (HO), Jerseys (JE) and HO×JE crosses. Traits in the genetic evaluation were divided based on analysis module: production traits (PROD), cow fertility (CFERT), heifer fertility (HFERT), calving traits (CES), livability (LIV), wellness traits (WT), and calf wellness traits (CWT). The traits in groups PROD, WT, and CWT were analyzed with a multivariate linear model while CES, CFERT, LIV, and HFERT were analyzed using a threshold-linear model, with one threshold and one or 2 linear traits. The number of records ranged from 1,582,060 for CWT to 16,320,968 for PROD. The variance components were estimated following the Gibbs sampling methodology using the BLUPF90 family program. Animals were classified as HO, JE and crossbreds based on their breed proportions. Besides animal, permanent environment, and trait-specific fixed effects, each model included a covariate effect of inbreeding and retained heterosis. Heritabilities (h^2) for the PROD group were 0.35, 0.29, 0.31, 0.13, 0.07, and 0.10 for milk, fat, and protein yield, somatic cell score, daughter pregnancy rate, and productive life, respectively. In the fertility groups, the h^2 for cow and heifer conception rate were 0.04 and 0.02, respectively and age at first calving was 0.10. In the CES group the h^2 were 0.06 for calving ease and stillbirth and 0.17 for gestation length. The livability h^2 was 0.03. In the WT group, h^2 ranges from 0.003 for milk fever and cystic to 0.06 for mastitis and CWT values were 0.11, 0.13, and 0.19 for respiratory disease, scours, and calf livability, respectively. The analyses conducted showed the feasibility of using multibreed procedures for estimation of covariance components in multibreed field dairy data set.

Key Words: dairy cattle, multibreed, heritability

1086M Single-step genomic BLUP prediction in Holsteins, Jerseys, and their crosses in the United States. G. Vargas*, N. Vukasinovic, D. Gonzalez-Peña, T. Passafaro, Z. Ahmed, A. Kulkarni, C. Przybyla, and D. Nkrumah, *Zoetis Genetics, Kalamazoo, MI.*

Zoetis Genetics has recently launched Herdity, an evaluation for Holstein (HO) and Jersey (JE) crossbred animals based on ssGBLUP methodology. The aim of this study was to obtain multibreed genomic predictions for 27 traits and compare them with predictions from single breed evaluation (Clarifide Plus) and the national genetic evaluation. Seven multitrait blocks were used: production traits (PROD); cow fertility (CFERT); heifer fertility (HFERT); calving traits (CES); livability (LIV); wellness traits (WT) and calf wellness traits (CWT) under 2 scenarios. The traits in PROD, WT, and CWT were analyzed with a multivariate linear model while other groups were analyzed using a threshold-linear model. The number of records ranged from 1,582,060 for CWT to 16,320,968 for PROD. The 2 scenarios contained genotypes for 181,379 JE, and 53,799 HO×JE crosses and 1,905,292 (all) and 810,944 (relevant) HO genotypes, respectively. The algorithm for proven and young (APY) was used with a random core of 30K animals. Genomic predictions from the 2 scenarios were compared; predictions for WT and CWT were also compared with predictions from Clarifide Plus, and with health trait predictions from the US national genetic evaluation. The correlations between genomic predictions from 2 scenarios were above 0.93 for all traits, indicating that using only relevant instead of all HO genotypes provided comparable results and reduced computational requirements. Positive correlations between the traits in WT and CWT and Clarifide Plus ranged from 0.36 for metritis to 0.89 for mastitis for JE, and from 0.67 for calf livability to 0.96 for metritis for HO. Correlations with traits in the national evaluation were negative for age at first calving for HO, JE, and HO×JE (−0.41, −0.23, and −0.29, respectively). Moderate to high positive correlations were obtained for other traits. The results indicate that large-scale ssGBLUP evaluation is feasible for commercial multibreed populations. Strategies to select animals using information from Clarifide Plus and Herdity may vary depending on the breeding goal.

Key Words: dairy cattle, multibreed, single-step genomic best linear unbiased predictor

1087M Identification of signatures of selection within a structured crossbreed population. M. A. Jaafar*[1], B. J. Heins[2], C. Dechow[3], and H. J. Huson[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]West Central ROC, University of Minnesota, Morris, MN, [3]109 Almquist Research Center, The Pennsylvania State University, University Park, PA.*

The mechanisms of population differentiation and divergence can be clearly understood by detecting genomic signatures of selection. Furthermore, it can help us comprehend the genetic effects of traits that are subject to selection and the inheritance of loci, particularly in crossbreeding systems. Excessive selection on particular genes or lineages may cause the loss of beneficial haplotypes in the following generation or potentially the entire population. Therefore, the purpose of this study is to identify the rotational cross, ProCross' (PC) selection trend between Viking Red (VKR), Holstein (HOL), and Montbeliarde (MON) ancestry and its relationship to production traits. Producing fertile cows with improved milk protein yield, carcass value, and health performance is the main aims of this cross. Genotype data were produced from 365 PC cattle. The genetic distance matrix was then calculated before the population was divided into 3 subgroups using Ward's method of cluster analysis. Each cluster was then phased to its parental genotype before genetic selection was performed using the integrated haplotype score (iHS), a test based on linkage disequilibrium (LD). Finally, production traits were associated with each genetic signature that had been discovered. The first and second clusters, which contained a mixture of VKR- and MON-sired individuals, showed a significant signature of selection on BTA 6, 9, 12, 19, and 21. Significant signals from the third cluster, which consisted of HOL- and VKR-sired individuals, who have a significantly higher milk, fat, and protein performance were visible on BTA 2, 7, 9, 19, and 21. Further investigation revealed the presence of functionally significant genes in these areas, including *PROP1* and *SPOCK1* on BTA7, and *ITPK1* and *FBLN5* on BTA21. Previous studies reported these genes are linked to 39 traits, mostly production traits. Overall, the findings of this study show how artificial selection factors have an impact on the dynamic genetic architecture and selection of genes and ancestry in the crossbred animals.

Key Words: signature of selection, ProCross, Ward clustering

1088M First-lactation crossbreds of Holstein with Jersey, Montbéliarde, Normande, and Viking Red compared to pure Holsteins for 305-day production and somatic cell score in a pasture production system. B. J. Heins* and K. T. Sharpe, *University of Minnesota, Morris, MN.*

The objective of this study was to compare 305-d milk, fat, and protein production and SCS of Holstein (HO) and crossbred cows during their first lactation. Holstein cows (n = 145) were compared with Grazecross crossbred cows (n = 251) composed of the Normande, Jersey, and Viking Red breeds and ProCross crossbred cows (n = 355) composed of the Montbéliarde, Viking Red, and HO breeds from DHIA test days. Cows were housed at the University of Minnesota West Central Research and Outreach Center, Morris, Minnesota, a grazing herd, and calved from March 2017 to November 2021. The dairy herd is managed as 2 separate herds: an organic herd and low-input conventional herd. The 2 herds were analyzed separately. Best Prediction was used to calculate actual production (milk, fat, protein, and SCS) for 305-d lactations with adjustment for age at calving, and records less than 305 d were projected to 305 d. Independent variables for statistical analysis were the fixed effects of genetic group (HO versus Grazecross crossbred versus ProCross crossbred) and herd-year nested within genetic group. For the conventional herd, HO cows (6,474 kg) had greater ($P < 0.05$) milk production than ProCross (6,007 kg) and Grazecross (5,153 kg) crossbred cows during first lactation. Combined fat plus protein production was similar ($P > 0.05$) for HO (458 kg) cows and ProCross (432 kg) cows. Across first lactation, the ProCross (3.06) were similar to HO cows (3.07) for SCS; however, Grazecross crossbreds had greater SCS (3.61). For the organic herd, HO (6,056) cows had greater ($P < 0.05$) milk production than ProCross (5,418 kg) and Grazecross (5,012 kg) crossbred cows during first lactation. Fat plus protein production was similar for HO (426 kg) cows and ProCross (390 kg) cows, but the Grazecross (364 kg) cows were lower during first lactation. The SCS was greater ($P < 0.05$) for Grazecross (3.56) cows compared with HO (3.19) cows and ProCross (3.15) cows. Three-breed crossbreds may have advantages in a rotational crossbreeding system in a pasture production herd.

Key Words: crossbreeding, pasture, Montbéliarde

1089M Understanding the production of beef from dairy systems in the UK: An analysis of trends. J. Gordon*[1,2], [1]*SRUC, Edinburgh, United Kingdom,* [2]*University of Edinburgh, Edinburgh, United Kingdom.*

This paper draws on comprehensive data of the UK cattle sector to analyze how dairy beef cross (DBX) production has changed over time, providing insights into risks and opportunities for future development of the cattle industry. Utilizing data in the Cattle Tracing System from 2010 to 2020, in combination with interview data from 47 semi-structured interviews with cattle farmers and stakeholders in the UK. This interdisciplinary approach provides a lens to identify trends in the sector and understand the underlying influencing factors. This study has found the number of DBX animals in the UK herd grew significantly between 2010 and 2020 (34% and 47% of calves born from dairy were DBX, respectively, in those years). The DBX production comes from all dam breeds with 2 main beef breeds gaining increasing importance recently: in 2010, the Limousin ranked first, followed by Aberdeen Angus and British Blue. While, annual Limousin DBX numbers dropped by 36k, Aberdeen Angus and British Blue grew by 133k and 128k, respectively. Focusing on DBX animals from black and white dams we found that the British Blue DBX were slaughtered at the same age as pure British Blue (29 mo average). Aberdeen Angus DBX were slaughtered on average at 30 mo compared with 31 mo for the pure Aberdeen Angus. Contrastingly, Limousin DBX had an increased average age of slaughter of 31 mo compared with the 28 mo of pure Limousin. DBX animals are not only used for meat production, but in breeding too, though over the 2010–2020 period we found a slight reduction of 3% of DBX cows that had offspring. The growth of the DBX sector is reliant on 2 key breeds. The large frame of the British Blue may benefit meat quantity, whereas the popularity of Angus may be due to its premium price. Reliance on 2 key beef breeds brings a concern about diversity within the system. In terms of opportunities, just as for pure beef animals, a reduction in slaughter age could improve environmental efficiency of the system.

Key Words: breeding, breed selection, beef on dairy

1090M Impact of using sexed semen and beef semen on genetic progress and economic benefits. Y. Gong*[1], K. F. Reed[2], and V. E. Cabrera[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Cornell University, Ithaca, NY.*

The improved reproductive performance and the use of sexed semen have resulted in an oversupply of heifer replacements, presenting an opportunity to use beef semen to produce crossbred calves of higher value for the beef market in combination with sexed semen to expedite the genetic progress. To quantify genetic progress and economic benefits of different semen-use strategies, a simulation study was conducted using a version of the Ruminant Farm Systems model modified to represent genetic inheritance through a net merit (NM) trait assigned to all animals. The study considered 2 heifer semen use strategies: sexed semen used on the top 50% of heifers (H1) or all heifers (H2), and 2 cow semen use strategies: sexed semen used on the top 25% of cows, with beef semen used on the bottom 25% of cows (C1), or sexed semen used on the top 50% of cows, with beef semen used on the remaining cows (C2). Five scenarios were evaluated: (1) conventional semen used for all eligible animals (control), (2) H1C1, (3) H1C2, (4) H2C1, and (5) H2C2. We simulated a herd of 1,000 cows that was maintained through a monthly purchase and sale of springers. Male and crossbred calves were sold while female calves were retained for breeding. To assign NM for animals at the start of the simulation we randomly selected values from CDCB NM percentile tables for year 2022 and adjusted the values downward based on the average linear increase in NM over the animals' lifespan. We defined genetic progress as the simulated change in NM over a 10-year period. The NM increase of top sires and market replacements was assumed to be the same as the average rate during the past 5 years. Compared with the control, the 4 mixed semen strategies increased the NM annual rate of increase for cows and heifers by $24 to $30 and $22 to $26, respectively. Furthermore, the H1C2 strategy covered the extra reproduction cost through the income generated by selling crossbred calves, without including the positive impact of genetic progress on herd performance. These results highlight the value of using sexed semen and beef semen to enhance the genetic progress and economic benefits of the herd.

Key Words: sexed semen, beef semen, genetic progress

1091M A meta-analysis of selection intensity, effective population size and inbreeding in sheep and goat populations. K. A. Sokoloff*[1], C. M. Rochus[1], J. L. Ellis[1], and C. F. Baes[1,2], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Department of Clinical Research and Veterinary Public Health, University of Bern, Bern, Switzerland.*

Selection of livestock to improve production traits has resulted in genetic gain but also caused a reduction in the genetic diversity within populations, which can lead to inbreeding depression. Because intense directional selection relies on genetic variation, genetic diversity of populations is crucial, ensuring they continue to be both productive and healthy. Inbreeding can be quantified via various measures, including the inbreeding coefficient (F_{ROH}) which is calculated based on runs of homozygosity (ROH). These are regions of the genome where the haplotype inherited from each parent is identical. Another way to quantify the genetic diversity of a population is effective population size (N_e), which is the number of animals that would have the same rate of inbreeding if they were an ideal population (following Hardy-Weinberg assumptions). A comparison of livestock selection strategies differing in intensity would allow for a deeper understanding of the relationship between selection intensity, N_e and F_{ROH}. Sheep and goat populations are a good reflection of the variability available in livestock populations, with several estimates of N_e and F_{ROH} available. Our objective was to study this relationship in ruminant populations, using estimates from peer-reviewed literature in a meta-analysis approach. Estimates were collected through literature searches in Web of Science (30), Science Direct Elsevier (29), PubMed (21) and Agricola (13), as well as via hand searching. The Boolean search logic included ("effective population size") AND (sheep OR goat) AND ("inbreeding" or "inbreeding coefficient") AND ("Runs of Homozygosity" OR "ROH"). The exclusion criteria used for refining searched articles included: the species is not sheep or goat, the article is a review, and inbreeding was not quantified with F_{ROH}. A mixed model, treating study as a random effect, will be applied to examine the relationship between inbreeding and selection intensity across the published literature. Once completed, the results of this study will be informative in the development of tools for breeders to manage inbreeding and identify populations in need of conservation.

Key Words: inbreeding, ruminants

1092M Genetic parameters and candidate genes for lifetime milk yield in Chinese Holsteins. H. Zhang[1], A. Wang[1], S. Mi[1], L. Brito[2], G. Guo[3], Q. Yan[4], S. Chen[4], and Y. Wang*[1], [1]*China Agricultural University, Beijing, Beijing, China,* [2]*Purdue University, West Lafayette, IN,* [3]*Beijing Sunlon Livestock Development Company Limited, Beijing, Beijing, China,* [4]*Dairy Association of China, Beijing, Beijing, China.*

Lifetime performance is a trait that combines production and longevity, which is one of the traits of primary interest for dairy cattle breeders. This study was conducted to 1) estimate the lifetime milk yield in the Chinese Holstein population; 2) estimate the genetic parameters for lifetime milk yield traits, including their heritability, genetic correlation and the correlations with production, longevity and type traits; 3) identify the important genes associated with lifetime milk yield by integrating the genomic and transcriptomic analyses. The lifetime milk yield was calculated based on the 3,365,612 test-day milk yield records of 134,029 cows, and it averaged 24,800.8 kg. A total of 6 lifetime milk yield traits were defined, including one trait covering full lifespan and 5 traits covering partial lifespan. Genetic parameters for lifetime milk yield traits were estimated using records from 20,565 cows. Heritabilities for lifetime milk yield ranged from 0.073 to 0.137. The relatively high genetic correlation between lifetime milk yield and productive life (0.868) was found when comparing the 305d milk yield in the first lactation (0.468). Most genetic correlations between lifetime milk yield and type traits were low (less than 0.3). Genome-wide association study (GWAS) and weighted gene co-expression network analysis (WGCNA) were performed to identify strong candidate genes for lifetime milk yield based on genotype data from 3,424 cows and peripheral blood RNA-seq data from 23 cows, respectively. Some SNPs and gene co-expression modules significantly associated with lifetime milk yield were found by GWAS and WGCNA, respectively. Enrichment analyses of the candidate genes identified by GWAS and WGCNA revealed some important immune-related pathways, energy utilization and metabolism-related pathways, and the FoxO signaling pathway involved in longevity. In conclusion, this study first performed the genetic analysis for lifetime milk yield in Chinese dairy cattle and revealed the important candidate genes by integrating the genome and transcriptome, which is beneficial for balanced dairy breeding.

Key Words: lifetime milk yield, genetic parameter, candidate gene, genome-wide association study, weighted gene co-expression network analysis

1093M Genome-wide association studies of lifetime performance index in Canadian Holstein cattle. B. McIlquham*[1], E. Etten[1], S. Chen[2], L. Brito[2], FS Schenkel[3], and M. Melka[1], *[1]University of Wisconsin–River Falls, River Falls, WI, [2]Purdue University, West Lafayette, IN, [3]University of Guelph, Guelph, ON, Canada.*

The production (LPI_PROD) and durability (LPI_DUR) components of Lifetime Performance Index play an important role in ranking the genetic merit of animals for overall lifetime performance. This index enables dairy producers to select animals that have high genetic merit for production and durability. The goal of this research was to identify gene loci and pathways associated with these 2 components of LPI. A Genomewide Association Study (GWAS) of LPI_PROD and LPI_DUR was conducted using 8,624 and 8,828 animals, respectively. A total of 1,711,646 and 1,699,277 whole-genome imputed SNPs were interrogated for association with LPI_PROD and LPI_DUR, respectively. Genes within +/− 100kb of the significant SNPs were identified and run through the KOBAS software (http://kobas.cbi.pku.edu.cn) for annotation and pathway enrichment analyses. Genomic regions that were found to be significantly associated with LPI were then run through the CattleQTL database to identify QTL and economically important traits associated with the genomic regions detected in this study. A total of 109 SNPs were found to be significantly associated with LPI_PROD ($P < 5$ x10–8) and a total of 3 SNPs were significantly associated with LPI_DUR ($P < 5$ x10–8), after Bonferroni correction. The most significant SNPs associated with LPI_PROD and LPI_DUR were found to be located on chromosomes 14 and 29, respectively. Several pathways were found to be significantly associated with the LPI_PROD ($P < 0.05$). Some of the identified pathways were prolactin signaling pathway, endocrine resistance, dopaminergic synapse and T cell receptor signaling pathway. According to the QTL database analysis results, significant SNPs were found to be associated with QTL/traits of economic importance to the dairy industry, such as milk fat percentage, milk fat yield, milk protein yield, calving ease, length of productive life and daughter pregnancy rate. The identified SNPs, genomic regions, biological pathways and the associated economically important traits that they are associated with reveal the genetic background of the production and durability components of LPI index.

Key Words: genome-wide association, dairy, lifetime performance

Dairy Foods 1: Microbiology and Cheese

1094M Effect of skim milk powder and whey protein concentrate addition on the manufacture of probiotic mozzarella cheese. M. Hamouda*, A. Sharma, R. Joshi, and P. Salunke, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Mozzarella cheese is extensively used in baked foods such as pizza, burgers, etc. Ingredients used in the manufacture of mozzarella cheese play an important role in its composition, yield, and functionality. The objective of this experiment was to study the effect of adding skim milk powder (SMP) and whey protein concentrate (WPC80) on probiotic whole milk mozzarella cheese manufactured using *Bifidobacterium bifidum*. The pasteurized cow milk was heated to 55°C for reconstituting SMP and WPC80 and divided into 5 parts: T1 (control), T2 (1% SMP), T3 (1.5% SMP), T4 (1% WPC-80), and T5 (1.5% WPC-80). After mixing powders in respective concentrations in the treatments, the milk was cooled to 35°C, and *Bifidobacterium bifidum* culture (5%) was added. Mozzarella cheese was manufactured using the standard manufacturing protocol. The experiment was replicated 3 times. Proximate analysis, titratable acidity (%), calcium, soluble nitrogen (SN), total *Bifidobacterium bifidum* count, cheese yield, and Schreiber melt test was carried out at 0, 7, 15, 21, and 30 d of storage. An internal sensory panel was used for sensory analysis (color, appearance, texture, and flavor). Obtained data were statistically analyzed using ANOVA and mean comparison was done using Tukey. WPC-added mozzarella cheese samples (T4 and T5) had higher acidity, total solids, and protein content than SMP-added mozzarella cheese (T2 and T3) and control samples (T1) during storage. The cheese yield was significantly ($P < 0.05$) higher in the SMP-added cheese than in the control and WPC-added mozzarella cheese. The addition of WPC80 led to a significant increase ($P < 0.05$) in the count of *Bifidobacterium bifidum* during storage at 5°C. WPC-added cheeses (T4 and T5) had increased meltability and higher browning on pizza compared with SMP-added mozzarella cheese (T2 and T3). Sensory evaluation showed that WPC80 addition increased sensory scores compared with SMP addition. As storage time progressed, there was a significant ($P < 0.05$) increase in the numbers of *Bifidobacterium bifidum*, meltability, and sensory scores of mozzarella cheese in all treatments. In conclusion, a small addition of SMP or WPC80 to milk can be helpful in increasing yield, probiotic count, and certain functional characteristics of probiotic mozzarella cheese.

Key Words: mozzarella cheese, probiotic, chemical and microbiological analysis

1095M Lactic acid bacteria isolated from Minas artisanal cheese: Probiotic potential, safety and viability under conditions of the gastrointestinal tract. I. C. N. Coelho[1], T. B. A. Miranda[1], S. M. Fafá[2], L. M. M. Magalhães[1], I. M. Costa[1], J. E. G. Gomes[3], D. C. S. Assis[1], L. M. Fonseca*[1], C. F. A. M. Penna[1], E. H. P. Andrade[1], M. R. Souza[1], and B. M. Salotti-Souza[1], *[1]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [2]Vila Velha University, Vila Velha, ES, Brazil, [3]Laboratory of Microbiology, Enzyme Technology and Bioproducts - Federal University of Agreste de Pernambuco, Garanhus, PE, Brazil.*

For the selection of new probiotic, it is necessary to evaluate the functional, technological and safety properties of the strains. The objective of this work was to evaluate the probiotic potential of 10 strains of lactic acid bacteria (LAB), isolated from the manufacture of artisanal Minas cheese in the Campo das Vertentes, MG, Brazil. The following strains: *Lactobacillus plantarum* (3), *Lactobacillus curvatus* (2), *Lactobacillus paracasei* (1), *Lactococcus lactis* (1), *Leuconostoc mesenteroides* (2) and *Leuconostoc pseudomesenteroides* (1) were evaluated for antimicrobial susceptibility by the disc diffusion test. Additional tests included autoaggregation (4°C, 37°C, 42°C), and hydrophobicity by solvent adhesion of the strains and absorbance measurement at 580nm. The in vitro survival test was used to assess the probiotics survival in a simulated condition alike the gastrointestinal tract (GIT). Analysis of variance followed by Tukey's test was applied to detect statistical differences ($P < 0.05$) between strains. All LAB evaluated were sensitive to ampicillin, 90% clindamycin, 80% erythromycin, 70% chloramphenicol, 40% tetracycline, 30% ceftazidime and streptomycin and 20% gentamicin, but all were resistant to kanamycin and vancomycin. The autoaggregation results at the evaluated temperatures did not show variation. In general, the LAB strains showed low hydrophobicity values, with the *Lactobacillus paracasei* (GV17) and *Lactococcus lactis* (GV103) presenting the highest results, 32.88% and 45.60%, respectively. The *Lactobacillus paracasei* (GV17), *Lactococcus lactis* (GV103) and *Leuconostoc mesenteroides* (GV15) were less affected by the gastric and intestinal phases in the survival test and their population at the end of these phases was greater than 8 $\log_{10}$ cfu/mL. Considering the in vitro analysis of the probiotic potential of the autochthonous cultures of Minas artisanal cheese that were analyzed, the GV17 and CV103 strains were considered safe and potentially probiotic, with promising properties for future applications in functional fermented products.

Key Words: beneficial effects, dairy, safety

1096M Microbiological characterization of Minas artisanal cheese from the Campo das Vertentes region (Brazil) during ripening in rainy and dry seasons. G. L. C. Valente[1], R. C. Figueiredo[1], R. F. Brito[1], L. M. Fonseca*[1], A. P. Madureira[2], A. M. Silva[3], B. M. S. Souza[1], C. F. A. M. Penna[1], and M. R. Souza[1], *[1]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]Universidade Federal de São João del-Rei, São João del-Rei, Minas Gerais, Brazil, [3]Universidade Federal de São João del-Rei, Sete Lagoas, Minas Gerais, Brazil.*

Minas artisanal cheese (MAC) is a traditional Brazilian product from Minas Gerais state. Due to its artisanal character, some factors such as extensive manipulation, the use of raw milk and the addition of endogenous starter cultures may contribute to the presence of undesirable microorganisms in the final product. On the other hand, lactic acid bacteria (LAB) found in cheese contribute to the food safety. Thus, the aim of this survey was to evaluate the microbiological characteristics of MAC, from the Campo das Vertentes region, throughout ripening at dry and rainy seasons. In each season, duplicate samples of MAC were collected from 4 registered cheeseries, at 1, 7, 14, 22 and 60 d of ripening. The analyzed parameters were: most probable number of coliforms; counts of LAB, coagulase-positive *Staphylococcus* and molds and yeasts; and presence of *Salmonella* spp. The Friedman test was used to compare the results among seasons and ripening periods, with a significance level of 5%. The most prevalent microorganisms in MAC were LAB, regardless of the period of ripening and season. Coliforms had high counts in MAC from 1 to 14 d of ripening in the rainy season, probably due to high temperatures and environmental humidity. Despite the coliform counts showing a decreasing trend throughout the ripening, there were abrupt increases suggestive of recontamination (such as at 22 d in the rainy season). Coagulase-positive *Staphylococcus* was detected in MAC from d 1 to d 14 in the rainy and dry seasons, respectively. Mold and yeast counts were statistically higher during the dry period, probably due to milder environmental temperatures and greater dispersion of spores in the air. *Salmonella* spp. was not detected in any MAC sample. The results of sanitary evaluation of MAC were in line with the legislation at 60 (rainy season) and 22 (dry season) days of ripening. Hygienic failures in handling the MAC may have resulted in contamination, which made it difficult to determine an earlier ripening period according to current legislation.

Key Words: raw milk cheese, food safety, foodborne pathogens

1097M Investigating the source of microorganisms causing discoloration on the surface of artisanal washed-rind cheeses.

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Washed-rind cheeses can be greatly impacted by factors such as contamination from the manufacturing process, as well as temperature and relative humidity within aging rooms, which determine the balance of bacteria and fungi of the surface microbiota. The aim of this study was to evaluate the resident microbiota of an artisanal cheese-making facility to identify the source of microorganisms potentially causing rind defects. After sanitation, sampling was carried out twice per month for 6 mo from June to November. Serial dilutions of samples (environmental swabs, liquid samples, freshly prepared cheese curd, rind of aging cheeses) were plated on M17 agar supplemented with 0.5% lactose (LM-17), De Mann, Rogosa and Sharpe (MRS) agar supplemented with 0.1% vancomycin (MRS-V), Yeast, Glucose and Chloramphenicol agar (YGC), and tryptic soy agar supplemented with 4% sodium (TSAS). Isolates were collected from LM17, YGC and TSAS plates from rinds of 5 cheeses of 5 age categories for identification by MALDI-TOF. Significantly higher total aerobic counts were obtained from aging planks ($P = 0.05$) and brine ($P = 0.02$), while drains and sanitized equipment were only sporadically contaminated. Accumulation of yeast and mold were seen to be highest on aging planks and in brine. Cheese rinds were classified by the presence or absence of black markings which would make them undesirable for sale. Isolates from rind with no discoloration were identified as *Brevibacterium* spp. and *Brachybacterium alimentarium*. However, *Scopulariopsis* spp. isolates were obtained from cheese rinds with brown discoloration and *Auerobasidium* spp. with black discoloration. Further work is required to link the development of discoloration with the microbiota of the aging planks and brine, as well as with the humidity conditions in the ripening rooms.

Key Words: washed-rind cheese, aging, discoloration

1098M Inclusion of pasture in dairy cow's diet: Fatty acid profile of Danbo-type cheese.

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The aim was to evaluate the impact of partially replacing a TMR with fresh alfalfa in lactating cows on the Danbo cheese's fatty acid profile (FA). In a crossover design experiment, 12 cows were randomly assigned to 1 of 2 feeding strategies: (T1) 100% TMR and (T2) 60% TMR + fresh alfalfa grazed without limit of quantity. Each experimental period lasted 26 d (21 d adaptation + 5 d sampling). The milk was collected during the morning milking and the cheeses were made individually on the same day from the milk of each cow (n = 12 cheeses/period). After 90 d of maturation, the FA profile was determined by gas chromatography. Data were analyzed by ProcMixed and means separated by LSMeans (SAS). There were differences between the periods, but the sequence of treatment application did not influence the study. According to the results and comparing the treatments, T2 presented increased levels of rumenic (CLA, 18:2 *c*9, *t*11; $P < 0.001$), vaccenic (TVA, 18:1 t11; $P = 0.004$), and α-linolenic acid (18:3 *c*9, *c*12, *c*15, $P = 0.004$), all of which are beneficial for health. Although the SFA:UFA ratio did not differ between groups, the total *trans*-FA was raised by almost 20% in T2 (Table 1). In summary, these findings indicate that partially replacing a

Table 1 (Abstr. 1098M). Danbo-type cheeses fatty acid profile (g/100 g FA)

						P-value	
Item	T1[1]	T2[2]	P1[3]	P2[4]	SEM	T[5]	P[6]
14:0	12.26	11.88	12.38^a	11.77^b	0.282	0.113	0.019
16:0	34.81	35.73	34.92	35.62	0.919	0.051	0.122
18:0	8.59^a	8.06^b	8.67^a	7.98^b	0.312	0.008	0.002
18:1 *c*9	16.26	16.27	16.09	16.45	0.477	0.979	0.359
18:1 t11	1.59^b	1.91^a	1.67	1.83	0.063	0.004	0.106
18:2 *c*9, *c*12	1.78	1.72	1.76	1.75	0.072	0.210	0.737
18:2 *c*9, *t*11	0.62^b	0.79^a	0.66^b	0.75^a	0.027	<0.001	0.028
18:3 *c*9, *c*12, *c*15	0.41^b	0.55^a	0.50	0.46	0.029	0.004	0.205
∑ 18:2 *trans*	2.46^b	2.85^a	2.54^b	2.77^a	0.082	0.003	0.041
SFA	71.57	70.90	71.62	70.85	0.618	0.194	0.142
MUFA	19.01	19.06	18.82	19.26	0.472	0.905	0.300
PUFA	2.20	2.27	2.26	2.20	0.091	0.214	0.278

[a,b]Statistical differences ($P \leq 0.05$).

[1]T1 = Treatment 1 (100% TMR).

[2]T2 = Treatment 2 (60% TMR + fresh alfalfa).

[3]P1 = Period 1.

[4]P2 = Period 2.

[5]T = Treatment.

[6]P = Period.

TMR for fresh pasture in cow's diet could be an option to increase the contribution of FA with beneficial repercussions in human health, such as anticarcinogenic effects provided especially by CLA.

Key Words: CLA, cheese fat, dairy products

1099M Molecular characterization of diarrheagenic *Escherichia coli* strains in bulk raw milk samples from Northern Tocantins, Brazil.

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Escherichia coli is a human and animal pathogen that can be transmitted through consumption of raw milk and dairy products. Among the *E. coli* strains found in milk, diarrheagenic strains have the greatest public health impact, as they can cause serious illnesses. This study quantified and identified enteropathogenic (EPEC), Shiga toxin-producing (STEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), and enteroaggregative (EAEC) *E. coli* in bulk raw milk samples from dairy farms in Augustinópolis, Tocantins, Brazil. Five samples were collected from pooled raw milk on 189 dairy farms at different times between September and November 2022 and transported to the Food Microbiology Laboratory of the Universidade Federal do Norte do Tocantins in Araguaína/TO. The samples were serially and decimally diluted in a saline and peptone solution, inoculated on Compact Dry EC (Nissui Pharmaceutical), and incubated at 35°C for 24 h according to the manufacturer's instructions. All blue colonies, indicative of *E. coli*, were purified on MacConkey agar. The DNA was extracted and was analyzed by multiplex polymerase chain reaction (PCR) to detect genes encoding virulence factors specific for EPEC (*eaeA*), STEC (*stx1* and *stx2*), EIEC (*ipaH*), ETEC (ST and LT), and EAEC (probe CVD). Detection of EHEC was defined as a simultaneous detection of *eaeA* with *stx1* and/or *stx2*. To differentiate typical (human) and atypical (non-human) EPEC isolates, they were analyzed by PCR to detect *bfpA*. The mean (±standard deviation) *E. coli* count in the milk samples was $2.1 \times 10^6 \pm 6 \times 10^5$ cfu/mL. Overall, 58 *E. coli* strains were recovered from all the samples; 9 (15.5%) were identified as EPEC, 2 (3.4%) as EIEC, and 5 (8.6%) as EAEC. Each pathotype originated from a distinct sample, and all EPEC isolates were atypical. The high *E. coli* counts present in the milk samples indicate a lack of proper hygiene practices during production, storage, and/or transportation. The presence of different diarrheagenic *E. coli* pathotypes could pose a risk to consumers if the raw milk is not pasteurized.

Key Words: enteroaggregative, enteroinvasive, enteropathogenic

1100M Camel milk production and supply chain hygienic practice with isolation of *Escherichia coli* strains from selected pastoral community in Ethiopia.

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Background: Raw milk consumption is a common trend in pastoral African communities either due to absence of preservation technology and lack of knowledge in milk born infection and intoxication. Methods: Camel milk in the supply hygienic practice with isolation of and antimicrobial resistance test on *Escherichia coli* strains was assessed in Doba district pastoral community, Eastern Ethiopia. Using cross-sectional study, various samples along the chain (camel udder to market point) were aseptically collected. Samples were examined using conventional microbiological and biochemical test procedures for *E. coli* strains isolation. *Escherichia coli* O157:H7 strains were screened from the *E. coli* isolates using a latex agglutination test (Oxoid). Antimicrobial resistances were tested using disc diffusion techniques. Using STATA 11, chi-squared r was calculated from the data. Significance of the studied variables was determined at *P*-values <0.05 considering 95% confidence interval. Results: Out of 544 samples, 117 (21.51%) and 26 (4.78%) were positive for *E. coli* and *E. coli* O157:H7, respectively. Of the total samples, *E. coli* was 99 (20.37%) and 18 (31.03%) from milk sample but *E. coli* O157:H7 were 20 (4.12%) and 6 (10.34%) from milk equipment swab and milker's hand swab samples. A significant difference in the occurrences of *E. coli* O157:H7 at *P*-value 0.036 was observed among sample sources. Antimicrobial resistance test results showed that both *E. coli* and *E. coli* O157:H7 isolates were 100% resistant to penicillin, vancomycin, and amoxicillin. About 97.4%, 74.8%, and 70.2% of *E. coli* and 100%, 84.5%, and 74.8% of *E. coli* O157:H7 were resistance to ampicillin, tetracycline, and oxytetracycline, respectively. A total of 13 (81.25%) *E. coli* and 24 (92.3%) *E. coli* O157:H7 isolates were resistant to 4 or more classes of antibiotics. Conclusion: The presence of *E. coli* and *E. coli* O157:H7 including multidrug-resistant isolates in raw milk highlights how the current milking practices and handling practices are unhygienic. Therefore, strategies in the prevention and control of foodborne infections that could be caused by multidrug-resistant strains will depend on how much hygienic milking and handling practices of milk improve.

Key Words: *E. coli*, *E. coli* O157:H, antimicrobial resistance, raw milk, hygiene

1101M Milk fat globule membrane enhances neurotransmitter synthesis of lactic acid bacteria.

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Serotonin, dopamine, and GABA are neurotransmitters (NT) or communication molecules beneficial for mental and physical health. NT can be produced in the gut by enzymatic conversions of amino acid precursors. These NT from the gut send signals to the brain via the gut-brain axis. This bidirectional link involves major body systems that influence physiological responses to the environment and maintain internal symbiosis. To complement the host's NT production, probiotic lactic acid bacteria (LAB) present in the gut offer distinct benefits, including NT synthesis and maintenance of gut health, and are naturally present in dairy foods. The milk fat globule membrane (MFGM) is a bioactive component of milk that positively influences brain function and the gut microbiota. Therefore, the objectives of this work were to characterize NT production by relevant LAB and determine the influence of MFGM on NT production. Four LAB strains (*Pediococcus acidilactici*, *Lactobacillus helveticus*, *Limosilactobacillus reuteri*, and *Lacticaseibacillus rhamnosus*) were selected based on genomic data containing genes for NT production and probiotic properties. All strains were grown overnight before incubation with appropriate NT amino acid precursor to activate enzyme production and the cell-free supernatants were collected. Serotonin and dopamine samples were filtered using a C_{18} column with methanol elution. All samples were concentrated using vacuum evaporation and measured using a spectrophotometer following reactions with respective indicator reagents. The optimal precursor concentration for each strain and neurotransmitter was determined before supplementing with MFGM and repeating the protocol. The results of this project show no GABA detection by any strain. Dopamine production was unaffected by MFGM supplementation for all strains. Serotonin production was significantly enhanced with MFGM supplementation by all strains, including a 5-fold increase by *Lacticaseibacillus rhamnosus*. These findings indicate a novel application of MFGM as a functional ingredient in dairy foods, and when combined with probiotic bacteria has great potential to enhance human health.

Key Words: gut microbiota, probiotics, human health

1102M Effect of stretching temperature on the growth of four dairy pathogen bacteria in raw-milk pasta filata cheese manufacture.

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Recently, the consumption of raw milk cheeses has been associated with foodborne diseases. This study aimed to evaluate the effect of stretching temperatures on the growth of 4 dairy pathogens during the manufacture of Sicilian raw-milk stretched cheeses. Cheese production was carried out with raw cow's milk started with commercial lactic acid bacteria (LAB) strains following the traditional transformation process that includes curd scalding and stretching phase in hot deprotenized whey. Experimental cheeses were contaminated with 10^3 colony-forming units (cfu)/mL of *Escherichia coli* O157 ATCC 25922 and *Staphylococcus aureus* ATCC 33862 and 30 cfu/mL of *Listeria monocytogenes* ATCC 7644 and *Salmonella* Enteritidis ATCC 13076. The experimental plan involved 3 trials with 3 different stretching temperatures: trial A, 74.5°C for 15 min; trial B, 83.6°C for 20 min; and trial C, 90.2°C for 20 min. Microbiological analyses were performed on raw milk, coagulated milk, curds, acidified curds, stretched curds, cheeses after brining, and ripened cheeses at 30, 60, and 90 d. All samples were subjected to the decimal serial dilution procedure and the microbial suspensions were used for the plate counts of the pro-technological, spoilage and pathogenic populations. Data on microbial growth were statistically evaluated using a mixed model to test the effect of the temperature. During milk coagulation, the levels of inoculated pathogens increased significantly ($P < 0.05$) and progressively decreased during curd cooking and acidification. After the stretching phase, the loads of added pathogens drastically reduced (with concentrations $<3 \log_{10}$ cfu/g), in trials B and C, and remained below the detection limit from brining and during the entire ripening. The levels of mesophilic and thermophilic coccus and rod LAB of curds were comparable among the 3 trials and reached values above 6 $\log_{10}$ cfu/g in cheeses at 90 d of ripening. This study showed that stretched cheese making technology was effective in limiting the growth of the 4 selected dairy pathogens, but the best temperature and time combination to be applied during stretching is 83.6°C for 20 min.

Key Words: raw-milk cheeses, stretched temperature, pathogen inoculation

1103M Characterization of a novel gene cluster encoding multiple bacteriocins in the dairy starter culture *Streptococcus thermophilus.*

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Streptococcus thermophilus is a yogurt and cheese starter culture capable of producing antimicrobial peptides called bacteriocins. Applications for these antimicrobials have not been explored as the molecular mechanisms regulating their production and bactericidal activity remain largely undefined. In this study, comparative genomics and a bacteriocin prediction program were used to identify and characterize a genomic region encoding multiple small peptides in *S. thermophilus* ST134 as a candidate for its bactericidal activity. Deletion of this region resulted in a complete loss of ST134 antimicrobial activity. Additional deletion mutants identified an essential 2-component regulatory system and bacteriocin transporter; and suggested ST134 produces 2 bacteriocins encoded within 3- and 4-gene operons respectively. Deletion mutants were screened for bacteriocin activity against 31 *S. thermophilus* strains, which identified 4 strains resistant to both bacteriocins, and an additional 4 strains resistant to the bacteriocin encoded by the 4-gene operon. Strains naturally resistant to the bacteriocin encoded by the 3-gene operon were not detected. Complementation assays of individual genes and gene combinations into a heterologous *S. thermophilus* (ST109) host revealed that the 3-gene operon encodes for a 2-peptide class II bacteriocin. Additional studies are needed to characterize the bacteriocin encoded by the 4-gene operon and to identify the bacteriocin target molecule on susceptible strains. Our long-term goal is to understand the specificity between the bacteriocin and its target, generate allelic variants with diverse specificity, and explore their utility for both strain-specific and broad activity antimicrobials.

Key Words: *Streptococcus thermophilus*, bacteriocin

1104M A previously undescribed lactococcal CEP is proposed to be a new CEP type designated as group i.

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Cheese flavor is influenced by the bacteria added during cheese production. A bitter taste in Cheddar cheese can arise during aging and has been linked to lactococcal starter lactic acid bacteria (SLAB) cellular envelope proteinases (CEPs) and their ability to generate bitter peptides. Genomic sequence analysis is used to screen potential starter cultures for CEP types associated with bitterness. The DNA from 18 single strain lactococcal SLAB was isolated, sequenced, assembled, and annotated into draft genomes. The genomes were then analyzed using BLAST to compare the CEP genes with established bitter and non-bitter producing CEP amino acid sequences. Based on the amino acid sequences in the active and binding sites, these strains grouped into 7 individual CEP classifications. Seven strains were identified as having non-bitter group d type CEPs, 2 were identified as non-bitter group b, 1 as bitter group e, 1 as bitter group g, 1 as group c, 1 without a CEP gene, and 5 as having a novel CEP not previously described. We propose a new CEP group i for this CEP. Like group h, group i differs from all the other CEP classifications due to the presence of a glycine residue at position 166 in the substrate binding region. Group i differs from group h at position 177 where it contains a leucine residue whereas group h has isoleucine. Given that group i is closely related to group h it is likely that it will behave similarly and produce bitterness in Cheddar cheese. The isoleucine to leucine substitution should not influence the functionality of the binding site since both are hydrophobic aliphatic amino acids. However, we cannot say definitively if this is the case. Because isoleucine is branched at the β-carbon and leucine is not, they influence protein structure differently, with isoleucine preferring β-sheet structure and leucine preferring α-helix structure. The structure of the proposed group i protein at position 177 is alpha-helical (UniprotKB). Thus, if the isoleucine does change the structure of the binding site to a β sheet, it could influence CEP functionality or binding specificity.

Key Words: Cheddar cheese, bitterness, lactococci

1105M Synergistic antimicrobial activity of bovine lactoferrin and cannabidiol (CBD) isolate from hemp (*Cannabis sativa*) against foodborne pathogens.

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Foodborne illness gives rise to numerous health problems every year worldwide. The aim of this study was to determine the antimicrobial activity of bovine lactoferrin in combination with cannabidiol (CBD) isolate from hemp (*Cannabis sativa*) against *Escherichia coli* and *Staphylococcus aureus* in trypticase soy broth. A broth microdilution assay using optical density to monitor bacterial growth was used to determine the synergistic antimicrobial activity of bovine lactoferrin and CBD isolate against the 2 foodborne pathogens. Effective combinations of these antimicrobial substances were further evaluated for their bactericidal activity by monitoring growth over time using the pour plate method. The data of time kill assays were evaluated by ANOVA and the means were separated using the Tukey's test to determine significant differences ($P < 0.05$). The results indicated a difference ($P < 0.05$) in the growth of these bacteria. Bovine lactoferrin at a concentration of 500 µg/mL was significantly ($P < 0.05$) inhibitory to 3 strains of *Staphylococcus aureus.* When bovine lactoferrin (500 µg/mL) was combined with 264 µg/mL of CBD isolate, the minimum inhibitory concentration (MIC) for the 3 strains of *Staphylococcus aureus* decreased by 3-folds. Synergistic bactericidal activity of bovine lactoferrin and CBD isolate caused significant ($P < 0.05$) inhibition of the 2 strains of *Escherichia coli.* These results indicated that the use of bovine lactoferrin in combination with CBD isolate from hemp may preserve food and beverage products against *Escherichia coli* and *Staphylococcus aureus* pathogens.

Key Words: bovine lactoferrin, CBD isolate, foodborne pathogen

1106M Biofilm formation capability of *Listeria monocytogenes*' food-associated isolates.

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The capability of *Listeria monocytogenes* to form biofilm is considered to be one of the major factors contributing to the organism's persistence in the food processing environment (FPE). This study aimed to examine the genetic underpinning of biofilm-forming capacities in *L. monocytogenes* isolated from foods and food processing environments. A collection of 150 *L. monocytogenes* strains isolated from a range of food products, food-processing environments, and clinical sources were available from the Teagasc Culture Collection. Each strain was screened for the ability to form biofilm using the crystal violet method in microliter plates with incubation at 37°C. The association of biofilm formation with phylogenic lineage, clonal complex (CC) and serogroup at pangenome level were also investigated using whole-genome sequences (WGS). Of the 150 strains evaluated, 16.67% exhibited strong biofilm formation, i.e., one standard deviation above the mean, in particular, ANOVA with respective groups revealed that isolates sources from seafood, serogroup 1/2a, 1/2b-3b-7 and CC101 ($P < 0.05$). Pangenome-wide association analysis identified 524 candidate genes that are associated with strong biofilm formation, many of which (78.05%) were of unknown function (hypothetical). Comparative analysis of the genome sequences of the isolates for a complement of genes previously shown to have a role in biofilm formation (i.e., *actA*, *lmo0435*, *lmo0673*, *luxS*, *inlL*, *lmo2504*, *prfA* and *recO*) revealed that all 150 of the isolates carried *actA* and *recO*. However, the presence of the remaining genes, namely *lmo0435*, *lmo0673*, *luxS*, *inlL*, *lmo2504* and *prfA*, were found to be not statistically significant in the ability to form biofilm. The intricate mechanisms behind biofilm formation will be better understood with further research on the genes highlighted by pangenome analysis that have unknown functions.

Key Words: *Listeria monocytogenes*, biofilm, comparative genomic

1107M Link between virulence, biofilm and antimicrobial-resistance genes and specific clonal complex types of *Listeria monocytogenes*.

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Precise classification of foodborne pathogen *Listeria monocytogenes* is a necessity in efficient foodborne disease surveillance, outbreak detection, and source tracking throughout the food chain. The aim was to perform comparative genomic analysis of *L. monocytogenes* isolates to determine specific genetic markers associated with presence of gene previously shown to have a role in virulence, stress tolerance, biofilm formation and antimicrobial resistance. A total of 150 *L. monocytogenes* isolates whole-genome sequences (WGS) from various food products, food processing environments and clinical sources were investigated for variation in presence of specific gene and their possible link to genetic markers, namely clonal complexes (CC), serogroups as well as phylogenetic relationships. Pangenome-wide association analysis by Scoary using Fisher's exact test identified 11 genes specifically associated with clinical isolates. Screening for the presence of antimicrobial and virulence genes using the ABRicate tool uncovered variation in presence of *Listeria* pathogenicity islands (LIPI) and other known virulence genes. Specifically, the distribution of *actA*, *ecbA*, *inlF*, *inlJ*, *lapB*, *LIPI-3* and *vip* genes across isolates were found to be significantly CC dependent while the presence of *ami*, *inlF*, *inlJ*, and *LIPI-3* was associated with clinical isolates specifically. In addition, Roary-derived phylogenetic grouping based on antimicrobial-resistance genes (AMRs) revealed that thiol transferase (*FosX*) genes were present in all of lineage I isolates, presence of lincomycin resistance ABC-F type ribosomal protection protein (lmo0919_fam) were also genetic lineage dependent. More importantly, the genes found to be specific to CC-type were consistent when a validation analysis was performed with fully assembled, high-quality complete *L. monocytogenes* genome sequences (n = 247) extracted from the National Center for Biotechnology Information (NCBI) microbial genomes database. This work highlights the usefulness of MLST-based CC typing using WGS as a tool in classifying isolates.

Key Words: *Listeria monocytogenes*, pangenome-wide association

1108M Growth behavior of *Listeria monocytogenes* in a semi-soft, rind-ripened artisanal cheese at cold chain and abuse temperatures.

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Predictive growth modeling data has largely been derived from deliberately adjusted liquid culture medium to mimic a true food matrix. However, there are undeniable disparities between predictions made in a laboratory broth model and those made in a real food matrix. In addition, there is a need for effective predictive models that simplify risk assessment. The aim was to examine the growth behavior of *Listeria monocytogenes* on a semi-soft, rind-ripened cheese made from pasteurized cow's milk at a range of cold chain temperatures and to develop a dynamic predictive model in accessing temperature abuse scenario

for this product type. The study was conducted in accordance with EU Reference Laboratory (EURL) guidelines for undertaking challenge studies to determine the growth of *L. monocytogenes*. The entire retail size (225 g) of the semi-soft, rind-ripened cheeses were separately challenged with 3 strains of *L. monocytogenes* and their maximum specific growth rate (μ_{max}) were determined at refrigeration (3.9°C) and abuse conditions (12.5°C) respectively over the shelf-life (30 d). The strains were chosen to be representative of ongoing challenge studies of *L. monocytogenes* and dairy isolates namely *Lm*954 (Teagasc, Moorepark, Co. Cork, Ireland), 12MOB079LM (EURL) and F2365 (Jalisco cheese outbreak of 1985 in California). All 3 strains grew at both temperatures during shelf life, and the maximum number of 5.44 and 7.48 Log_{10} cfu/g for 3.9°C and 12.5°C respectively and strain variation in μ_{max} and Lag phases were observed. ANOVA tests on μ_{max} at both temperatures among tested strains revealed that CC14, 12MOB079LM strain grew significantly ($P \leq 0.05$) better than other tested strains. An additional dynamic modeling strategy, with temperature variations, is now being considered to assess the risk in real-life scenarios of temperature abuse effect. This study further highlights the ability of *L. monocytogenes* to proliferate in this product type during the downstream food chain at a realistic temperature and will help in assessing the risks of listeriosis.

Key Words: *Listeria monocytogenes*, challenge study, dynamic modeling

1109M A rapid stress test identifies post-pasteurization contamination in white and flavored fluid milk.

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Post-pasteurization contamination (PPC) in HTST fluid milk is typically caused by psychrotolerant gram-negative bacteria, such as *Pseudomonas*, and accounts for approximately 50% of fluid milk spoilage in the US. The rapid growth of gram-negative bacteria at refrigeration temperatures leads to high bacterial concentrations that often leads to development of sensory defects as the result of enzyme production. Providing the dairy industry with a method to rapidly detect PPC to eliminate the source of contamination and limit the negative impacts of premature spoilage is essential to improving fluid milk quality and consumer acceptance. To that end, we evaluated white and flavored commercially pasteurized milk samples collected during 2021 and 2022 (n = 777) for PPC using a total gram-negative test (Crystal Violet Tetrazolium Agar; CVTA) on d 14 of refrigerated shelf-life and a rapid stress test that consisted of incubating 100 mL of freshly pasteurized milk (i.e., 1–3 d after pasteurization) at 21°C for 18 h before plating on CVTA. When compared with the results from the d-14 CVTA count, the rapid stress test had an overall sensitivity of 71.9% and specificity of 92.6% which aligns with a pilot study that evaluated this rapid stress test on ~100 commercial milk samples. The white milk samples (n = 514) had a lower sensitivity of 68.9% compared with the flavored milk samples (n = 263) which had a sensitivity of 78.8%, but specificities for both milk types were similar at 92.5% and 92.8% for white and flavored samples respectively. The sensitivity of the rapid stress test is likely driven by the relatively small sample volume used for incubation and low initial contamination levels in the product. Compared with the d-14 CVTA count, the rapid stress test evaluated here offers processors a much faster method of PPC detection. Future studies should focus on evaluating the rapid test with larger volumes of milk (i.e., a half-gallon container) which would be more comparable to milk held by consumers throughout shelf-life and would likely improve the test sensitivity.

Key Words: fluid milk spoilage, post-pasteurization contamination

1110M Optimization of fermentation parameters to valorize byproducts from the dairy and poultry industries.

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The present work focused on 2 food waste products: acid whey and mechanically separated poultry (MSP). These by-products were combined using an innovative fermentation process to valorize them for application as food and feed ingredients. According to USDA, more than 775 million chickens were slaughtered first half of 2022. Significant waste, like MSP, is generated during slaughter and disposed of due to the lack of sustainable technologies for valorization. Acid whey is considered a waste from dairy industry due to its high acidity and mineral content. Our main objective was to define the parameters for optimal fermentation of these byproducts, and the time to control protein hydrolysis. The results of our experiments provided information on the hydrolysis yields as well as the characteristics and distribution of the peptides at different points in the fermentation. The MSP and acid whey were obtained from local companies. The blend used had 47.5% of acid whey, 50% poultry wastes and 2.5% wt/wt crude molasses. Inoculation with *Lacticaseibacillus rhamnosus* (OSU-PECh-69), a proteolytic strain, was performed. The fermentation was carried out using a laboratory scale of 1 L in a microaerophilic environment at 37°C for 10 d. The pH value, proteolytic activity, and bacterial counts were measured. Also, the soluble protein content and molecular weight distribution were determined. The collected data were fitted to a completely randomized design with 4 treatments and 3 replicates. ANOVA and mean differences were evaluated using the Tukey HSD test ($P < 0.05$). The initial pH of the mix of acid whey and MSP was 5.27 ± 0.02. All the treatments showed a significant ($P < 0.05$) drop in pH by d 2 3.70 ± 0.01 compared with the initial pH and remained constant until the last day of fermentation. The soluble protein indicated a significant ($P < 0.05$) decrease, while the concentration of the peptides increased significantly ($P < 0.05$). The adoption of next-generation biotechnologies offers possibilities to transform wastes into high-value products. To address the growing need for functional ingredients, some proteins can be recycled and converted into hydrolysates. These can be utilized in the food industry as bioactive peptides, emulsifiers, nutrients, flavor and water-bonding enhancers, while causing less environmental harm.

Key Words: acid whey, MSP, fermentation

1111M Effect of the microbial population in the milking system and farm practices on the culturable bacteria of raw milk.

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The objective of this study was to correlate raw milk quality on farm with the microbial contamination of tap water and the milking system. Approximately 120 cows were kept in tie stalls with sand bedding without access to pasture and milked by the herringbone parlor system. No tail docking was performed. Milking practices were documented, including wearing gloves, pre-dipping, post-dipping and teat drying as well as the daily change of the filter sock. After cleaning, 13 wet cotton swab samples taken along the milking system (2.5 cm × 10 cm on flat surfaces and 5 turns in pipe openings), 2 L of tap water and 25 mL of raw milk were collected twice a month from June to November in 2022 for microbial analysis. Swabs (n = 153), tap water (n = 12) and raw milk samples (n = 12) were plated and incubated on lactose-M17 agar at 30°C (48 h) for mesophilic lactose-utilizing bacteria (MLU) and on MRS-Vancomycin agar at 30°C (72 h) for anaerobic lactobacilli. Average counts of tap water were lower than 7.6×10^2 cfu/L on both media. Among the 15 sampling locations, the highest MLU average counts were detected in the proximal milk hose (7.8×10^5 cfu/swab) and distal milk hose (8.8×10^5 cfu/swab). Though the average MLU count of raw milk remained low (1.2×10^3 cfu/mL), monthly MLU counts followed the same trend as most of the swabs: low in June and July, increasing in August and September, and decreasing in October and November. Low levels of anaerobic lactobacilli were found in raw milk while sporadic high counts were detected in the claw, proximal milk hose and the proximal section of milk pipeline. Results showed that contamination in the first half of milking system in early summer tended to spread to the second half of the milking system in late summer and fall, even when milk quality was maintained through good milking practices. Regular replacement of milk hoses would help to avoid microbial accumulation in the milk pipelines which would require more intensive sanitization procedures for avoiding potential biofilm development that could end up in raw milk and impair product quality.

Key Words: raw milk, farm milking system, biofilm

1112M Incremental reductions in bulk tank spore levels are achieved through on-farm interventions. R. L. Evanowski*, S. I. Murphy, N. H. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Bacterial spores can enter the fluid milk system at the farm level, survive HTST pasteurization, and cause fluid milk spoilage late in shelf-life. Spoilage due to sporeforming bacteria is observed in about 50% of HTST fluid milk and is one of the biggest hurdles to extending its shelf-life. We conducted an intervention study to determine the impact of (i) a training intervention (i.e., a one-time classroom style training for milking parlor employees as well as infographics that outlined the training posted in common areas near the parlor), and (ii) a towel intervention (i.e., washing towels with chlorine bleach and drying) on bulk tank raw milk spore counts and shelf-life of pasteurized product manufactured from this raw milk. Four New York State dairy farms were enrolled in the study; 2 farms were randomly assigned the towel intervention and 2 farms were randomly assigned the training intervention. Study design included collecting bulk tank raw milk samples once each week for 3 wk for baseline data followed by 4 wk of sample collection after the interventions were implemented ($n = 28$). Spore tests were performed on each sample, and samples were also pasteurized for further shelf-life analysis. The mean psychrotolerant, mesophilic, and thermophilic spore counts before the application of interventions were 0.2, 1.6, and 1.6 cfu/mL respectively and 0.03, 0.8, and 0.9 cfu/mL respectively, after the interventions were applied. However, only mesophilic spore counts were significantly reduced after the interventions were initiated and a reduction of 48.8% (0.79 cfu/mL) was observed using a mixed effects linear regression model. There was no significant difference between the type of intervention applied (i.e., towel intervention or training intervention). Furthermore, no significant difference in microbial growth was observed throughout shelf life of fluid milk after either intervention was implemented. Future studies should verify that these interventions reduce spores in raw milk on dairy farms with different management practices (e.g., pasture based, larger farms, etc.) and should verify the impact of combined interventions on product shelf life.

Key Words: sporeforming bacteria, dairy farm, milk quality

1113M Valorization of underutilized dairy waste residues: Production of lactic acid through microbial fermentation. C. R. Surana*[1,2,3], E. Byrne[1], M. Callanan[2,3], and O. McAuliffe[1,3], *[1]Department of Food Biosciences, Teagasc Food Research Centre, Fermoy, Co.Cork, Ireland, [2]Department of Biological Sciences, Munster Technological University, Cork, Co.Cork, Ireland, [3]VistaMilk SFI Research Centre, Teagasc Agricultural Food Research Center, Fermoy, Co.Cork, Ireland.*

Annually, approximately 190 million tonnes of liquid waste or co-products is generated by the dairy sector in the EU. These waste streams are nutrient-rich and currently underutilized which gives scope to transform them through microbial fermentation to produce economically valuable products and reduce their negative environmental impact. Such an approach can contribute to the circular bioeconomy by making food production systems more sustainable by reducing the burden of these waste streams on the environment. In this study, whey from acid casein hydrolysis (acid whey) and salty whey from Cheddar cheese manufacture were evaluated as feedstocks for production of lactic acid. Lactic acid has a variety of uses in the food industry such as flavoring, curing and preservation and synthetic production of polylactic acid. A total of 466 lactic acid bacteria strains were screened on differential agar for homofermentative production of lactate, which was observed in 263 strains. Quantification via HPLC revealed a lactate yield of >18 g/L for 40 of these strains in MRS, with the highest yield (26.48 g/L) observed for *Lacticaseibacillus paracasei* DPC6583. Eleven high-lactate producing strains grew well ($OD_{600} > 1$) in salty whey but lactate yields were substantially lower in salty whey (7.29 g/L) compared with MRS (24.44 g/L) in a controlled batch fermentation. Preliminary evaluation of acid whey suggests it is better substrate for growth than salty whey. Higher lactate yield was also observed for some strains, such as *L. paracasei* DPC 2021, with a yield of 9.25 g/L lactate in an uncontrolled batch fermentation. An LC-MS analysis of acid whey and salty whey fermentates has been conducted and may reveal other potential high-value end products. Harnessing the power of microorganisms is an approach that shows great potential for biotransformation of waste streams and co-products.

Key Words: biotransformation, dairy waste-stream, lactate

1114M *Saccharomyces cerevisiae* and *Lachancea thermotolerans* for milk acidification: Effects of yeast strain and glucose. P. Gamboa-Moreno*, T. DeMarsh, and S. Alcaine, *Cornell University, Ithaca, NY.*

Representing important staples of the dairy industry, cultured milk products are mostly fermented with lactic acid bacteria (LAB). Lactic acid bacteria are susceptible to bacteriophage contaminants, which can inhibit fermentation, contributing to food waste and economic loss. This study evaluates the effects on milk acidification of 5 yeast strains, alone and in combination with glucose addition, attempting to provide a way of developing LAB-free cultured dairy products. Due to their capability to synthesize lactic acid, 4 strains of *Lachancea thermotolerans* and a commercial bioengineered strain of *Saccharomyces cerevisiae* were tested. Two bottles with 200 mL of UHT milk were prepared for each strain. Both were treated with lactase (120 acid lactase units per gram of lactose); one received supplemental glucose (50 g/L), and the other did not. All samples were inoculated with 10^8 cfu/mL of yeast and were incubated at 30°C for 24 h. Plate counts were executed at hours 0 and 24 using potato dextrose agar with chloramphenicol (25 mg/L); pH was measured every 15 min; and final titratable acidity (TTA) was assessed. Cell counts for each strain were compared at the beginning and the end of the fermentation, using a *t*-test. Glucose's effect was analyzed with a *t*-test for each strain, comparing final pH and TTA between the treatments with and without glucose addition. Strains' effect was analyzed with an ANOVA and Tukey's HSD for final pH and TTA of all treatments. All tests used a 0.05 significance level. Only 1 of the 10 treatments displayed significant differences in cell counts before and after fermentation ($P <$

0.05), showing a 1 log reduction. Glucose addition resulted in a lower final pH and a higher TTA for each strain ($P < 0.05$). *Saccharomyces cerevisiae* strain's fermentates (with or without glucose) had the lowest final pH values and highest TTA. Two strains of *L. thermotolerans* also reduced the pH below the isoelectric point of caseins (pH 4.6) when supplemented with glucose. These results confirm that some of the tested yeast strains effectively acidify milk, a starting point for the further development of novel LAB-free fermented dairy products.

Key Words: fermented dairy, yeast

1115M Machine learning models suggest farm management practices and weather conditions only account for a small proportion of variance in spore levels of organic raw milk.

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High level of mesophilic and thermophilic spores in raw milk can lead to quality issues of dairy powders. As these sporeformers can originate from farm environments, it is important to understand contributions of farm-level factors to spore levels in raw milk. The objective of this study is to investigate the impact of farm management practices and weather conditions on levels of different sporeformers in raw milk by leveraging machine learning models. In this study, organic raw milk from US dairy farms (n = 102) located in 11 states were tested for mesophilic spore count (MSC) and thermophilic spore count (TSC). Milk was heat treated (HT) at 80°C/12 min, then plated on standard methods agar (SMA) and incubated at 32°C and 55°C for MSC and TSC, respectively. At each sampling date, a survey was collected from each farm to obtain structured data about farm management practices. Weather conditions related to temperature, precipitation, solar radiation, and wind were obtained on the same date from an open-source website. A random forest model with 1,000 trees was trained to predict logMSC and logTSC in each raw milk sample. Near-zero variance filter was used to remove imbalanced variables. The regression performance of random forest showed that only 21% and 23% variance (indicated by the R^2 for the cross-validated training data) of logMSC and logTSC, respectively, can be explained by the selected variables, suggesting that a large proportion of variance was unexplained. The random forest model consistently ranked that the farm-size-related variables (e.g., part-time employee number) and temperature-related variables among the top predictors for both logMSC and logTSC. The outcome of this study suggests that while weather conditions can account for some variance in different spore levels in raw milk, the effects of farm management practices can be potentially masked by the farm size. More objective evaluation of farm practices and additional geographical features at farm-level might be needed to allow more accurate prediction of spore levels in organic raw milk.

Key Words: sporeformer, raw milk, farm management

1116M Genomic insight into antibiotic-resistant *Bacillus paralicheniformis* LL32 with blood hemolytic phenotype.

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Bacillus paralicheniformis strains, as a close relative of *Bacillus licheniformis*, are proposed as commercial microorganisms in feed applications and biocontrol agents due to their biofilm production, antimicrobial and immunoregulatory activities. However, the safety of each strain must be evaluated as their genomes may encode virulence features and antimicrobial resistance. Virulence features and acquired antimicrobial genes among *Bacillus* strains may be transmitted to other bacteria, including pathogens. In this study, phenotypic and genomic approaches were used to assess the safety of *B. paralicheniformis* strain LL32, which can form biofilm, produce biosurfactant, and has antimicrobial activity against *Streptococcus uberis*, an environmental organism commonly found in animal bedding. The phenotypic evaluation indicated blood hemolytic activity and minimal inhibitory concentration (MIC) of >128 and 32 for erythromycin and clindamycin, respectively, indicating the possibility of acquired resistance in strain LL32, according to the European Food Safety Authority (EFSA) guideline. However, only the intrinsic chromosomal *ermD* gene linked to erythromycin resistance was detected and no genes associated with acquired clindamycin resistance were found within the genome. Moreover, the genome encodes the *licA*, *licB*, *licC*, and *licTE* genes of the lichenysin operon linked to biosurfactant production and to hemolytic activity. Therefore, we determined that clindamycin resistance is probably not acquired, and the high MIC value of clindamycin may possibly be due to the intact spermidine acetyltransferase gene (*speG*), which neutralizes toxic compounds by acetylation. The genome of strain LL32 encodes *fenC* and *fenD* from the fengycin operon, supporting the classification with the species *B. paralicheniformis*. Finally, we conclude that due to the chromosomal location of *ermD*, lack of plasmid replication and transposon sequences within the genome, the transferability of *ermD* to other bacteria is unlikely to occur. Therefore, the biosurfactant producer *B. paralicheniformis* LL32 could be considered safe for further evaluation in industrial applications such as facilitating curd formation, enhancing cheese texture, and controlling the growth of pathogens and spoilage microorganisms.

Key Words: *Bacillus paralicheniformis*, food safety, antimicrobial resistance

1117M Molecular characterization of the toxigenic potential and antimicrobial resistance of *Staphylococcus aureus* isolates from Minas frescal cheese produced in Tocantins, Brazil.

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Minas frescal cheese (MFC) is a fresh, high-moisture dairy product and one of the main types produced in Brazil. During production, the cheese is heavily handled, which increases the chance of contamination with coagulase-positive *Staphylococcus* (CPS). This study analyzed *Staphylococcus aureus* contamination and characterized its toxigenic potential and antimicrobial resistance in not inspected MFC samples collected from fairs in Araguaína, Tocantins, Brazil. *Staphylococcus aureus* was isolated according to ISO 6888-1:1999. Isolates were confirmed by Gram staining and biochemical tests. *Staphylococcus aureus* isolates were subjected to PCR to detect exfoliative toxins A and B (*eta* and *etb*), toxic shock syndrome toxin (*tst*), and staphylococcal toxins A, B, C, D, and E (*seA* until *seE*). Susceptibility of toxigenic isolates to tetracycline, erythromycin, clindamycin, gentamicin, ciprofloxacin, sulfamethoxazole, trimethoprim, streptomycin, and enrofloxacin was determined according to Clinical and Laboratory Standards Institute guidelines. Overall, 355 CPS strains were isolated from 21 MFC samples; 177 (49.86%) were identified as *S. aureus*, which originated from 16 (76.2%) samples. Of these isolates, 54 (30.5%) contained toxin-related genes; *tst* (48.1%) and *seA* (33.3%) were the most common and *seD* was not detected. Eleven samples (52.4%) had at least one isolate with toxigenic potential. Thirteen (24%) of the 54 toxigenic isolates showed resistance to at least one class of antibiotics, and 11 (20.4%) were multidrug resistant (i.e., resistant to more than one class of antibiotics). Clindamycin resistance was most common (8/54; 14.8%), followed by tetracycline resistance (5/54; 9.25%). These findings show that informally produced MFC poses a risk and should be strictly regulated to prevent foodborne illness, when the product offers conditions for the multiplication and production of toxins by *S. aureus*.

Key Words: antibiotics, fresh cheese, staphylococcal toxins.

1118M Effect of refrigerated storage on the presence of *Listeria monocytogenes* in Brazilian mozzarella cheese.

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Dairy products must be monitored for *Listeria monocytogenes* contamination, which must be controlled by quality management. *Listeria monocytogenes* is a biofilm-producing psychrotrophic pathogen that poses a significant contamination risk in foods, particularly refrigerated. Refrigerated storage of mozzarella cheese (for up to 6 mo) may increase the growth of psychrotrophic microorganisms posing a potential health risk, particularly because Brazilians consume mozzarella raw. This study evaluated mozzarella for the presence of *L. monocytogenes* both immediately after production and after 120 d of storage at 5°C. In December 2021, 2 samples from 5 batches of mozzarella were collected from a dairy factory in Tocantins State immediately after packaging. The samples were transported to the Food Microbiology Laboratory of the Universidade Federal do Norte do Tocantins. One sample from each batch was immediately evaluated, while the other was stored at 5°C for 120 d (the stated product shelf life). Sample aliquots were collected aseptically, placed in sterile plastic bags, and homogenized in Half-Fraser broth. After pre-enrichment (35°C, 24 h), selective enrichment and differential growth were performed according to Brazilian standard methods. Isolates on Oxford agar with typical characteristics were selected and purified. Genomic DNA was extracted and subjected to PCR to detect the *L. monocytogenes*-specific gene *lmo0733* along with positive and negative controls. On d 1 post-production, 30 isolates suggestive of *L. monocytogenes* were recovered from 2 samples; none were confirmed by PCR. After refrigerated storage, 62 isolates suggestive of *L. monocytogenes* were obtained from all batches, and 8 (12.9%) were confirmed by PCR. All 8 isolates were from the same batch or sample (20%). This indicates that the occurrence of *L. monocytogenes* in mozzarella produced in the tropical region of Brazil may increase during refrigerated storage, indicating the need for better quality control to identify the source and control contamination and regulatory guidelines to provide safer food for consumption.

Key Words: biofilms, food safety, psychrotrophic

1119M Upcycling whey permeate into an acetic-acid beverage with *Brettanomyces claussenii*, a preliminary study.

D. G. Hauser*, V. K. Rivera Flores, and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Dairy byproducts such as whey permeate (WP) are an environmental and economic burden for the industry and are produced in large quantities annually. *Brettanomyces claussenii* is a food safe yeast that can utilize lactose, and alternately produce ethanol or acetic acid (AcOH) depending on environmental conditions, namely the presence of oxygen. Prior work in our laboratory group has shown that the addition of exogenous β-galactosidase to whey substrates results in *B. claussenii* preferentially utilizing glucose over galactose, a low-glycemic-index sugar, facilitating the production of an alcohol-free AcOH beverage enriched in galactose. Therefore, the following experiment was undertaken as a preliminary step to understand the impact of time and agitation on fermentation outcomes, toward optimizing the production of AcOH and the retention of galactose during aerobic fermentation of WP by *B. claussenii*. Whey permeate powder was reconstituted at 20% solids (168 g/L lactose) and treated with a commercial β-galactosidase at 40 IU/g lactose. Duplicate 500-mL fermentations were carried out using 10^5 cells/mL and incubated at 30°C with agitation at 50 or 200 RPM for 32 d. Galactose was measured enzymatically every 4 d, and glucose, lactose, and AcOH were measured enzymatically at the beginning and end of the experiment. Ethanol was measured via GC-FID at the beginning and end of the experiment. Glucose was completely depleted in the 50-RPM treatments but remained in low concentrations in the 200-RPM treatments. In all treatments, galactose was retained and increased in concentration over time, likely due to evaporation, ranging from 105.3 to 116.6 g/L at the end of the fermentation. We found that AcOH was produced in all treatments but was higher at 200 RPM, although this difference was not statistically significant (14.5 ± 0.6 versus 7.1 ± 3.1 g/L, $P = 0.08$ via 2-tailed *t*-test). Ethanol content reached 1.6 ± 0.7 and 0.5 ± 0.3% ABV in the 50- and 200-RPM treatments, respectively. These results demonstrate the impact of agitation on fermentation outcome and serve as an initial step in optimizing the production of a novel acetic-acid beverage from WP.

Key Words: upcycling, whey permeate, fermentation

1120M Survey of sodium gluconate content in retail Cheddar cheese.

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Addition of sodium gluconate as a processing aid to Cheddar cheese curd has been used as a means of preventing calcium lactate crystal formation in aged cheese. Gluconate is metabolized by *Paucilactobacillus wasatchensis* to ribulose-5-phosphate by 6-phosphogluconate dehydrogenase with liberation of carbon dioxide. The presence of gluconate in cheese has now been added to the list of confirmed risk factors for unwanted gas production by *Pa. wasatchensis* or similar NSLAB. In prior research, gluconate was only found in cheese when it had been added as a processing aid. The objective of this study was to survey commercial cheeses to determine the use of sodium gluconate as a processing aid. Fifty-three retail packs of cheese were purchased from stores in Utah (USA) as either blocks, shredded cheese, sliced cheeses and as cracker-sized cuts and sticks, and were labeled as either mild, medium or sharp. Cheeses samples were measured for gluconate content using the enzyme analysis kit K-GATE (Megazyme Inc.) with all samples tested in duplicate. A level of 0.02% (wt/wt) or less measured by this assay was considered to be negligible. For the 14 cheeses labeled as mild, 4 cheeses had gluconate levels ranging from 0.14 to 0.45% (wt/wt). Three cheeses were shredded and the other was a sliced cheese. Of the 16 cheeses labeled as medium that were analyzed, one shredded cheese contained 0.36% sodium gluconate, 2 cheeses contained low gluconate levels (0.07 and 0.09%), while the other 13 cheeses were below 0.02%. Of 16 cheeses labeled as sharp, 2 cheeses show evidence of added sodium gluconate and contained 0.10 to 0.11% gluconate. In summary, 7 of the 53 cheeses tested contained gluconate at levels (≥0.1% wt/wt) that imply the sodium gluconate was being added to curd during salting as a way to prevent or delay formation of calcium lactate crystals. These cheeses were mainly converted into shreds and were made by 3 out of 14 companies listed as the manufacturer or distributer on the cheese package. This level of residual gluconate is a risk factor for gas production in these cheeses by *Pa. wasatchensis* or similar NSLAB.

Key Words: Cheddar cheese, gas production, sodium gluconate

1121M Bacterial contamination of soft Wagashi cheese. M. Muntari*[1,2], P. K. Karikari[1], and J. S. Stevenson[3], *[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Texas A&M University, College Station, TX, [3]Kansas State University, Manhattan, KS.*

Although milk is a healthy human nutritional component, it can present a favorable environment that promotes growth and development of microorganisms. In most parts of Ghana as in other countries in West Africa, raw rather than processed milk is the major source of fluid milk consumption in most households. Wagashi is a locally adopted name used to refer to a traditionally processed cheese in the West African region. Wagashi is also an important economic commodity in the West African region that serves as an inexpensive source of animal protein in most households. The rate at which this cheese variety is consumed and the way it is produced in Ghana prompted this study to determine the *Escherichia coli* bacterial concentration in Wagashi samples and possible sources of that contamination in milk at the farm milk to the Wagashi production stage. Data were collected by survey questionnaire from both milk and Wagashi producers, laboratory analyses and visual observation of the market environment in which the samples were sold. A total of 15 samples of Wagashi were randomly collected from 3 different suburbs in the Ashanti region of Ghana. To learn more about potential causes of Wagashi contamination, 20 questionnaires were given out to milk and Wagashi producers (milk producers, n = 10; Wagashi producers, n = 10). Laboratory analyses were carried out on the cheese samples to determine the pH, water holding capacity, total viable count, and the concentration of *E. coli*. We used SPSS (IBM Corp., v. 20) to analyze data using descriptive statistics such as frequencies, percentages, and mean. *Escherichia coli* was found in 40% of the total Wagashi samples. Bacterial counts were also high (mean count of 10^8 cfu/mL) in all samples. No significant differences ($P > 0.05$) were detected in the pH, water holding capacity, and total microbial counts in the Wagashi samples among the 3 suburbs. The results of the survey indicated that 80% of the milk producers are uneducated about the relevance of sanitizing the udder and teats of the cow before and after milking. Some of the milk producers add water to milk to increase the volume. Some of the Wagashi producers (20%) indicated they do not wash the plant extract they use as coagulants for the preparation of Wagashi. Some of these unhygienic practices by milk and Wagashi producers may have been the possible sources of bacterial contamination in the milk product and Wagashi products. It was concluded that Wagashi produced in the Ashanti region of Ghana may be a high-risk food, given the high bacterial content and the presence of *E. coli* found in the samples.

Key Words: Wagashi, *E. coli*, contamination

1122M Effects of ultrafiltration followed by heat or high-pressure treatment on camel and bovine milk cheeses. M. Mbye*, M. Ayyash, and A. Kamal-Eldin, *United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates.*

The combined treatment of ultrafiltration (UF) and high-pressure processing (HPP) could enhance the coagulation properties of camel milk cheese and improve its chemical and physical properties. Therefore, the aim of this study was to assess the effects of ultrafiltration (UF) combined with high-pressure processing (HPP) or heat treatment on the quality of soft cheese produced from camel milk (CM) in comparison to bovine milk (BM). Milk was concentrated by UF (2- and 4-fold) before treatment with HPP at 350 MPa or 550 MPa for 5 min at 4°C or by pasteurization at 65°C for 30 min or at 75°C for 30 s. The cheese was produced using starter cultures (Direct Vat Set cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 1:1) to reduce pH before camel chymosin was added. The yield, composition, texture profile, rheological properties, and o-phthalaldehyde (OPA) assay were determined, one-way ANOVA was used to test the significance ($P \leq 0.05$), and the Tukey test was used to compare the mean values between groups. The lowest pH value (5.1) was observed in camel cheese 2-fold UF treated at 75°C for 30 s, which was significantly different from the other treatments, and the highest pH value (5.5) was reported in bovine milk cheese treatment with 4-fold UF combined with HPP at 550 MPa. The highest yield of BM cheese (26%) was observed under the treatment with 4-fold UF combined with HPP at 550 MPa. The CM cheese had the highest storage and loss moduli as well as a total solid of (45.42%) which was more significant ($P \leq 0.05$) than other treatments. According to SDS-PAGE electrophoresis and OPA assay results, CM cheeses were more susceptible to proteolysis by showing a lower molecular band, which could be a result of HPP treatment and the involvement of some active enzymes in CM cheeses. In conclusion, UF combined with HPP can improve the textural profile and total solid content of CM cheese when compared with CM cheeses that have undergone heat treatment.

Key Words: camel and bovine milk cheese, ultrafiltration, structure of cheese

1123M Effect of polyphenol caffeic acid on the functionality of processed cheese product. A. Sharma*, R. Joshi, and P. Salunke, *South Dakota State University, Brookings, SD.*

The dairy sector is trying to develop functional dairy foods with health benefits due to widespread consumer demands. Caffeic acid (CA), a type of polyphenol is known to be present in most fruits and vegetables. However, there has not been any research done on their potential application in the manufacturing of processed cheese products (PCP). In this study, the effect of substituting emulsifiers and stabilizers (E&S: sodium citrate and disodium phosphate) with CA at varying concentrations on the functional properties of PCP was investigated. The PCP was formulated to have 49% moisture, 20% fat, 18% protein, and 2% salt. In PCP formulation, CA was added at 4 concentrations, 0.25%, 0.5%, 0.75%, and 1%. The PCP was prepared using 2 milk protein ingredients: milk protein concentrate and milk protein isolate for each treatment, and the experiment was replicated twice. The control was prepared with E&S. The ingredients were blended in a Thermomix at 1,100 rpm at 90°C for 5 min, and the PCP was stored in cups at 4°C. After 48 h, the PCP samples were analyzed for pH and unmelted (penetration test) and melted properties [rapid viscoanalyzer (RVA), modified Schreiber test (MST), rheology]. Statistical analysis was conducted in R software using 2-way ANOVA, and Tukey's HSD test ($P < 0.05$) was used to compare means. The composition was similar in all treatments; however, the pH of samples decreased from 5.94 to 5.09 as the concentration of CA increased. The hardness of PCP with 0.75% CA was significantly lower ($P < 0.05$) as compared with the control whereas there was no significant difference between 0.25% CA, 0.5% CA, and 1% CA. The MST results indicated no significant difference between the control and treated samples. The frequency sweep of all the samples including the control resulted in more solid-like behavior [storage modulus (G') > loss modulus (G'')]. There was no significant difference between treated samples for RVA-Hot viscosity; however, the control sample has significantly ($P < 0.05$) higher RVA-Hot viscosity indicating CA addition resulted in PCP with high flowability. In conclusion, 100% replacement of E&S in PCP with polyphenols at low concentrations is possible.

Key Words: processed cheese product, caffeic acid, functionality

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1124M Effect of dosage level of fibrolytic enzyme and incubation time on in vitro rumen degradability of intercropped whole plant oat with whole plant faba bean silage in dairy cows. C. Nagy, V. H. Guevara Oquendo, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada.*

The objectives of this study were to study the effect of dosage level of fibrolytic enzyme and incubation time on in vitro rumen degradability of intercropped whole plant oat with whole plant faba bean silage in dairy cows. The intercropping oat and faba bean were grown in 3 fields and were cut at the growth stage with whole oat plant at the soft dough stage and whole faba bean plant at the late pod stage. The rumen degradability was estimated using in vitro technique with rumen liquid from rumen cannulated lactating cows. The in vitro rumen degradability of DM (DMD), NDF (DNDF) and ADF (DADF) was determined at both short and long incubation times (6, 24, and 48 h). The treatment design was a 5 × 3 factorial design with 5 dosage levels of fibrolytic enzyme (0, 0.075, 0.15, 0.225, and 0.3 mL/L) and 3 incubation times. The experimental design was a RCBD with the dosage level as a fixed effect and animals and in vitro batches as random block effects. The data were analyzed using the Mixed model procedure in SAS 9.4 with the analysis RCBD model. The Orthogonal Polynomial Contrast of SAS was used to study relationship between dosage levels or incubation times and in vitro degradability. The results showed that there was an interaction effect between dosage levels and incubation time ($P < 0.01$) for DDM, DNDF, and DADF. There was a cubic relationship between dosage levels and DDM ($P = 0.02$), a tendency for a linear relationship with DNDF ($P = 0.06$), and quadratic relationship with DADF ($P = 0.04$). The average of DDM of intercropped silage was 36%, 55%, and 63% at 6-, 24-, and 48-h incubation, respectively. The average of DNDF of intercropped silage was 6%, 28% and 41% at 6-, 24- and 48-h incubation, respectively. The average of DADF of intercropped silage was 15%, 27% and 50% at 6-, 24- and 48-h incubation, respectively. In conclusion, the dosage level of fibrolytic enzyme and incubation time had a significantly synergistic effect on in vitro rumen degradability of the intercropped oat and faba bean silage.

Key Words: intercropping oat and faba bean silage, dosage level of fibrolytic enzyme, degradability of nutrients

1125M The association of *Lentilactobacillus buchneri* and *Lentilactobacillus hilgardii* affects the starch degradability in snaplage. H. Scardini Junior, L. Lima, M. Cardoso, R. Ferreira, and T. Bernardes*, *University of Lavras, Brazil.*

The aim of this study was to assess the fermentation, aerobic stability, and starch degradability of snaplage inoculated with a newly released inoculant. Treatments included no additive and the addition of *Lentilactobacillus buchneri* NCIMB 40788 and *Lentilactobacillus hilgardii* CNCM I-4785, both bacteria at a rate of 150,000 cfu/g of FM. Whole ears (38% of moisture) were processed and ensiled (snaplage) in 5-L jars for 30, 60, and 120 d, totaling 36 experimental units. Measurements included microbial counts, fermentation end products, aerobic stability, and ruminal in situ starch degradation at 7 h ($isSD_{7h}$). The trial was set up as completely randomized using a 2 × 3 factorial design (inoculation and conservation time). Data were analyzed using the MIXED procedure of SAS, followed by a Tukey test at $P \leq 0.05$. Inoculated silages had lower concentration of lactate and greater concentration of acetate, propionate, ethanol, and 1,2-propanediol than control silages. Lactic acid bacteria were greater in the treated snaplage (7.16 vs. 5.32 log cfu/g), and yeasts were lower in those silages than untreated snaplage (1.72 vs. 2.79 log cfu/g). Aerobic stability increased over time in both silages. At 60 d of conservation, treated snaplage showed 117 h of stability against 49 h in the control silages. At 120 d of conservation, the aerobic stability was 102 h and 168 h for control and treated snaplage, respectively. Starch concentration did not differ between treatments, and it did not change over time (on average, 52% of DM). The $isSD_{7h}$ was similar between treatments at 30 d of fermentation ($P > 0.05$). At 60 d and 120 d of conservation, inoculated silages had greater $isSD_{7h}$ (91.5%) than control (82.5%). Overall, the combination of *L. buchneri* and *L. hilgardii* changes snaplage fermentation, improving the aerobic stability and the starch degradability from 60 d of conservation.

Key Words: inoculant, snaplage, starch degradation

1126M Effects of ensiling method, microbial inoculation, and storage length on the fermentation profile and aerobic stability of whole-plant corn silage. M. R. Pupo*[1], C. Heinzen Jr.[1], M. S. Souza[1,2], and L. F. Ferraretto[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Federal University of Amazônia, Belém, PA, Brazil.*

The trial was a completely randomized design with a 2 (ensiling method) × 2 (microbial inoculation) × 2 (storage length) factorial arrangement. Whole-plant corn forage was harvested at 36% DM, inoculated with 300,000 cfu/g of WPCS of *Lentilactobacillus buchneri* AHLB02, and *Pediococcus pentosaceus* AHPP02 (LBPP) or distilled water (CON), and ensiled in 20-L plastic buckets at a density of 195 kg of DM/m^3 or in nylon-polyethylene standard barrier vacuum pouches (900 g fresh of material). Silos were randomly assigned to be stored for 30 or 90 d. Each treatment combination was prepared in quadruplicates. Data were analyzed using PROC GLIMMIX of SAS with fixed effects of ensiling method, microbial inoculation, storage length, and all their interactions with significance at $P \leq 0.05$. Silage pH was greatest ($P = 0.001$) for LBPP silage in bucket, intermediate for LBPP silage in vacuum pouches, and lowest for CON silage in bucket. Lactic acid was 0.5 percentage-units lower ($P = 0.001$) whereas acetic acid was 0.6 percentage-units greater ($P = 0.001$) for LBPP than for CON silage. Acetic acid ($P = 0.001$) and ethanol ($P = 0.01$) concentrations were greater at 90 d than 30 d. Ethanol concentration was 0.3 percentage-units greater ($P = 0.02$) for buckets than vacuum pouches. The 1,2-PD ($P = 0.001$) was greatest for LBPP silage in vacuum pouches (0.44% DM), intermediate for LBPP silage in bucket (0.36% DM), and lowest for CON silage regardless of ensiling method (0.15% DM). Likewise, ammonia-CP was greatest ($P = 0.01$) for silage in bucket and vacuum pouches for 90 d, intermediate for silage in vacuum pouches for 30 d, and lowest for silage in bucket for 30 d. Total acids concentration was 0.6 percentage-units greater ($P = 0.001$) for 90 d than 30 d. The aerobic stability (measured only in buckets) was greatest ($P = 0.02$) for WPCS fermented for 90 d regardless of microbial inoculation (240 h), intermediate for LBPP silage fermented for 30 d (126.7 h), and lowest for CON silage fermented for 30 d (65 h). Microbial inoculation improved fermentation of WPCS but this response was more pronounced at 90 d. Both methods are adequate for corn silage fermentation research.

Key Words: fermentation, inoculant, corn

1127M Effects of ensiling method, microbial inoculation, and storage length on the fermentation profile of high-moisture corn. M. R. Pupo*, E. C. Diepersloot, C. H.P. Camisa-Nova, and L. F. Ferraretto, *University of Wisconsin–Madison, Madison, WI.*

The study was a completely randomized design with a 2 (ensiling method) × 2 (microbial inoculation) × 2 (storage length) factorial arrangement. High-moisture corn (HMC) was harvested at 74.1% DM, inoculated with 450,000 cfu/g of HMC of *L. buchneri* AHLB02, and *Pediococcus pentosaceus* AHPP02 (LBPP) or distilled water (CON), and ensiled in 3.785-L plastic buckets at a density of 792.5 kg of DM/m^3 or in nylon-polyethylene standard barrier vacuum pouches (1,300 g of fresh material). Silos were randomly assigned to be stored for 30 or 90 d. Each treatment combination was prepared in quadruplicates. Data were analyzed using PROC GLIMMIX of SAS with fixed effects of ensiling method, microbial inoculation, storage length, and all their interactions with significance at $P \leq 0.05$. Silage pH was 0.2 percentage-units greater ($P = 0.001$) for LBPP than CON. Lactic acid concentration was 0.7 percentage-units greater ($P = 0.001$) for CON than LBPP. Acetic acid concentration was 0.5 percentage-units greater ($P = 0.01$) for LBPP than CON at 30 d. At 90 d, acetic acid concentration was greatest ($P = 0.01$) for inoculated HMC silage in bucket (1.2% DM), intermediate for LBPP in vacuum pouches (1.1% DM), and lowest for CON regardless of ensiling method (0.2% DM). Ethanol concentration was greatest ($P = 0.001$) for silage in buckets for 30 d, intermediate for silage in vacuum pouches regardless of storage length, and lowest for silage in bucket for 90 d. The 1,2-PD concentration was greatest ($P = 0.001$) for LBPP at 90 d (0.7% DM), intermediate for LBPP at 30 d (0.5% DM), and lowest for CON regardless of storage length (0.1% DM). Likewise, ammonia-CP concentration was 2.5 percentage-units greater ($P = 0.001$) for LBPP at 90 d compared with other treatments. Total acid concentration was 0.1 percentage-unit greater ($P = 0.001$) for CON than LBPP. Total acid concentration was 0.1 percentage-unit greater ($P = 0.001$) for 90 d than 30 d. No treatments reached aerobic stability up to 240 h. Microbial inoculation improved fermentation of HMC with a more pronounced response at 90 d of storage. Minor differences in ensiling method suggest that both methods are suitable for laboratory silage research.

Key Words: inoculation, laboratory silos, silage

1128M The effects of a microbial inoculant on the fermentation of triticale silage harvested at two dry matters. X. Liu*, C. Mellinger, G. Weiner, and L. Kung, *University of Delaware, Newark, DE.*

Triticale is often harvested when wilting conditions are suboptimal leading to ensiling at relatively high moisture contents. This can lead to dominant fermentations by undesirable *Clostridia* and *Enterobacteria*. The objective of this study was to evaluate the effect of adding lactic acid bacteria (LAB) to triticale ensiled at 2 levels of DM. Triticale was direct cut (LOW) or wilted (HIGH) and was treated with water (CTRL) or an additive (FRTE) comprised of *Lactiplantibacillus plantarum*, *Pediococcus acidilactici*, and *Lactococcus lactis* (300,000 cfu/g fresh matter of forage; Provita Supplements Inc.) and vacuumed and heat sealed in 25 × 36 cm polyethylene bags. Five individually replicated bags were prepared for each treatment and stored at 22°C for 1, 3, 5, 7, 21 and 90 d. The experiment was a randomized design with a factorial arrangement of treatments with main effects of additive treatment, DM, and days of fermentation and their interactions. Data were analyzed with JMP Pro 16, and differences among means were tested using Tukey HSD when significant at $P \leq 0.05$. The LOW and HIGH triticale DM were 25.65% and 33.88% DM, respectively. The pH at ensiling was 6.11 for LOW and 6.37 for HIGH. Numbers of LAB were higher for FRTE treatments at 1, 5 and 7 d, causing treatment with FRTE to decrease in pH more rapidly than CTRL regardless of DM, starting at 1 d through 90 d. Treatment with FRTE reduced the numbers of ENT more quickly in LOW-FRTE than in LOW-CTRL from d 3 through 7 and prevented an increase in their numbers on d 3 for HIGH-CTRL. Treatment with FRTE resulted in higher lactic acid than in CTRL from d 3 through 90 and it also had lower concentrations of NH_3-N than CTRL from d 3 to 90. Treatment with FRTE also prevented the production of butyric acid after 90 d of ensiling regardless of the moisture content. The inoculant used in this experiment can be used to produce clostridial-free silage even when the moisture content is high.

Key Words: dry matter, triticale silage, lactic acid bacteria inoculants

1129M Evaluating the effects of maturity at harvest, microbial inoculation, and ensiling durations on nutritive value, and fermentation characteristics of sorghum hybrids. S. Farooq*[1], M. Wallau[1], F. Amaro[1], C. Cornejo[2], R. Trump[1], J. Portuguez[1], C. A. Niño de Guzmán[1], L. Mu[1], K. Arriola[1], H. Sultana[1], L. Ferraretto[3], and D. Vyas[1], *[1]University Of Florida, Gainesville, FL, [2]National Agrarian University La Molina, Lima, Perú, [3]University of Wisconsin, Madison, WI.*

Sorghum is considered an important forage because of its morpho-physiological adaptation to water scarcity with greater biomass yield despite uneven rainfall distribution. Sorghum hybrids available for the livestock producers differ in length of maturity, nutritive value and biomass potential; however, not many studies have looked at differences in ensiling characteristics of sorghum hybrids. We aimed to analyze the effects of types of hybrids (H1, H2, H4, H5), maturity at harvest (Low and High DM), microbial inoculation (CON, INO), and ensiling duration (30 d, 90 d) on ensiling characteristics, fermentation profile and nutritive value of sorghum silage. We hypothesized that hybrid specific differences will be observed on ensiling characteristics; however, microbial inoculation will increase aerobic stability, regardless of hybrids used. Thirty-two treatments (2 maturities × 4 hybrids × 2 inoculants × 2 ensiling durations) with 4 replicates per treatment combination (total 128 mini-silos) were used for the study. Hybrids were grown on 4 replicate plots in each of 4 blocks. Sorghum hybrids harvested were inoculated without or with mixture of *Lactobacillus buchneri* and *Lactococcus lactis* containing 150,000 cfu/g applied at 30 mL/kg of forage and ensiled. Data were analyzed using the MIXED procedure of SAS. Microbial inoculation increased aerobic stability after 30 d (317 vs. 62 h) and 90 d of ensiling, and these effects were attributed to lower yeast and mold counts in inoculated silage after 30 and 90 d of ensiling; however, maturity at harvest had no effect on these parameters. Acetic acid increased with microbial inoculation after 30 d and 90 d of ensiling duration. In conclusion, microbial inoculation increased aerobic stability and acetate concentration while yeast and mold counts were lower.

Key Words: homolactic bacteria, heterolactic bacteria, yeast

1130M Relationship between sorghum silage berry processing score measured with different sieves. E. C. Diepersloot*[1], K. Raver[2], J. P. Goeser[1,2], J. M. Piñero[3], and L. F. Ferraretto[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [2]Rock River Laboratory Inc., Watertown, WI, [3]Texas A&M Agrilife Extension, Texas A&M University System, College Station, TX.*

Sorghum silage is an important forage source for ruminants, especially in drought-prone areas. However, small, hard sorghum berries limit processing and hence starch digestibility. A berry processing score (BPS) using a 1.70-mm sieve was initially developed, but additional research suggested its replacement with a 2.36-mm sieve. Therefore, the objective of this study was to evaluate the relationship and understand differences of sorghum silage BPS measured using 1.70- and 2.36-mm sieves (BPS1.70 and BPS2.36, respectively). Three independent sorghum silage trials comprised a data set of 95 samples. Samples had BPS measured in a vertical shaker with both sieves (percentage of starch passing through either sieve). The difference (BPS2.36 − BPS1.70) between BPS methods was calculated for each sample. Relationship between BPS1.70 and BPS2.36 or difference between methods was analyzed with Proc Reg of SAS (9.4), and the concordance correlation coefficient (CCC) was calculated with the DescTools package of R CRAN (4.1.2). A comparison of the difference between methods for each quartile of the data set based on BPS1.70 (Q1: 0.8–5.3%; Q2: 5.4–13.6%; Q3: 14.0–19.7%; Q4: 20.1–28.7% starch passing through the sieve) was conducted with Proc Glimmix of SAS. The BPS1.70 averaged 13.1 ± 7.91% starch, while BPS2.36 averaged 25.5 ± 14.37% starch. A positive relationship was observed ($P = 0.001$; $R^2 = 0.91$; RMSE = 4.30) between BPS methods with the following equation: $y = 1.73x + 2.79$. Despite being highly correlated, the CCC (0.51) analysis highlighted a lower agreement between BPS methods. A positive relationship between the BPS1.70 and difference between methods was also observed ($P = 0.001$; $R^2 = 0.65$; RMSE = 4.30) with the equation $y = 0.73x + 2.79$. The moderate correlation of the variables supports the moderate agreement by CCC (0.45). The difference in BPS was lowest ($P = 0.001$) for Q1 (6.2% starch), followed by Q2 (8.9% starch), then Q3 (13.7% starch), while Q4 (21.0% starch) was greatest. Despite the high correlation between methods, BPS 2.36 is recommended, as BPS 1.70 appears to be more restrictive as the degree of processing increases.

Key Words: starch, starch digestibility, processing

1131M Effects of kernel type, processor adjustment, and fermentation time on starch characteristics of corn silage. A. M. Wilder*[1], J. Lawrence[2], and R. J. Grant[1], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*Cornell University, Ithaca, NY.*

Silage starch must be digestible and accessible to rumen microbes for optimal ruminant utilization. Our objective was to test corn with vitreous (VI) and floury (FL) kernels at moderate (MP) and heavy (HP) processing. Two corn hybrids that differed in kernel type (one with floury mutation) were grown in adjacent fields. Roller gap was adjusted at harvest to create 2 processing levels (2.5 mm-MP;1.5 mm-HP). Forage material was ensiled in bucket minisilos for 0, 45, 90, and 135 d (FT). Physical and chemical starch analyses were conducted to determine starch content, in vitro digestibility (7 h; starchD), processing score (CSPS; dry sieve, 4.75 mm), and soluble starch (50-µm wet sieve). The experiment formed a 2 × 2 × 4 factorial arrangement that was analyzed via a standard least squares linear model in SASJMP. Starch content differed significantly by kernel type ($P < 0.01$; 35.3-FL vs. 38.7-VI) but was not affected by processor setting. StarchD improved over time ($P < 0.01$) and was 6.2 percentage points higher for FL corn on average ($P < 0.01$). A 3-way interaction was present ($P = 0.04$) in which starchD of MP corn increased sooner than HP at early FT. The CSPS was not affected by FT or processor setting, but was higher in VI corn than FL corn (69.9 vs. 56.7%; $P < 0.01$). A significant ($P < 0.01$) increase in corn silage soluble starch was observed with greater FT with the FL corn higher overall (72 vs. 65%; $P < 0.01$). Aggressive processing in HP treatment improved soluble starch ($P = 0.01$) in relation to MP. A significant ($P < 0.01$) processor setting × FT interaction revealed that this difference was primarily detected at the start of fermentation. Differences in kernel type can have a considerable impact on corn silage starch characteristics, and processor settings above the recommended range may result in negligible benefits with higher harvest costs. While CSPS and soluble starch are both indicators of starch accessibility, these metrics did not agree on the accessibility of VI and FL starch in this study. Soluble starch assessment may be useful on silages with differing kernel types and may also prove to be an indicator of starchD.

Key Words: floury kernel, roller gap, soluble starch

1132M Fermentation characteristics of pure and mixed sugar beet silages. E. M. V. Hvas*[1], M. Larsen[1], L. Andersen[2], and M. R. Weisbjerg[1], [1]*Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Aarhus University, Tjele, Denmark,* [2]*KWS SCANDINAVIA A/S, Vejle, Denmark.*

The aim was to study co-ensiling of sugar beets with various effluent absorbing feeds by measuring fermentation weight loss and key parameters. Sugar beets were cut and ensiled either with or without an ensiling partner in vacuum bags for 6 mo. A total of 10 silages were made including 1 pure sugar beet silage (SB), and 9 mixed silages. The ensiling partners were: grass silage (GS), maize silage (MS), grass hay (GH), dried beet pulp (BP), soybean hulls (SH), rapeseed meal (RSM), wheat bran (WB), sunflower meal (SM), and wheat distillers grain (DDGS). These were selected based on their water-holding capacity which was tested beforehand to calculate the mixing ratio of the silages. Data were analyzed using linear models with ensiling partner, time, and their interaction as fixed effects. Loss of effluent after 6 mo of ensiling were only observed for SB (306 ± 17.4 g/kg DM). Total gaseous weight loss (GWL) was greatest for SB ($P < 0.01$; mean ± SEM; 282 ± 7.43 g/kg DM) and differed among the mixed silages depending on ensiling partner. Of the mixed silages GWL was greatest for WB and DDGS and lowest for MS (184.5 vs. 91.4 ± 7.43 g/kg DM). Ethanol concentrations were greater for SB ($P < 0.01$; 345 ± 17.1 g/kg DM) than the mixed silages. Of the mixed silages DDGS had the greatest ethanol concentrations, and RSM and MS had the lowest ethanol concentrations ($P < 0.01$; 203 vs. 98.5 ± 17.1 g/kg DM). Total GWL of the silages was closely related to the ethanol content ($R^2 = 0.92$). Both MS and GS had a greater pH than SB ($P < 0.01$; 3.94 vs. 3.56). Acetate concentrations were lower for DDGS than for SB ($P < 0.01$; 12.2 vs. 22.4 ± 1.38 g/kg DM). Butyrate concentrations were low for all silages, but greater ($P < 0.01$) for MS (0.22 ± 0.01 g/kg DM) and GS (0.36 ± 0.01 g/kg DM) than for SB and the remaining mixed silages (0.00 ± 0.01 g/kg DM). We found that MS, BP, DDGS, and GH all had a lower L-lactate concentration than SB ($P < 0.01$). In conclusion, co-ensiling sugar beets with other feeds reduced the gaseous losses and ethanol concentrations of the silages and eliminated the loss of effluents.

Key Words: gas losses, fermentation products, ethanol

1133M Variation of the alfalfa energy-to-protein ratio to maximize nitrogen use efficiency by rumen microbes. A.-A. Poulin*[1,2], F. Hassanat[2], G. F. Tremblay[2], D. Ouellet[3], R. Petri[3], M. Thériault[2], A. Claessens[2], A. Bertrand[2], and É. Charbonneau[1], [1]*Université Laval, Quebec, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Quebec, QC, Canada,* [3]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

In the rumen, imbalance between rapidly fermentable energy, namely from NFC, and highly degradable CP leads to an inefficient use of N for microbial protein synthesis, and an accumulation of NH_3. This study evaluated the effects of an improvement in the alfalfa NFC/CP ratio on the in vitro rumen degradation attributes. A total of 36 alfalfa forages contrasting for their NFC/CP ratio (Ratio + averaged 2.61 vs. Ratio − averaged 1.40; $P < 0.01$) and similar for their neutral detergent fiber concentrations (averaged 341 g/kg DM) were compared using 6 in vitro 24-h incubations with the ANKOM^{RF} gas production system. Data were analyzed using the MIXED procedure of SAS with treatment (Ratio + vs. Ratio −) as fixed effect and incubation day as random effect in the model. Significant effect was declared at $P \leq 0.05$. Apparent degradability of DM (57 vs. 53%; SEM = 0.6; $P < 0.01$) and total gas production (263 vs. 228 mL; SEM = 4.6; $P < 0.01$) were higher when Ratio + rather than Ratio − forages were incubated. Total VFA concentration (48 vs. 42 m*M*; SEM = 1.7; $P < 0.01$) in ruminal fluid after 24 h of incubation was greater, while the methane concentration of the gas produced (7.6 vs. 8.2 mL/100 mL of gas; SEM = 0.35; $P < 0.01$) was lower when Ratio + rather than Ratio − forages were incubated. The NH_3 concentration in ruminal fluid was lower (6.4 vs. 12.0 m*M*; SEM = 0.68; $P < 0.01$), while the microbial N use efficiency (69 vs. 56 g of microbial N/100 g of N incubated; SEM = 1.7; $P < 0.01$) was greater when Ratio + rather than Ratio − forages were incubated. An improvement in the alfalfa NFC/CP ratio, resulting from a concomitant increase in NFC and a decrease in CP concentrations, increased the microbial N use efficiency, decreased the production of NH_3, and is expected to decrease N release in the environment.

Key Words: alfalfa, non-fiber carbohydrates to crude protein ratio, in vitro

1134M Effect of harvesting time on the nutritional quality of small grains' forage for silage. H. Galyon*, M. Schultz, and G. Ferreira, *Virginia Tech, Blacksburg, VA.*

This study evaluated the effect of harvesting time on the yield and the nutritional composition of small grains' forage. Eight different small grain varieties were planted in plots at 3 locations in Virginia [Blacksburg (BB), Blackstone (BS), and Orange (OR)] in 2021 and 2022, therefore representing 6 environments. Within each environment, 2 varieties of barley, 2 varieties of rye, and 4 varieties of triticale were planted according to a randomized complete block design with 3 replicates or blocks. Plots were 2.74 m long and 1.20 m wide. Half of the plots (n = 144) were harvested at the boot stage of maturity (defined as the stage when the head was within the sheath of the flag leaf), and the other half of the plots (n = 144) were harvested at the soft-dough stage of maturity (defined as the stage when no milky endosperm was evident when grains were pressed between fingers). The forage biomass was cut 8 to 10 cm above ground using a hedge trimmer, weighed in the field, cut at 12.7-mm length using a lettuce cutter, and frozen in a cooler with dry ice. After thawing, drying (55°C), and grinding (1-mm screen) forage samples were analyzed to determine the concentration of ash, CP, aNDFom, ADL, and starch. Data were analyzed using the MIXED procedure of SAS, and the model included the random effect of environment, the fixed effect of variety, the fixed effect of maturity, the variety × maturity interaction, the random effect of block nested in environment, and the random residual error. Harvesting at the soft-dough stage yielded substantially more DM than harvesting at the boot stage (12.0 vs. 5.1 Mg/ha; $P < 0.01$). Relative to harvesting at the boot stage, harvesting at the soft-dough stage decreased the concentration of ash (7.1 vs. 4.0%; $P < 0.01$), decreased the concentration of CP (12.3 vs. 6.6%; $P < 0.01$), increased the concentration of aNDFom (50.5 vs. 60.7%; $P < 0.01$), increased the concentration of ADL (5.5 vs. 8.5; $P < 0.01$), and increased the concentration of starch (5.7 vs. 6.6; $P < 0.01$). In conclusion, harvesting at the soft-dough stage of maturity yielded substantially more forage of poorer nutritional quality, mainly on CP and aNDFom but not starch.

Key Words: barley, rye, triticale

Lactation Biology 1

1135M Single cell multi-omics analysis reveals the underlying regulatory mechanism for lactation tailoring by hypothalamus-pituitary-mammary gland axis in dairy cows. C. Zhang* and H. Liu, *Zhejiang University, Hangzhou,Zhejiang, China.*

For milk production in mammals, the mammary gland undergoes a series of complicated changes (development, remodeling, and regeneration) during the lactation cycle, which require the action of hormones from hypothalamus-pituitary gland and local sites. In dairy cows, hypothalamus-pituitary-mammary gland axis systems interact in a very sophisticated and complicated manner, but many of the underlying interactive and integrated mechanisms remain not quite clear until now. Here, we apply single-nucleus RNA and spatial transcriptomics to generate an atlas of hypothalamus-pituitary-mammary gland axis during lactation of dairy cows. We obtained 30,654 high-quality single nuclei, among which 8,071 were from hypothalamus, 6,686 from pituitary, and 15,897 from mammary gland cells. Moreover, we identified the oligodendrocyte, astrocytes, and microglia, as well as the neuronal subtypes in the hypothalamus. Analyses of cell-type-specific transcriptomics showed the dynamic gene expression profile associated with oligodendrocyte differentiation, and more striking, glutamatergic and GABAergic neurons, actively functioning in energy metabolism, and stress responses, were presumed to exert a remarkable effect on lactating mammary gland. Besides, the hormone-oriented interactions of specific cell types in the hypothalamus toward the pituitary provided feedback for the endocrine process. Cluster analysis further demonstrated the different hormone-producing cell types in the pituitary. For the mammary gland, we define the major cell subtypes as well as an alveolar cell subpopulation with high HSP90 expression (heat stress marker). Aside, by employing the integrative analysis of single-nucleus and spatial transcriptomics, we generate an in situ map of cell types, which would provide a comprehensive understanding of functional tissue organization and cell-to-cell interaction. Together, our study offers a rich resource to elucidate lactation regulation tailoring by hypothalamic-pituitary-mammary gland axis in dairy cows.

Key Words: dairy cows, single-nucleus RNA, spatial transcriptomics

1136M Characterization of bioactive lipids that promote lipid synthesis in bovine mammary epithelial cells. M.-C. Guesthier*[1,2], T. Kustova[1], P. Piantoni[2], G. Shroeder[2], and S. A. Burgos[1], *[1]Department of Animal Science, McGill University, St-Anne-de-Bellevue, QC, Canada, [2]Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN.*

We previously developed a high-throughput screening method to measure lipid droplet (LD) accumulation in primary bovine mammary epithelial cells (BMEC). This method allowed us to identify bioactive lipids (BL1 and BL2) that increased accumulation of lipid droplets in BMEC. The objective of this study was to validate and characterize the effect of BL1 and BL2 in our cellular model using high-content microscopy, gas-chromatography and RT-qPCR. Isolated BMEC were grown to confluency using growth media and were induced to lactogenic differentiation with lactogenic media for 4 d. During the last 16 h of d 4, BMEC were treated with 10 μM of BL previously conjugated to BSA. For the microscopy assays, BMEC were fixed, permeabilized, and stained with BODIPY 493/503, a specific fluorescent probe for neutral LD, as well as the nuclear (DAPI) and cytoskeletal (Phalloidin-tetramethylrhodamine) counterstains. The BMEC were imaged using a high-content cell imaging microscope. Each plate contained vehicle control, BL1 and BL2 in triplicates. Data were analyzed by ANOVA with PROC MIXED in SAS. Validation assays showed an increase in LD of 95% for BL1 and 92% for BL2 ($P \leq 0.05$) compared with vehicle control. The MTT assay showed a 50% cytotoxic concentration at 49 μM for BL1 and 68 μM for BL2. In dose-response experiments, LD accumulation was maximal at 60 μM for BL1 whereas LD increased linearly with BL2 up to 125 μM treatment. The combination of the compounds did not show a synergistic or additive effect. Profiling of fatty acids (FA) from total lipid BMEC extracts showed that BL2 increased ($P \leq 0.05$) the proportion of de novo FA (≤14 carbons) by 32% compared with non-treated BMEC. To identify the potential mechanism of action influenced by BL, we measured mRNA abundance of genes involved in lipogenesis by RT-qPCR. We found that BL1 showed a 2-fold increase and BL2 a 2.7-fold increase (both $P \leq 0.05$) in the mRNA abundance of *ACACA*, which encodes for the acetyl-CoA carboxylase 1 enzyme. However, the compounds did not affect the expression of *SREBF1* or other lipogenic genes tested (*FASN*, *DGAT1*, *SCD1* and *FABP3*). These results indicate that the effect of bioactive metabolites was partially caused by the stimulation of lipogenic pathways in BMEC.

Key Words: lipogenesis, gene expression

1137M Prepartum infusions of 5-HTP and EGTA are similarly effective in preventing postpartum hypocalcemia. H. H. Hanling*, A. Vang, W. Frizzarini, T. Cunha, H. Fricke, and L. L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

Hypocalcemia is a common postpartum disease in dairy cattle caused by a lack of circulating calcium at parturition. Around the time of calving, the mammary glands require calcium for milk production. If unprepared for such a demand, blood calcium will decline rapidly and cause paresis. Previous studies showed that serotonin and a calcium chelator administered prepartum can prevent postpartum hypocalcemia by causing a transient state of the disease. The objective of this study was to determine whether the prepartum intravenous infusion of the precursor to serotonin, 5-hydroxytryptophan (5-HTP), or the calcium chelating agent, ethylene-glycol-tetraacetic acid (EGTA), was more efficient at preventing postpartum hypocalcemia. A total of 24 multiparous cows were assigned to 1 of 4 infusion treatments (n = 6 per treatment): 5-HTP, EGTA, 5-HTP+EGTA, or saline (control). Treatments were administered via jugular catheter starting 7 d before expected calving date. Infusions lasted 6 h/d, and rates were adjusted to hold blood ionized calcium (iCa) between 0.7 and 0.8 mM in cows that received EGTA to induce subclinical hypocalcemia. Treatment significantly affected prepartum and postpartum iCa ($P < 0.05$). The EGTA- and 5-HTP+EGTA-treated cows had the lowest blood iCa prepartum compared with other treatments ($P < 0.0001$). The 5-HTP treated cows also had significantly lower blood iCa than the control ($P = 0.0008$). Cows that received EGTA alone required greater infusion rates to lower their blood iCa levels compared with cows that received 5-HTP+EGTA ($P = 0.0014$). Postpartum, 5-HTP, EGTA, and 5-HTP+EGTA treatments had greater iCa compared with the control—thus a reduced risk of hypocalcemia. Two control cows required postpartum clinical hypocalcemia treatment. There were no differences in iCa between 5-HTP-, EGTA-, or 5-HTP+EGTA-treated cows postpartum, but these 3 treatments significantly increased blood iCa compared with the control. Therefore, 5-HTP and EGTA are similarly effective in preventing postpartum hypocalcemia.

Key Words: hypocalcemia, serotonin, calcium

1138M Characterization of immune cellular heterogeneity in milk from healthy bovine mammary glands.

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Cells present in milk, known as somatic cells (SC), are a heterogeneous and intricate population composed of several subsets of cells. However, a complete understanding of immune cell heterogeneity in cow milk remains elusive. The objective of this study was to characterize immune cell subpopulations in milk from healthy dairy cows. Using a single milk collection (3.8 L) from 2 multiparous Holstein cows (≥300 DIM, $\leq 1.62 \times 10^5$ SC/mL, and MY ≥45 kg/d), we isolated hematopoietic cells present in milk using fluorescence activated cell sorting (FACS) with CD45 as cell surface marker. A total of 600,000 individual, nucleated, viable hematopoietic cells were sorted and collected from each milk sample. From this, a single-cell suspension of approximately 1,000 cells was prepared from each cow for single cell RNA sequencing. Gel beads and barcodes were generated, cDNA amplified, cDNA sequencing libraries were constructed and sequenced. Data analysis and clustering were carried out using the Seurat Package from R (4.2.2). After data normalization, scaling, and filtering control, the final data set included 923 and 851 single $CD45^+$ cells containing 17,771 and 12,156 features. Uniform Manifold Approximation and Formation (UMAP) analysis revealed 7 and 8 distinguishing clusters in each sample that differed in gene expression profile. Based on marker expression, most immune cells present in the samples were T cells (CD3E and PTPRC). Three different T cell subpopulations were revealed: helpers (CD4), cytotoxic (CD8A and CD8B), and regulatory T cells (IL2RA). The remaining 4 clusters were composed of granulocytes (neutrophils, eosinophils, and basophils; TLR4 and CXCL8), macrophages (PTPRC, CD14, CD68, TL2, IL1B), and a small population of B cells (CD19, CD22 and MS4A1). The study and characterization of the populations of hematopoietic cells present in milk provide a basis for developing greater understanding of the immune response of the bovine mammary gland during lactation. This work was supported by USDA-NIFA Hatch Project VA-13995

Key Words: immune cells, bovine milk, scRNA sequencing

Physiology and Endocrinology 1

1139M Brown Swiss and Holstein dairy cows have different inflammatory and immune responses to their first calving. L. Cattaneo*[1], M. Sfulcini[1], V. Lopreiato[2], F. Piccioli-Cappelli[1], A. Catellani[1], A. Minuti[1], and E. Trevisi[1], *[1]Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy, [2]Department of Veterinary Sciences, Università di Messina, Messina, Italy.*

Brown Swiss (BS) is a dairy breed selected mainly for milk composition and cheese yield, whereas Holsteins (HF) are the most productive cows. We hypothesized that different inflammatory and immune responses between these breeds exist during the transition period. Late-gestation heifers approaching their first calving of BS breed (n = 10) or HF (n = 11), raised in the same pen and fed the same diet, were enrolled. Blood was harvested at −21, −7, 3, 7, 14, 21, and 28 d relative to calving to assess the inflammatory and hematological profiles, and to measure mRNA expression in leukocytes of genes related to inflammation and immune system with PCR. Data were analyzed with repeated measures mixed models (proc GLIMMIX in SAS). Milk yield was higher in HF during the first month of lactation ($P = 0.01$). Hematocrit and primary leukocyte population counts were higher in HF ($P \leq 0.05$). Plasma albumin ($P = 0.02$) and paraoxonase concentrations ($P = 0.06$) were higher in HF. Ceruloplasmin was lower ($P < 0.01$) and myeloperoxidase tended to be lower in HF ($P = 0.10$). At −21 d, HF had a higher mRNA abundance of *CD14*, *TLR4*, and *CD16* compared with BS ($P \leq 0.05$). Among genes related to the inflammatory cascade, *IL6R* and *NLRP3* were upregulated ($P \leq 0.05$) but *TNFA* was downregulated ($P = 0.02$) in HF. After calving, HF had lower expression ($P \leq 0.04$) of migration and cell adhesion genes (*CCR2* and *ITGAL*) and antimicrobial-related genes (*MMP9* and *MPO*). Regarding inflammation-related genes, *IL18*, *ALOX5*, and *ALOX15* were downregulated ($P \leq 0.05$) in HF. Transcript abundance of antioxidant system genes (*SOD2* and *NOS2*) was downregulated in HF ($P \leq 0.05$) but *SOD1* was downregulated at −7 and upregulated at 28 d ($P = 0.02$). These differences highlight the role of breed in the modulation of the immune and inflammatory responses at calving. Our data could suggest that BS cows experience a more severe inflammatory response at calving but, at the same time, might have a more active immune system.

Key Words: transition period, dairy breed, inflammation

1140M Effects of phase feeding an acidified close-up ration for improved plasma calcium status in immediate postpartum period. M. B. Samarasinghe*[1], L. E. Hernández-Castellano[2], N. B. Kristensen[3], and M. Larsen[1], *[1]Department of Animal and Veterinary Sciences, Aarhus University Viborg, Foulum, Tjele, Denmark, [2]Animal Production and Biotechnology group, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Arucas, Spain, [3]SEGES Innovation P/S, Aarhus, Denmark.*

This study aimed to investigate the effects of phase-feeding acidified corn silage-based close-up rations compared with a single ration dry cow feeding strategy on blood pH and ionized Ca concentration (iCa). A total of 28 Holstein dry cows were fed a common far-off ration (i.e., grass based; DCAD of 300 mEq/kg DM) and randomly allocated to 4 experimental treatments in the close-up period. One group continued far-off ration (FAR, n = 7). The other 3 groups were phase-fed with a corn silage-based close-up ration acidified with: (1) $MgCl_2$ (MGC-70, DCAD of −70 mEq/kg DM, n = 7), (2) MGC-70 added with NH_4Cl (MGC-100, DCAD of −100 mEq/kg DM, n = 7), or (3) a 7-d transition diet [grass silage-diluted MGC-70 (20:80 DM basis)] followed by feeding MGC-100 in last 14 d of close-up period (OVE, n = 7). After calving all cows received a common lactation TMR. Blood samples were collected by puncture of an ear artery at −12, +0.5, +1.5, and +2.5 DIM, and, pH and iCa were measured in fresh blood. The OVE treatment was intended to test effects on feed intake during the diet shift; thus, blood data were analyzed by contrasts: (1) FAR vs. MGC-70, MGC-100, OVE, and (2) MGC-70 vs. MGC-100 and OVE. Arterial blood pH was lower for the 3 acid treatments (7.43 ± 0.01) compared with FAR group (7.5 ± 0.01) at −12 DIM ($P < 0.01$). However, pH was increased by acid treatments (7.52 ± 0.01) compared with FAR (7.47 ± 0.01) at +1.5 DIM ($P < 0.01$) and at +2.5 DIM ($P < 0.01$). Furthermore, MGC-70 group (7.50 ± 0.01) had lower pH than the MGC-100 and OVE (7.53 ± 0.01) at +2.5 DIM ($P = 0.03$). The iCa was similar at −12 DIM; however, acid treatments had greater iCa (1.03 ± 0.02 m*M*) than FAR group (0.92 ± 0.04 m*M*) at +0.5 DIM ($P = 0.02$) and tended to be greater at +2.5 DIM ($P = 0.09$). However, iCa concentrations between the acid treatments were not different. In conclusion, the study showed that a potential pH overshoot in arterial blood soon after calving might challenge the ability of the cow to maintain the iCa activity in blood, which was probably an effect of sudden shift from acidified close-up rations to the lactation TMR.

Key Words: acidification, ionized calcium, periparturient cows

1141M Systemic transcriptional analysis in lactating cows with elevated peripheral serotonin. V. L. Pszczolkowski[1,2], A. M. Larsen[1,3], J. Laporta[1,2], L. L. Hernandez[1,2], W. Li[3], and S. I. Arriola Apelo*[1,2], *[1]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [2]Endocrinology and Reproductive Physiology Graduate Training Program, University of Wisconsin–Madison, Madison, WI, [3]USDA Dairy Forage Research Center, Madison, WI.*

The objective of this study was to investigate the systemic transcriptional response to elevated peripheral serotonin in lactating cows. In a crossover design, multiparous Holstein cows (187 ± 34 DIM, n = 8) were intravenously infused with saline or the serotonin precursor 5-HTP (1 mg/kg bodyweight) on d 1–3 and 8–10 of each period, for 1 h/d, starting at 0700 h. Adipose tissue (WAT), liver, and mammary were biopsied 6 h after d-10 infusion. Extracted RNA was sequenced, mapped to the cattle reference genome (ARS-UCD 1.2), and analyzed with DEseq2 for differentially expressed genes (DEG) with cutoff of mean read-count ≥5, fold-change ≥2, and P-value <0.01. We found that 5-HTP downregulated 71 and upregulated 14 identified genes in WAT, 59 genes were downregulated and 95 upregulated in the liver, and 43 downregulated and 29 upregulated in mammary tissue. Expression patterns were analyzed by QIAGEN Ingenuity Pathway Analysis (IPA) with absolute Z-score ≥2 as cut-off. Based on IPA prediction, 5-HTP decreased activity of 54 upstream regulators of the DEG in WAT, including insulin, PPAR gamma, and FOXO-1; and increased activity of 17 regulators. In line, synthesis and metabolism of triacylglycerol and uptake of monosaccharides were predicted to be inhibited in response to 5-HTP. In the mammary glands, 5-HTP increased the activity of 90 upstream regulators and decreased activity 220 of them. In addition, IPA predicted that 5-HTP increased mammary lipids trafficking. In the liver, 90 upstream regulators had decreased activity, including insulin receptor, PKC, FOXO-1 and 2, and SREBP-1 and 2; and 61 had increased activity, including the cholesterol synthesis inhibitors INSIG-1 and 2.

In line, the superpathway of cholesterol biosynthesis was predicted to be inhibited by 5-HTP in the liver. In conclusion, lactating cows with elevated peripheral serotonin developed a systemic response, altering the expression of genes in energy metabolic pathways, including reduced lipid synthesis in WAT, reduced cholesterol synthesis in the liver, and increased lipid trafficking in the mammary tissue. Together, these changes may function to shift energy partitioning toward milk synthesis.

Key Words: adipose, liver, mammary

1142M Transition milk feeding shows marginal effects on blood metabolome of dairy calves.

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Transition milk is a rich source of bioactive components that may improve intestinal development and health in calves. The objective of this study was to evaluate the effects of feeding transition milk on the metabolome in blood plasma of dairy calves. German Holstein calves (30 females, 20 males) were fed either 6 L of milk replacer (MR, n = 25, 140 g/L) or transition milk (TRANS, n = 25) from their dams twice daily. The calves were blocked in pairs by sex. All calves were bottle-fed with the first colostrum (3.5 to 4 L) within 2 h of birth, followed by 1.5–2 L of colostrum 11.5 h later, and subsequently received the MR or the TRANS diet for 5 d. Calves (2–3 h after birth) were housed in individual hutches bedded with straw for the first 14 d of life. From d 15, calves were housed in group pens and all received MR via automatic milk feeders (max. 12 L/d), and starter feed, hay, and water were freely available. All calves were weaned at 14 wk of age. Targeted metabolomics using the MxP Quant 500 kit with liquid chromatography-tandem mass spectrometry was performed in plasma samples collected at wk 2, 6, and 14 of life. Among the metabolites (n = 301) used in the analysis, hexoses, lactic acid, cholesterol esters (CE 18:2), CE (18:3), Gly, Gln, Ala, Lys, Arg, Met, phosphatidylcholine diacyl (PC aa) C34:2, PC aa C36:2, betaine, Pro, and Ser were the metabolites with the highest concentrations. Statistical analysis of the metabolite data was performed using MetaboAnalyst 5. Significant (fold change >1.5, FDR <0.05) metabolites were identified by volcano plots using log-transformed data. At wk 2, 7 metabolites [PC aa 36:6, PC aa 36:5, PC aa 40:5, PC ae 40:6, cholesterol esters (CE) 20:5; CE 15:0 and CE 17:1] were higher in calves fed TRANS than in those fed MR. No significant metabolites were found between treatments at wk 6 and 14. Overall, our results suggest that feeding transition milk for 5 d had only little and transient effects on the plasma metabolome of dairy calves. It remains to be seen whether the TRANS-induced metabolic deflection in the second week of life may exert sustained effects on animal health.

Key Words: calf, colostrum, transition milk

1143M Divergence in feed efficiency yields differences in tissue-level gene expression.

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Our objective was to characterize liver and muscle tissue transcriptome changes in mid-lactation cows with divergent feed efficiency status with or without rumen-protected choline treatment. Multiparous Holstein cows (161 ± 3 DIM; n = 64) were assigned randomly to either rumen-protected choline (RPC; 17.7g choline ion; Balchem Corp.) or control (CTL; 0 g choline ion). Liver and muscle tissue were collected from the highest and lowest 16 cows (n = 32) in the final week, as determined by a mid-point RFI calculation. The RFI was determined as DMI regressed on milk energy, DIM, metabolic BW, ΔBW, and treatment. Final RFI was calculated and the lowest and highest 12 cows (n = 24) were retained as LowRFI (more feed efficient) and HighRFI (less feed efficient). Liver and muscle tissue RNA sequencing was performed to identify differentially expressed genes (DEG; $P \leq 0.05$; fold change ≥1.5; mean read count ≥10) between LowRFI and HighRFI cows. DEseq2 (R; v.4.2.1) was used to identify DEG by RFI group, controlling for treatment. Gene ontology (GO) analysis was performed in DAVID (v.2021) with KEGG pathways and GO functional classification using a Benjamini-Hochberg FDR-adjusted P-value ≤ 0.05. In muscle tissue, a total of 315 DEG were identified, in which 163 were downregulated (DR) and 152 were upregulated (UR) in LowRFI cows. The UR genes were enriched in GO terms related to actin filament binding ($P = 0.01$), extracellular space ($P < 0.01$), and extracellular matrix ($P = 0.02$). The DR genes were enriched in GO terms related to the nucleus components ($P < 0.01$). In liver tissue, a total of 268 DEG were identified, in which 88 were DR and 180 were UR in LowRFI cows. Genes that were UR were primarily enriched in GO terms associated with cell division ($P < 0.0001$), cell cycle ($P < 0.0001$), and microtubule binding ($P < 0.0001$), and DR genes showed an enrichment in GO terms related to cellular response to copper ion ($P < 0.001$). Significant transcriptome changes were observed in cows with divergent feed efficiency, though the mode of gene expression regulation in muscle and liver tissue is not yet clear.

Key Words: RNAseq, residual feed intake

1144M Effects of feeding a body-cooling feed additive and chromium on milk and embryo production in lactating dairy cows.

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The aim was to evaluate the effects of an osmolyte-mineral body-cooling feed additive (ICE, Cargill Animal Nutrition) added or not with Cr (chromium propionate, Kemin Industries) on milk and embryo production in lactating dairy cows. The study was conducted on a commercial freestall dairy herd located in the state of Sao Paulo, Brazil, from May to December 2022. The final data set included 58 multiparous Holstein cows randomly assigned to treatments: (1) no additive, used as control (CTL; n = 20), (2) 50 g/d of ICE (ICE; n = 20), or (3) 50 g/d of ICE plus 6 mg/d of Cr (ICE+Cr; n = 18). Treatments were mixed into an iso-energetic supplement top-dressed at 300 g/d individually using headlocks once daily during a.m. feeding from dry-off until 80 DIM according to treatment design. All cows were individually identified by color-coded ear tags, grouped together within dry and lactation pens, and fed TMR diets twice daily. Farm staff, including support veterinarian, were blind to treatments. All cows underwent a standard 4-d FSH superovulation protocol, followed by AI performed twice at 12 and 24 h after induced ovulation using semen from a single fertility-proven sire (total of 2 sires used, balanced across treatments; single batch per sire); uterine flushes occurred between 70 to 80 DIM. Milk production was recorded daily, and 2-d a.m./p.m. milk samples were collected weekly for component and SCC. Statistical analyses were performed with the GLIMMIX procedure (SAS 9.4); significance was declared at $P \leq 0.05$ and tendency at $P \leq 0.10$. Both ICE and ICE+Cr had greater proportion of viable embryos (54.2 and 65.9 vs. 40.8 ± 5.8%; $P < 0.05$) and less degenerated embryos (39.1 and 28.5 vs. 59.2 ± 5.9%; $P < 0.05$) compared with CTL cows. These effects were more pronounced in ICE+Cr than in ICE ($P < 0.05$). In addition, milk production tended to be higher in ICE and ICE+Cr compared with CTL (39.9 and 40.0 vs. 38.9 ± 1.3 kg/d; $P = 0.08$). Treatments had no effect on milk components or SCC. In conclusion, feeding ICE overall increased embryo quality and milk production. Moreover, the combination of ICE and Cr had additive effects toward embryo viability in lactating cows.

Key Words: osmolyte, hydration, embryo quality

1145M Lipolysis modulates the biosynthesis of palmitoyl- and oleaoyl-ethanolamines in bovine adipocytes.

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Adipose tissue (AT) lipolysis during the periparturient period and other stages of negative energy balance increases availability of FA that are substrate for endocannabinoid biosynthesis. Among the latter, N-acylethanolamines, including N-palmitoylethanolamine (PEA) and, N-oleoylethanolamine (OEA), are activators of ion channels and PPAR nuclear receptors and therefore modulate AT metabolic and inflammatory responses. Currently, the effect of lipolysis activation on the production of PEA and OEA in adipocytes is unknown. The objective of this study was to determine the direct effect of lipolysis activation through the classic and inflammatory lipolytic pathways on the biosynthesis of PEA and OEA. Preadipocytes from subcutaneous AT (right flank) of nonlactating non-gestating multiparous dairy cows (n = 6) were induced to differentiate for 7 d. Adipocytes were preincubated 12 h with saline (SAL) or inhibitors of the classic (niacin, NIA, 100 µ*M*) and inflammatory (flunixin meglumine, FM, 10 µ*M*) lipolytic pathways. Lipolysis was induced by β-adrenergic stimulation with 1 µ*M* isoproterenol (lSO), or lipopolysaccharide (LPS, 1 µg/mL) alone or combined with NIAFM. Basal (BAS) condition without ISO or LPS was used as control. After 7 h cells/media were collected and the effect of lipolysis on acylethanolamines biosynthesis was evaluated using HPLC-MS/MS targeted lipidomic analysis. The statistical model included the fixed effect of lipolysis, inhibitor, and the random effect of cow. Values are nmol of acylethanolamines in media/µg protein. The PEA media content during ISO conditions (0.042) was lower than BAS and LPS (0.09; 0.08 ± 0.02; $P = 0.05$). We found that OEA increased during LPS (0.10) vs. ISO (0.071) and BAS (063 ± 0.02). Further, FM and NIAFM increased PEA in LPS and ISO conditions compared with NIA or SAL. In contrast, OEA content was decreased by NIAFM in LPS and BAS conditions compared with NIA and SAL. Inhibiting the classic or inflammatory pathway alters PEA and OEA biosynthesis. The impact of treating cows with NIA and FM during stages of negative energy balance where endocannabinoids modulate metabolic functions warrants further investigation.

Key Words: endocannabinoids, lipolysis, adipocytes

1146M Association between postpartum systemic inflammation and serum calcium in healthy multiparous dairy cows—An exploratory observational analysis.

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Our objective was to investigate the association between serum total Ca concentration (tCa) and postpartum systemic inflammation (SI) in dairy cows. We hypothesized that the tCa-SI association varies in the first 8 DIM. This exploratory analysis used data from a field trial in which cows were randomly assigned to receive oral Ca supplementation after calving (OSCa) or not. Healthy multiparous cows (n = 101) from 2 farms in Ontario were included. The inflammatory markers assessed were serum concentrations of haptoglobin (Hp), albumin (ALB; negative acute phase protein), and serum amyloid A (SAA). Albumin, Hp, and tCa were assessed at 0 (<12 h after calving), 0.5, 1, 2, 4, 6, and 8 DIM; SAA was assessed at 0, 2, and 4 DIM. We built linear regression models accounting for repeated measures for each marker. Full models included tCa, tCa^2, DIM, parity (2 vs. ≥3), OSCa, the interactions of tCa × DIM, tCa^2 × DIM, tCa × parity, tCa^2 × parity, and farm as a random effect. The lowest ALB was observed after 2 DIM while Hp and SAA were highest at 2 DIM. The association of ALB with tCa varied quadratically over time (tCa^2 × DIM: $P = 0.03$). At 0, 0.5, 1, and 2 DIM, as tCa decreased, ALB increased but at 4, 6, and 8 DIM as tCa decreased, ALB also decreased. For Hp and SAA, tCa, tCa^2 ($P < 0.07$), and DIM ($P < 0.001$) covariates remained in the models. At tCa <1.7 mmol/L (zenith; only observed at 0, 0.5, and 1 DIM) tCa and Hp were positively associated but when tCa >1.7 there was a negative relationship with Hp. For SAA, at tCa <1.9 (zenith) tCa and SAA were positively associated but when tCa >1.9 there was a negative relationship with SAA (tCa at 0 DIM: 2.08 ± 0.19 SD; at 2 DIM: 2.24 ± 0.18; and at 4 DIM: 2.40 ± 0.14). The associations between tCa and markers of SI changed within the first 8 DIM in clinically healthy cows. Potential interventions to modulate hypocalcemia or SI should consider their interactions. Future studies should further explore the association of the degree and duration of hypocalcemia and SI to better understand the direction and mechanisms of this relationship.

Key Words: hypocalcemia, inflammatory markers, homeorhesis

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1147M Effect of feeding management during the first 21 days postpartum, on direct and residual productive response and adaptation to grazing of multiparous Holstein dairy cows. C. Rivoir*[1], G. Mendina[2], L. Adrien[2], and P. Chilibroste[1], [1]*Universidad de la Republica, Facultad de Agronomía, EEMAC, Paysandú, Uruguay,* [2]*Universidad de la Republica, EEMAC Facultad de Veterinaria, Paysandú, Uruguay.*

Intensification of dairy production systems in Uruguay has involved the implementation of strategies that includes confinement with total mixed rations (TMR) either in dry lots or in low-cost barns. Questions have emerged about the productive impact of its use in short periods during early lactation (e.g., first 21 d postpartum) in multiparous dairy cows. An experiment was carried out at the Research Station "Dr. Mario A. Cassinoni" of the School of Agronomy (Paysandú, Uruguay) to study the productive response of multiparous dairy cows after a period of contrasting feeding management during the first 21 d in milk (DIM). Two treatments were compared: T21 (n = 10) were fed a TMR diet ad libitum (29.9 ± 3.5 kg DM cow^{-1} day^{-1}) and T0 (n = 13) cows started grazing the day after calving and were fed a TMR (13.3 ± 0.5 kg DM cow^{-1} day^{-1} plus 1 grazing session). At d 22 the T21 cows were moved to T0 treatment until 60 DIM. Data were analyzed as a complete randomized block design using the GLIMMIX procedure of SAS OnDemand software with a mixed model that included treatment, week and the interaction as fixed effect and block as random effects. Milk production on T21 was higher than T0 (40.1 vs. 35.1 kg cow^{-1} day^{-1}; $P = 0.007$) during the differential feeding period (0–21 DIM). During the residual period (22–60 DIM) T21 cows tended to produced more milk than T0 (39.8 vs. 38.3 kg cow^{-1} day^{-1}; $P = 0.07$). No differences were found in grazing behavior between treatments (T0: 237, and T21: 239 min^{-1} grazing day^{-1}, $P = 0.86$). It seems that these differences in milk production might be related to a carryover effect of higher dry matter intake during the transition period as well as changes in selectivity (Chilibroste et al., 2015; Menegazzi et al., 2021). Changes in feeding management during the first 21 DIM had an impact on milk production of multiparous dairy cows. Further research is required to better understand the mechanisms involved in the direct and residual responses.

Key Words: fresh dairy cow, feeding strategy, multiparous cows

1148M Characterization of heat stress in lactating Holstein cows at different lactation stage using productive performance, physiological indicators, blood, and milk characteristics based on South Korean climate conditions. J. H. Jo*[1], J. G. Nejad[1], J. S. Lee[1], M. K. Choi[1], Y. R. Kim[1], M. S. Ju[1], S. H. Keum[1], T. Z. Liu[1], S. Y. Maeng[1], H. R. Kim[2], and H. G. Lee[1], [1]*Department of Animal Science and Technology, Sanghuh College of Life Sciences, Konkuk University, Seoul, Republic of Korea,* [2]*Animal Nutrition and Physiology Team, National Institute of Animal Science, RDA, Wanju, Republic of Korea.*

Heat stress effects on lactating Holstein cows in South Korean climate conditions were investigated by analyzing productivity, physiological, blood, and milk characteristics in 2 studies. A 2-way ANOVA was performed to analyze the data using the mixed procedure of SAS. The first study tested the effect of 3 consecutive days of different temperature-humidity index (THI) ranges of moderate (85–87 and 82–84), mild (78–80 and 75–77), and comfort (72–74 and 69–71) on 80 mid-lactating cows (parity: 2.08 ± 0.13; DIM: 147.01 ± 3.34). The results indicated that mid-lactating Holstein cows exposed to an environment where the THI decreased from 85–87 to 69–71 had increased milk protein, fat, solids-not-fat (SNF), and milk urea nitrogen (MUN) levels, as well as increased hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration, and glucose concentrations ($P < 0.05$). Conversely, milk yield, β-hydroxybutyrate, nonesterified fatty acid, blood urea nitrogen, total protein, calcium, cortisol, and rectal temperature levels were decreased in the same THI range ($P < 0.05$). The second study compared various THI levels (comfort, mild, and moderate) across 3 lactation stages (early, mid, and late) in 160 lactating Holstein cows (parity: 1.77 ± 0.06; DIM: 231.10 ± 9.84). The results indicated that THI and lactation stage had significant impacts on milk characteristics such as milk fat, protein, SNF, and MUN, while lactation stage influenced milk yield ($P < 0.05$). Lymphocyte, hemoglobin, hematocrit, and MCH were influenced by both THI and lactation stage ($P < 0.05$). In conclusion, these findings suggest that lactation stage is not the only factor influencing milk parameters, and THI has a significant impact on most parameters regardless of the stage of lactation. Additionally, implementing appropriate summer breeding management practices is important to minimize the negative effects of heat stress and to optimize milk production and quality.

Key Words: heat stress characterization, lactation stage, milk characteristics

1149M Back to basics: Precision while mixing total mixed rations and its impact on milking performance. A. Bach*[1,2], [1]*Marlex Research and Education, Barcelona, Catalonia, Spain,* [2]*ICREA, Barcelona, Catalonia, Spain.*

Details from every mixing load of TMR fed to ~8,000 cows distributed in 72 pens from 19 farms, along with individual milk yield of each cow in every pen and farm was collected from a feeding and management system (algoMilk; www.algoMilk.com) between 2020 and 2022 on a daily basis to assess the impact of quality of mixing TMR on animal performance. Divergence between expected and actual amounts of ingredients mixed in every load was calculated and expressed as a percentage relative to expected amounts. Ingredients were classified as: (1) energy grains (i.e., corn, wheat), (2) protein sources (i.e., soybean meal, canola meal), (3) hays (i.e., alfalfa hay), (4) grain silages (i.e., corn silage), (5) non-grain silages (i.e., alfalfa silage), (6) minerals (i.e., salt), and (7) straw (i.e., wheat straw). Milk yield was averaged within farm and pen on a weekly basis, and mixing divergences were also averaged by load or by ingredient type and week within pen and farm. The weekly standard deviation (SD) of mixing divergences was calculated for every pen and farm. A 2-degree polynomial mixed-effects model accounting for the random effects of farm and pen within farm, and the continuous effect of the weekly mixing divergences their weekly standard deviations (SD) was run. The average ± SD divergence of the total amount of TMR prepared was 1.55 ± 2.27%, which means that, in general, mixing errors were caused by adding an excess of one or more ingredients. Energy grains (1.0 ± 3.79%), grain-silages (2.1 ± 2.25%), hays (2.06 ± 4.08%), and protein sources (0.14 ± 3.5%) were mixed in excessive amounts; whereas non-grain silages (−1.44 ± 3.65%) and straw (−0.41 ± 7.77%) were mixed in lower amounts than expected. Divergence in the total amount of TMR and its weekly SD was negatively and quadratically ($r = -0.16$; $P < 0.001$) or negatively correlated ($r = -0.11$; $P < 0.0001$) with milk yield, respectively. The SD of the divergence in the amount of grain or protein sources was quadratically and negatively ($r = -0.10$; $P < 0.001$) or negatively correlated ($r = -0.14$; $P < 0.001$) with milk yield, respectively. Minimizing mixing errors may have positive effects on milking performance.

Key Words: composition, feeding, management

1150M Characterization of heifer raising costs in Quebec Holstein herds. L. Laflamme-Michaud*[1], R. A. Molano[1,2], É. Charbonneau[1], O. Brassard[2], S. Binggeli[1], R. Roy[2], D. Warner[2], É. Paquet[1], and D. E. Santschi[2], *[1]Département des sciences animales, Université Laval, Québec, Québec, Canada, [2]Lactanet, Canadian Network for Dairy Excellence, Ste-Anne-de-Bellevue, Québec, Canada.*

The rearing of replacement animals is a significant cost for dairy production. These expenses are rarely characterized and analyzed. The aim of this study was to characterize the costs of raising replacement heifers in the province of Quebec, Canada. A total of 93 dairy farms was recruited. Selected herds were mainly composed of Holstein animals and registered to the official DHI. Farms were visited at least once from April 2021 to May 2022. A sample of heifers of all ages and mature cows were weighed using a weight tape and a survey to producers was done about their rearing practices, time for each task, and feeding costs. Heifer rearing-related expenses were obtained through management reports for 86 herds or by the producers. Feeding and labor costs were uniformized from on-farm grouping to phase, using a proportion of BW gain. Data were analyzed using a mixed model with the effect of phase as fixed effect and the random effect of farm. Mean age at first calving was 24.4 ± 1.4 mo among farms. Preliminary results indicate that the mean total cost of raising heifers is CAN$4,912 ± 1,062 and that costs are significantly different through phases (Table 1). Considerable variability was observed in total raising cost and how expenses were distributed among rearing phases. This information could serve producers to optimize their raising costs.

Key Words: heifer, costs

1151M Climatic conditions, production, and composition of milk in 8 years of a pasture-based dairy farm in Southern Brazil. K. Frigeri[1], M. Deniz*[2], K. De-Sousa[1], and F. Vieira[1], *[1]Biometeorology Study Group, Federal University of Technology, Paraná, Dois Vizinhos, Paraná, Brazil, [2]School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo, Brazil.*

This study assessed the relationship between climatic conditions, production, and composition of milk. For this, we linked a climatic database with a longitudinal database (from August 2012 to August 2019) of a commercial pasture-based dairy farm in Rio Grande do Sul, Southern Brazil. The climatic database with relative humidity, air temperature, wind speed, and precipitation data were obtained from the National Institute of Meteorology (INMET). Milk production per cow and tank milk composition (protein, fat, lactose, somatic cell count, and total bacterial count) were evaluated monthly by the commercial dairy farm. To perform data analysis, we determined 2 groups of data: group 1, climatic conditions and milk production; and group 2, climatic conditions and tank milk composition. After that, we determined the linear correlation between each variable within each group; there, we determined the canonical loadings of the variables with the most significant impact within the group. All procedures were performed through the PROC CANCORR available on SAS software. Wind speed and precipitation were the most critical climatic conditions for both groups. A canonical correlation (rc = 0.41; $P = 0.003$) between climatic conditions and milk production was found. Canonical loadings (cl) showed that the wind speed (cl = 0.43) and precipitation (cl = 0.53) affected milk production per cow (cl = −0.54). Overall, the climatic conditions explain 17% of the variation in milk production per cow ($r^2c = 0.17$). There was a canonical correlation (rc = 0.51; $P = 0.017$) between climatic conditions and tank milk composition. The wind speed (cl = 0.76) and precipitation (cl = −0.62) affected protein (cl = 0.46), fat (cl = 0.73), somatic cell count (cl = −0.62), and total bacterial count (cl = 0.74). Overall, the climatic conditions explain 26% of the variation in tank milk composition ($r^2c = 0.26$). Our findings showed that precipitation presented the highest canonical correlation with the production and composition of milk.

Key Words: dairy cows, milk quality, biometeorology

1152M The impacts of lameness on reproduction and milk yield in lactating dairy cattle. L. P. Bielamowicz*[1] and B. W. Jones[1,2], *[1]Tarleton State University, Stephenville, TX, [2]Texas A&M AgriLife, Stephenville, TX.*

Lameness and infertility are in the top 3 reasons for culling in the dairy industry. Lameness can stem from injury, nutrition, housing, management, and genetics. Lameness impacts milk yield, dry matter intake, lying time, and reproductive efficiency. The objective of this study was to evaluate the impact lameness has on reproduction and milk yield in lactating dairy cattle. Data were obtained from AgriTech Analytics (Visalia, CA) from years 2020 to 2022 and included n = 70,848 cows. Lameness was determined on farms by farm personnel. Lameness was categorized as yes (n = 2,088), the cow was lame, or no (n = 68,760), they were not. Lactation number was categorized by lactation 1, 2, and 3 or greater. Somatic cell score (SCS) was determined by $SCS = \log_2(SCC/100) + 3$. All statistical analysis was carried out using SAS (Version 9.4, SAS Institute, Inc.). Significance was set at $P < 0.05$. Energy-corrected milk (ECM), SCS, and days open means were evaluated in SAS. A linear mixed model was used to evaluate lactation number, lameness category and days in milk on energy corrected milk, SCS and days open. The mean ECM was 14,514.09 kg ± 1,660.37 kg. The mean

Table 1 (Abstr. 1150M). Least squares means of raising costs (in CAN$) by phase[1]

Costs	Birth to weaning	Weaning to 6 mo	6–9 mo	9–12 mo	12–15 mo	15–18 mo	18 mo to precalving	Precalving	SE
Daily feed ($/d)	5.47^{b}	$2.59^{d,f}$	2.07^{f}	$2.33^{e,f}$	$2.77^{d,e}$	$3.02^{c,d}$	3.49^{c}	6.05^{a}	0.15
Daily labor ($/d)	4.47^{a}	1.04^{c}	0.58^{c}	0.65^{c}	0.72^{c}	0.66^{c}	0.73^{c}	2.32^{b}	0.21
Fixed ($/heifer)	113^{d}	177^{b}	144^{c}	145^{c}	146^{c}	147^{d}	256^{a}	63^{e}	7
Variable ($/heifer)	758^{a}	498^{b}	300^{e}	$330^{d,e}$	$423^{b,c}$	$397^{c,d}$	781^{a}	$346^{c,e}$	23
Total ($/heifer)	870^{b}	675^{c}	444^{e}	$476^{d,e}$	570^{d}	544^{d}	1036^{a}	409^{e}	25
Cost of gain ($/kg BW)	12.64^{b}	6.52^{d}	5.09^{d}	5.54^{d}	7.19^{d}	$7.92^{c,d}$	$11.15^{b,c}$	30.86^{a}	0.84

[a–f]Different superscripts in row indicate means that differ ($P < 0.05$).

[1]Fixed effect of phase was significant ($P < 0.01$) for all variables.

SCS was 2.36 ± 1.65. Days open mean was 104.84 d ± 69.18. Lame cows produced 60.78 kg less ECM than non-lame ($P < 0.0001$). Cows in lactation 1 had the greatest ECM, decreasing by higher lactation ($P < 0.0001$). Lameness was not associated with SCS. Lame cows were open 3.07 d longer than non-lame ($P < 0.0001$). Cows in lactation 3 and greater were open longer than lactation 1 or 2 ($P < 0.0001$). Overall, lameness negatively impacted days open and milk yield of these dairy cattle, while SCS was not affected. It is economically important for producers to detect and treat lameness because of these impacts. Further research can develop strategies to prevent lameness, therefore improving reproductive efficiency and milk yield.

Key Words: dairy, lameness, reproduction

1153M Buffalo milk quality: Somatic cell counts and microbial contamination. C. F. Viana[1], I. L. S. Gomes[1], E. H. P. Andrade[1,2], M. R. Souza[1], C. F. A. M. Penna[1], B. M. S. Souza[1], R. S. Conrrado[1,2], E. R. Campanha[1,3], G. Plefk[4], and L. M. Fonseca*[1,2], *[1]School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [2]Laboratory of Milk Quality Analysis, School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [3]Federal Institute of Minas Gerais, Bambuí, MG, Brazil, [4]Departament of Veterinary Medicine, Centro-Oeste State University of Paraná, Guarapuava, PR, Brazil.*

Buffalo milk represents about 11% of the total milk production in the world, and in some countries, it is the most consumed milk. However, knowledge of buffalo milk quality is still scarce. The objective of this work was to investigate quality parameters for buffalo milk in Brazil. A total of 837 samples of raw milk were collected in appropriate sampling 40-mL vessels from refrigerated tanks during 24 mo (July 2020 to July 2022). Analyzes included somatic cell counts (SCC) by flow cytometry (Somascope; Delta Instruments) and bacterial counting by flow cytometry, according to ISO 21187:2021 (Bactoscan FC, Foss Analytics). Results were expressed as CS/mL and cfu/mL, respectively, and were analyzed using descriptive statistics, Tukey test for multiple comparison of parametric data and Kruskal Wallis test for non-parametric data. Average trimonthly geometric SCC ranged from 225,000 during the winter, to 318,000 in the spring, with significant seasonal difference ($P < 0.05$). Spring and summer in Brazil are rainy seasons with high humidity and environmental temperatures, and usually these are propitious conditions for more udder contamination in the herds. Geometric bacteria counts ranged from 63,000 during the winter to 113,000 cfu/mL during the summer. These high counts are the result of poor milking hygienic conditions and environmental factors which facilitate contamination. These results show that seasonal variations are influential factors on buffalo milk quality. More studies are necessary to establish milk quality parameters for this species, as physiological reproductive and mammary differences are reported when compared with cows. Acknowledgments: FAPEMIG APQ-02740-17; FEPE proj. 882.

Key Words: buffalo milk, somatic cell count, microbial contamination

1154M Milk urea nitrogen as a predictor tool of nitrogen efficiency and nitrogen excretion in dairy cows. I. A. M. A. Teixeira*[1], M. G. Podda[2], D. Salis[2], D. Scoresby[1], M. Chahine[1], and S. A. Santos[3], *[1]University of Idaho, Twin Falls, ID, [2]University of Sassari, Sassari, Italy, [3]Universidade Federal da Bahia, Salvador, BA, Brazil.*

Nutritionists have used milk urea nitrogen (MUN) to monitor the protein nutritional status of dairy cows, as it is closely related to the urea nitrogen in the blood and the urinary nitrogen excretion. On the other hand, there is a general concern about the interpretation of MUN because there is a wide variation of MUN values between herds and within herds. This variation is due to many factors, such as feeding systems, diet characteristics, breed, lactation order, stage of lactation, among others. The objective of this study was to evaluate the effect of dietary nutrient composition and certain cow characteristics on MUN and ultimately to investigate the relationship between MUN and nitrogen excretion and efficiency of nitrogen utilization (NUE). A data set comprising 457 treatment means from 124 studies published in peer-reviewed journals from 2018 to 2022 was used. Relationships between MUN and diet and/or cow characteristics were developed using a meta-regression approach, considering the random effect of study. Backward stepwise selection was used to remove nonsignificant factors until all remaining factors in the model had a $P < 0.05$. We also considered the lowest AICc and independent variables that had variance inflation factor <10. We found that closer relationships existed between MUN and dietary factors when nutrients were expressed as absolute intakes, rather than concentrations in the diet. The MUN was significantly influenced by crude protein (or nitrogen), starch, and NDF intake. The inclusion of milk yield, energy-corrected milk, and days in milk associated with nutrient intake resulted in better predictions of MUN. The 5 best models showed high goodness of fit, with mean root mean square prediction error (RMSPE) lower than 8% of the MUN mean and CCC greater than 0.85. Furthermore, MUN associated with milk production and dry matter intake provided accurate and precise predictions of NUE, urinary N excretion, and N excretion in the manure (RMSPE <5%, CCC >0.90). It is concluded that nutrient intake and milk production play an important role in predicting MUN. In addition, we conclude that MUN can be used in prediction models of NUE and nitrogen excretion.

Key Words: manure nitrogen, MUN, urinary nitrogen

1155M Effect of yeast derivatives on somatic cell count and the immune function of dairy cows. A. Deliberalli[1], A. C. A. Abreu[1], B. Milla[1], S. N. de Oliveira[1], H. G. Bertagnon[1], M. A. Bonato[2], and W. L. S. dos Reis*[2], *[1]UNICENTRO, Guarapuava, Paraná, Brazil, [2]ICC, São Paulo, São Paulo, Brazil.*

Mastitis reduces milk yield and economic return in dairy farming. Yeast derivatives (YD) can improve milk production and quality by stimulating immunity and ruminal efficiency. Rich in β-glucans, YD are considered immunomodulators and act mainly on phagocyte cells. Yeast cytoplasm comprises metabolites, primarily amino acids, nucleotides, and vitamins, which impact ruminal microorganisms and then feed digestion and conversion. We evaluate the effects of YD supplementation for dairy cows on milk somatic cells count (SCC), blood leucocyte oxidative metabolism (Nitroblue tetrazolium blood test, NBT), and milk leucocyte profile. Multiparous Holstein cows, mid-lactation (average, 25 L/day), were distributed into 3 treatments: control group (CG, n = 8); autolyzed yeast rich in cell wall group (AY, n = 10; 15 g/animal/day, RumenYeast, ICC Brazil Company); and yeast rich in metabolites (YM, n = 7; 5 g/animal/day). Blood and milk samples were taken weekly (days: D0, D8, D16, D24, D32, D40, D48, D56). Initially, 30 cows were randomly assigned to 3 treatments; then SCC analyses were run in the week before D0. Cows above 200,000 cells/mL (subclinical mastitis, 5 cows) were excluded from the experiment since the aim was to study the immune response in healthy cows. The treatments (presence or absence of yeast derivatives) were given to cows from D0 to D56. The data were subjected to ANOVA with comparison of the means at 5% significance using the statistical software Instat GraphPad. After 40 d, significant differences in health status were noted. On D48 and D56, SCC was lower ($P < 0.05$) in AY cows than in CG; YM presented intermediate levels of SCC. This enhancement in health status may partly be explained by the results seen in blood analysis on D40, D48, and D56; both AY and YM cows had superior ($P < 0.05$) NBT compared with CG. This better immune status was also observed in the mammary gland, as noted in the leucocyte profile of milk. For instance, on D48 and D56, the percentual of milk macrophages was superior ($P < 0.05$) in AY than in CG or YM. Autolyzed yeast (RumenYeast) reduces SCC and stimulates the immune response, while yeast rich in metabolites (YM) had an intermediate effect in reducing SCC and enhancing the immune response.

Key Words: immunomodulators, somatic cell count, yeast derivatives

1156M Identifying on-farm factors associated with increased free fatty acids in bulk tank milk. H. Woodhouse*, D. Kelton, S. LeBlanc, T. DeVries, and K. Hand, *University of Guelph, Guelph, ON, Canada.*

Elevated concentrations of free fatty acids (FFA) in bulk tank milk may reduce milk quality. Triglyceride hydrolysis yields FFA and milk with ≥1.2 mmol FFA/100 g of milk fat is associated with undesirable characteristics, such as off-flavor, rancidity, reduced foam stability, and inhibited cheese coagulation. Previous research indicates that elevated FFA concentrations are multifactorial, and the objective of this study was to identify associated factors at the farm level on Canadian dairy farms. A cross-sectional study was conducted on 293 Canadian dairy farms in Ontario (n = 238) and British Columbia (n = 55). Farms were visited once to complete a survey, assess milking systems, and gather data on diets for lactating cows. Bulk tank FFA values for each farm were obtained from the provinces' milk marketing board and the monthly average FFA around each farm's visit date was used as the outcome variable. Univariable linear regression analyses were conducted for all explanatory variables, and those that were significant ($P < 0.1$) were included in the multivariable linear regression model. The factors identified as associated with increased FFA were those with $P < 0.05$ in the final multivariable model. A total of 71 of the farms were tiestall, 109 were freestall with milking parlors, and 113 were freestall with automated milking systems (AMS). The mean bulk tank FFA was 0.84 mmol/100 g of fat (SD = 0.40, range 0.27 to 3.67) and 10% (n = 29) of herds had a mean FFA of ≥ 1.2 mmol/100g of fat. In the final multivariable linear regression model, a milking frequency ≥3 times a day was associated with higher FFA in AMS ($\beta = 0.27$, $P < 0.01$) and tiestall milking systems ($\beta = 1.18$, $P < 0.01$) compared with parlors milking twice a day. Regardless of milking frequency, none of the farms with parlors had a mean FFA level ≥1.2 mmol/100 g of fat. For farms milking ≥3 times a day, changing the milk filter at least twice a day was associated with lower FFA ($\beta = -0.28$, $P < 0.01$). The absence of pre-cooling was also associated with higher bulk tank milk FFA ($\beta = 0.14$, $P = 0.04$). The final model adjusted R^2 of 29% indicates that there are more factors associated with bulk tank milk FFA that still need to be identified.

Key Words: milk fat, lipolysis, production

1157M Comparison of dry matter intake, production, and enteric methane emissions in dairy cows housed in respiration chambers vs. a head-chamber system (GreenFeed). X. Ma*[1], S. E. Räisänen[1], S. Amelchanka[2], K. Giller[1], MZ. Islam[1], Y. Li[1], R. Peng[1], M. Reichenbach[1], X. Sun[1], I. Müller[3], and M. Niu[1], [1]*Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH, Lindau, Zurich, Switzerland,* [2]*AgroVet-Strickhof, ETH Zürich, Lindau, Zurich, Switzerland,* [3]*Department of Animal Nutrition, DSM Nutritional Products, Kaiseraugst, Aargau, Switzerland.*

The open-circuit respiration chambers (RC) and GreenFeed System (GF; a head-chamber system) are commonly used techniques for measuring enteric methane (CH_4) emission from ruminants. This study compared the 2 techniques with 16 multiparous lactating dairy cows [(mean ± SD), 235 ± 68.4 d in milk, 29 ± 4.8 kg/d milk yield (MY)] receiving 1 of 4 dietary treatments: (1) positive control [CON: 3-nitrooxypropanol (3-NOP, Bovaer10)], (2) 3-NOP + tannin extract, (3) 3-NOP + essential oil, (4) 3-NOP + oilseed for a 25-d experimental period preceded by a 8-d covariate phase. Data from both covariate and experimental periods were used for the analysis. Dry matter intake (DMI) and MY were recorded daily. Methane emissions were measured during the last 5 d of the covariate and experimental periods. In the first 3 d, gas emissions were measured, and milk samples collected, in a tiestall barn using GF 8 times every 3 h, followed by 2-d RC period with 10-min measurement interval. The DMI (kg/d ± SE, 23.4 ± 1.89 vs. 24.6 ± 1.91), ECM yield (kg/d, 29.5 ± 3.75 vs. 30.3 ± 3.82), CH_4 production (g/d; 475 ± 90.4 vs. 455 ± 65.4), CH_4 yield (g/kg DMI; 20.4 ± 3.86 vs. 18.5 ± 2.32), and CH_4 intensity (g/kg ECM; 16.3 ± 3.15 vs. 15.2 ± 2.43) of cows in RC and GF were highly correlated, indicated by Pearson correlations: 0.55, 0.91, 0.85, 0.73, 0.82, respectively ($P < 0.01$). Prediction equations for CH_4 emissions in RC were developed in mixed models using the CH_4 measurements from GF as predictors. The interactions of treatment and CH_4 measurement techniques were removed from the model due to lack of significance. The final model included random effect of cow and treatment, and fixed effect of CH_4 emissions. For example, the CH_4 production equation $Y_{RC} = 248 + 0.4 \times X_{GF}$ was fitted with $R^2 = 0.82$, but further cross validation is needed. Overall, the results showed that the measurement of CH_4 parameters using RC and GF are highly correlated. In conclusion, the GF system can be considered a suitable alternative to the RC system and allows for a higher throughput by measuring more animals.

Key Words: GreenFeed, respiration chamber, methane

1158M Management and facility factors associated with milk yield in freestall facilities in the Midwest and Northeast USA. K. M. Luchterhand*, *Novus International Inc., St. Charles, MO.*

The objective of this study was to investigate pen- and farm-level facility and management factors associated with milk yield in freestall dairies across the Midwest and Northeast regions of the United States. The mature, high-production pen of 476 Holstein freestall-housed pens were used in the analysis. Data were collected from May 2013 to August 2022. Management data were collected from a questionnaire conducted with the owner or herdsman, and facility measures were collected during one visit to the dairy. The Mixed procedure of SAS 9.4 was used to individually test each variable on the outcome of pen milk yield (kg/cow/d). The random effect of pen nested within farm was used. Variables with $P < 0.30$ were available to the final model. Backward stepwise procedure was used until all variables had $P < 0.10$. Barn ventilation was significant ($P < 0.01$) with both cross-ventilated (CV; 47.1 ± 2.5) and tunnel-ventilated (TV) barns (41.7 ± 2.1) having greater milk yield than natural ventilation (NV; 39.7 ± 1.81; $P < 0.05$), and CV had greater milk yield than NV ($P < 0.01$). Farms that provided fans only (44.8 ± 1.9) or fans and soakers (45.0 ± 1.9) had greater milk yield than those with no heat abatement in the pen (38.7 ± 3.0; $P < 0.05$). No difference was shown between fans only or fans and soakers. Cows milked 2 times per day had 4.3 ± 0.9 and 6.8 ± 1.8 lower milk yield than cows milked 3 or 4 times per day, respectively ($P < 0.01$), with no difference between 3 or 4 times per day. Farms that housed fresh cows in a separate pen had 2.0 ± 0.8 greater milk yield than fresh cows housed in a pen for sick cows ($P = 0.02$) but did not differ from farms that did not have a fresh pen. Stall cleanliness (1 = clean, 2 = wet, 3 = soiled) was associated with milk yield ($P = 0.02$). Each 1-unit increase in stall cleanliness was associated with a reduction in milk yield by 1.9 ± 0.8. Providing heat abatement in the pen, milking more than 2 times per day, and providing clean stalls were associated with greater milk yield for freestall-housed cows.

Key Words: milk yield, management, freestall

1159M Changes in lactation curve parameters associated with age at first calving, parity, and calving season in dairy cows in tropical regions.

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This study aimed to model lactation curves of dairy cows under a tropical environment in El Salvador (mean temperature: 28°C, relative humidity: 78%) as a vital step in decision making for management and research in dairy farms. A database consisting of 95,189 fortnightly test day records from 2012 to 2021, (1,636 cows, 3,521 lactations) from 3 farms was collated. All farms had predominantly Holstein cows, under an intensive production system. The Dijkstra (1997) mechanistic model was fitted to individual lactations using a nonlinear procedure. Results were sorted by parity (1, 2, and 3+; 1,335, 878, 1,308 lactations, respectively), calving season (Q1: Jan to Mar; Q2: Apr to Jun; Q3: Jul to Sep; Q4: Oct to Dec), and age at first calving (AFC, 25.6 ± 3.74 mo; mean ± SD). Model parameters, including initial milk production (kg/d, M_0), rate of secretory cell proliferation (μ_T), decay (k), specific rate of mammary cell death (λ), and time to peak yield (Tp), milk yield at peak (MYp), and total 305-d milk yield (TMY305) were analyzed with a mixed model with parity, calving season, and AFC as fixed effects, year within farm as random effect, and cow as subject of the repeated measurements. There was no effect of AFC or season on M_0 and λ, but both parameters increased with parity ($P < 0.05$). Parity increased μ_T, which was smaller in Q3 compared with Q1 or Q2 ($P < 0.02$), with no effect of AFC. There was no effect of AFC on Tp (77.5 ± 6.6 d; mean ± SE), but decreased by 13 d in Q2, and by 20 d for parity 2 and 3+ ($P < 0.05$). No effect of AFC or parity was found for k, but k was smaller in Q3 and Q4 compared with Q2 ($P < 0.03$). Greater AFC increased MYp ($P = 0.01$), regardless of calving season, whereas MYp increased by 4.0 and 6.0 kg in parity 2 and 3+, respectively ($P < 0.05$). Average TMY305 were 5,261, 6,409, and 7,061 kg for parity 1, 2, and 3+, respectively ($P < 0.05$), with greater TMY305 in Q3 and Q4; AFC increased TMY305 in primiparous cows but decreased it in 2 or 3+ parity ($P = 0.02$). Clear effects of AFC, season and parity exist in lactation curve parameters of tropical dairy cows and are worth further exploring.

Key Words: lactation modelling, tropical dairy, calving season

1161M Impact of environmental toxin, perfluorooctanoic acid, on endometrial stromal cells.

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Per- and polyfluorinated substances (PFAS) are a large class of ubiquitous chemical compounds that persist in the environment. Exposure in dairy cows occurs through consuming contaminated feed or water. These chemicals cannot be metabolized and bioaccumulate. Previous work has found PFAS in plasma, milk, and skin samples of dairy cows. Detection of PFAS contamination has caused farms to cull cows and close their operations. In 2021, over $1.5 million was paid from the USDA to producers affected by PFAS. Further, these chemicals are detected in milk, posing a potential risk to human health. In humans PFAS is associated with an increased cancer risk, suppressed immune response in children, and decreased fertility in women. Our objective was to unravel the cellular response to PFAS in uterine biology. We hypothesized that perfluorooctanoic acid (PFOA) alters the endometrial stromal function and contributes to PFAS-induced subfertility in women. Immortalized human endometrial stromal cells (HESC) were cultured in vitro and treated with PFOA in concentrations ranging from 0.01 μ*M* to 1 m*M* or vehicle for 72 h to assess cell viability. In vitro decidualization assays were performed by exposing cells to 1 μ*M* PFOA for 2 h followed by decidualization media [estrogen, medroxyprogesterone acetate, db-cAMP (EPC)] for 72 h. Following EPC treatment, RNA was isolated, and sequencing performed. There was no difference in cell viability following exposure to PFOA for 72 h except at the highest dose (1 m*M*), where there was 100% decrease in cell number compared with vehicle. Based on transcriptome data, PFOA did not impair the ability of HESC to undergo decidualization, a critical step of pregnancy establishment in humans. However, there were 816 differentially expressed genes in PFOA exposed cells compared with vehicle. In summary, acute exposure to PFOA does not alter HESC viability but does induce differential gene expression independent of hormone response. Additional experiments to analyze the impact of long-term PFOA exposure on decidualization and the transcriptome are being conducted. Understanding the cellular mechanisms induced by PFAS is critical to both the dairy industry and human health.

Key Words: environment, milk quality, health

1162M Effects of polyphenolic compounds on enteric methane production and rumen fermentation in vitro.

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Supplementation with polyphenolic compounds (PC) could, through changes in the rumen microbiome, alter ruminal fermentation and reduce enteric CH_4 emission in dairy cows. This study investigated the effect of 9 PC contained in products from the food industry on rumen fermentation and methanogenesis in a batch culture in vitro system. Compounds were analyzed for their effect on cumulative gas production and composition and volatile fatty acid (VFA) and ammonia concentrations in an automated gas production system. Rumen inoculum was collected from 2 ruminally-cannulated lactating Holstein cows fed a standard 51% forage (corn silage-based) and 49% concentrate feeds diet. Dried and ground (through a 1-mm sieve) total mixed ration (TMR) fed to the donor cows was included as substrate in the incubations at 1% (wt/vol). The following PC were tested at 2% (TMR dry matter-basis) inclusion rate: chrysin, epigallocatechin-3-gallate (EGCG), epicatechin, fisetin, naringenin, quercitin and cherry, cocoa and green tea extracts. An incubation was carried out for 24 h at 39°C and duplicated (n = 6 bottles per PC). Gas production was continuously monitored, and headspace samples were analyzed for CH_4 concentration at 24 h. At 24 h, incubation medium samples were collected and analyzed for VFA and ammonia concentrations. Data were analyzed using the MIXED procedure of SAS with treatment in the model and incubation as random effect. When compared with the control (CON; TMR only), inclusion of PC did not affect ($P \geq 0.22$) pH, total gas production or CH_4 concentration or yield (i.e., per unit of substrate). Total VFA and ammonia concentrations were also not affected by treatment ($P \geq 0.10$). Relative to the CON, acetate-to-propionate ratio was increased ($P = 0.02$) by EGCG only. Fisetin, EGCG, naringenin and cherry, green tea, and cocoa extracts decreased ($P = 0.04$) the molar proportion of branched-chained VFA relative to CON. In this in vitro experiment, inclusion of PC at a rate of 2.0% of substrate dry matter did not have pronounced effects on ruminal fermentation and CH_4 production, except for some minor changes in VFA molar proportions.

Key Words: enteric methane, polyphenolic compound, in vitro

1163M Evaluation of plasma biotin and vitamin B_{12} concentrations of dairy cows fed varying concentrations of dietary vitamin D_3, vitamin E, Ca and Se under heat stress. M. Duplessis*[1], A. Ruiz-Gonzalez[2], and D. Rico[3], [1]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [2]*Université Laval, Québec, QC, Canada,* [3]*Centre de recherche en sciences animales de Deschambault, Deschambault, QC, Canada.*

Heat-stressed cows exhibit altered metabolism and ruminal microbiota composition, both of which can be modulated by dietary concentrations of some vitamins and minerals. As B vitamins have important roles in metabolism and are synthesized and utilized by the ruminal microbiota, the aim of this study was to assess plasma biotin (B8) and vitamin B_{12} (B12) concentrations under heat stress (HS) in dairy cows fed varying concentrations of vitamin D_3 (VITD) and E (VITE), Ca and Se. In a split-plot design, 12 multiparous Holstein cows [42.2 (SD: 5.6) kg of milk per day at 83 (SD: 27) days in milk] were used. The main plot was dietary VITE and Se supplementation: (1) Adequate: 11 IU/kg of dry matter (DM) of VITE and 0.55 mg/kg DM of Se (n = 6); (2) High: 223 IU/kg DM of VITE and 1.8 mg/kg DM of Se (n = 6). Within each main plot, cows were assigned to (1) HS (temperature-humidity index: 72–82) with adequate concentrations of VITD and Ca (1,012 IU/kg and 0.73%, respectively); (2) HS with high concentrations of VITD and Ca (3,764 IU/kg and 0.97%, respectively); (3) pair-feeding (PF) with cows in treatment 1 in thermoneutrality (temperature-humidity index: 61–64) with adequate concentrations of VITD and Ca in a duplicated 3 × 3 Latin square with 14-d periods followed by 7-d washouts. Blood samples were taken 4 h after feeding before the start of the trial and on d 14 of each period. The MIXED procedure of SAS was used with replication nested within the main plot and period as random effects. Before the beginning of the study, plasma B8 and B12 averaged 1.13 (SEM: 0.07) ng/mL and 222.4 (SEM: 23.0) pg/mL, respectively ($P > 0.48$). There were no plot and sub-plot effects on plasma B8 and B12 ($P > 0.15$) on d 14 of each period and respectively averaged 1.21 (SE: 0.09) ng/mL and 248.4 (SE: 25.4) pg/mL. Under the current conditions with PF cows, it can be concluded that HS did not impact plasma B8 and B12. Hence, it suggests that B8 and B12 supplements might not be necessary in mid-lactating cows under HS conditions combined to mineral and vitamin supplementation that can mitigate the impact of HS.

Key Words: cyanocobalamin, vitamin B_7, global warming

1164M Effect of pre-mowing pasture management on dairy cow behavior and production. J. St John*[1,2], T. J. DeVries[1], K. Schneider[1], and R. Bergeron[1], [1]*University of Guelph, Guelph, Ontario, Canada,* [2]*Lactanet, Montreal, Québec, Canada.*

Pasture access allows cows to perform grazing behavior and can improve dairy cow welfare as a result of reduced lameness and mastitis and adequate lying time. However, maintaining cow productivity in a more extensive pasture-based system requires efficient use of forage resources. One pasture management technique not widely explored is pre-mowing, defined as mowing grass and leaving it for cows to eat. Pasture quality has been reported to improve with pre-mowing, but whether this practice affects cow behavior and milk production remains unclear. For this study, 36 Holsteins were divided into 12 groups of 3 cows based on parity and days in milk. A crossover design was used to compare conventional (C) to pre-mowed (M) pasture, testing 4 groups per month. After 1 wk of adaptation, a week of data collection took place before switching treatments. Measures collected daily were: rumination and feeding behavior recorded by activity collars, 2×/d milk yield, and pasture utilization rate (PUR) recorded using a rising-plate meter for C pastures and by weighing grass samples pre- and post-grazing for M pastures. Milk samples were collected in a.m. and p.m., at the beginning, middle and end of the data collection week, to determine milk components including % fat, % protein, % lactose, urea, somatic cell count and fatty acid profile. The performance of each cow on C vs. M pastures was compared using a mixed model in SAS. We found that PUR was greater ($P < 0.001$) when cows grazed M pastures (63.5 ± 2.90%) compared with C pastures (21.5 ± 2.90%). In addition, dairy cows spent less ($P = 0.003$) time eating on M pastures (7.7 ± 0.16 h/d) than on C pastures (8.4 ± 0.16 h/d). Cows on M pastures tended ($P = 0.07$) to spend more time ruminating (33.4 ± 0.71% of time/d) than cows on C pastures (31.5 ± 0.71% of time/d). However, there was no detected effect on milk yield ($P = 0.62$) or milk components ($P \geq 0.05$). Overall, pre-mowed pastures may allow cows to graze more efficiently, but this difference may not have been large enough to affect milk production.

Key Words: pre-mowed, grazing, pasture utilization

1166M Prediction of orthophosphate in feces and manure from dairy cattle. J. L. Marumo*, P. A. LaPierre, A. F. Ortega, and M. E. Van Amburgh, *Cornell University, Ithaca, NY.*

Dairy cattle excreta are a valuable source of orthophosphate (Ortho-P), an inorganic form of phosphorus that is readily available for microorganisms, plant growth and development. There is, however, a growing environmental concern about the potential negative environmental impact of excessive amounts of Ortho-P excretion which can lead to eutrophication of water bodies. As a result, the development of mathematical equations to quantify and manage Ortho-P excretion on dairy farms could prove valuable for environmental sustainability. This study aimed to use literature data to develop empirical predictions for Ortho-P (g/kg) release using total phosphorus (total-P, g/kg) content of dairy cattle feces (Ortho-P_f) and manure (Ortho-P_m). Data sets from studies that evaluated and characterized the different forms of phosphorus in feces and manure from dairy cattle were compiled. After outlier exclusion, the final retained database for feces included 37 means from 5 studies while the manure comprised 23 means from 7 studies. A linear-mixed model was used to develop the predictive equations, incorporating the random effect of study. A leave-one-out cross-validation procedure was used to evaluate the predictive ability of the developed models, whereby studies were regarded as the folds. The fecal equation was determined as $\text{Ortho-P}_f\ (g/kg) = -2.447\ (0.572) + 0.966\ (0.083) \times \text{total-P}\ (g/kg)$ ($R^2 = 0.79$) and resulted in a root mean square prediction error as a percentage of mean observed value (RMSPE, %) of 32.8% and error due to random sources of 97.6%. Additionally, the manure equation was determined as $\text{Ortho-P}_m\ (g/kg) = -0.204\ (0.446) + 0.590\ (0.065) \times \text{total-P}\ (g/kg)$ ($R^2 = 0.77$) and had an RMSPE of 43.3% with random error of 93.9%. Both models revealed minimal mean and slope biases on feces and manure data. Findings suggest that these sets of equations can be used to easily predict excreted Ortho-P from total excreted P without the need for in vivo research, helping nutritionists and farmers to understand the impact of dietary P changes on the environment.

Key Words: orthophosphate, feces, dairy cattle

1167M Prediction of enteric methane emissions with and without monensin in dairy cattle. J. L. Marumo*, P. A. LaPierre, and M. E. Van Amburgh, *Cornell University, Ithaca, NY.*

The emissions of methane (CH_4) from enteric fermentation in ruminants pose a significant challenge due to its detrimental effect on climate change. However, in vivo CH_4 measurement techniques to quantify enteric emissions can be costly, making it necessary to develop predictive models. The objectives of the current study were to (1) compile a database of animal data that supplemented monensin and identify the principal dietary, animal and lactation performance input variables to predict enteric CH_4 production, (2) investigate the effect of monensin on CH_4 emissions, (3) develop empirical models to predict CH_4 production (g/d) and yield (g/kg DMI) in dairy cattle, and (4) evaluate the newly-developed models and published models in the literature. Monensin supplementation of 24 mg/kg DM significantly decreased CH_4 production and yield by 5.4% and 4.0%, respectively. Lack of robust models from the monensin database highlights the need for long-term in vivo research on the effect of monensin supplementation (<24 mg/kg DMI) on daily CH_4 production. The final retained database of 61 treatment means from combined data of lactating and nonlactating cows (COM) and a subset of 48 treatment means for lactating cows (LAC data) from 18 in vivo studies was used to develop CH_4 production and yield prediction models. Mixed-effects models were developed to predict CH_4 production and yield on COM and LAC databases with random effect of study. Leave-one-out cross-validation was performed to evaluate the predictive accuracy of the models. Feed intake (DMI) was the significant predictor of CH_4 production, and a DMI-only predictor model had similar root mean square prediction error (RMSPE; % of mean observed) value on COM and LAC data of 14.7 and 14.1%, respectively. An increase in model complexity across all databases improved prediction of CH_4 production, and CH_4 yield was best predicted by dietary forage only on COM data (RMSPE = 11.4%), while for LAC database was dietary forage, milk fat and protein yields (RMSPE = 7.4%). The new models showed improved predictions compared with published models for COM data [RMSPE = 10.7 vs. 13.2%; concordance correlation coefficient (CCC) = 0.95 vs. 0.93] and LAC data (RMSPE = 9.1 vs. 12.6%; CCC = 0.94 vs. 0.86); thus, they can be used to provide better CH_4 production and yield prediction in dairy cattle.

Key Words: monensin, methane emissions, empirical modeling

1168M Effects of weight loss on milk production of nulliparous Gyr cows. K. De-Sousa*[1], C. Gonçalves[1], GG.layk Vilela[1], A. Fernandes[2], J. Negrão[3], M. Deniz[4], and L. El Faro[1], *[1]Centro de Pesquisas de Bovinos de Corte, Instituto de Zootecnia, Serãotizinho, São Paulo, Brazil, [2]Associação Brasileira de Gir Leiteiro, Uberaba, Minas Gerais, Brazil, [3]Faculdade de Zootecnia e Engenharia de Alimentos – Universidade de São Paulo, Pirassunumga, São Paulo, Brazil, [4]Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.*

This study aimed to evaluate the relationship between postpartum weight loss and milk production of nulliparous Gyr cows. A total of 45 nulliparous dairy Gyr (average age of 35.4 ± 5.3 mo, range: 27–48 mo) were evaluated from prepartum (60 d before calving) to 305 d in milk (between 2013 and 2014) at the Brazilian Association of Dairy Gyr Breeders, in Uberaba, MG, southeast of Brazil. During the prepartum (60 d before calving) and postpartum (until d 35 of lactation) the cows were kept in an area of rotational pasture and received corn silage during the dry season (around 5 mo), and 6 kg of concentrate per day. After d 35 of lactation, the cows that produced more than 10 L/d receive 1 kg of concentrate for each 3 L of milk produced above 10 L. Body weight and milk yield (305 d) were registered monthly. The weight loss class was calculated by subtracting the body weight for each postpartum month from the prepartum weight; and divided into 3 categories: low, 0 to 27 kg; medium, 28 to 50 kg; and high, greater than 51 kg. Generalized linear models were used to determine whether weight loss influenced milk production. During the prepartum period (range: −60 d to 0 d), the cows showed on average (±SD) weight of 461.1 ± 55.5 kg (range: 343–582); while during lactation the average weight was 418 ± 51.5 kg (range: 296–594 kg). The average milk yield at 305 d was 10.4 ± 3.1 kg (range: 2–17 kg). Cows were likely ($P < 0.05$) to lose 5% of weight until 210 d in milk; after that, the cows were likely ($P < 0.05$) to increase 5% of weight by month. Most cows (81%) lost weight at the first stage of lactation (d 1–100), while 61% lost weight at the second stage (d 101–200) and 56% at the third stage of lactation (d 201–305). There was no difference ($P > 0.05$) on 305-d milk yield on cows from medium (9.9 ± 3.3, range: 3.3–15.2 kg), high (10.6 ± 2.9, range: 1.7–17 kg), and low weight loss (11.5 ± 2.8, range: 2.2–16.6 kg). Our findings suggest that under the conditions of this study, weight loss did not influence milk yield through 305 d in the first lactation of dairy Gyr cows.

Key Words: negative energy balance, lactation, Zebuine

1169M Transition management and cow performance in a sample of dairy farms in Argentina. P. Turiello*[1,2], R. C. Anton[3], M. Gorgerino[3], J. Prai[3], G. Ciepielak[3], N. Hajduczyk[3], L. Bussi[3], and C. Vissio[1,4], *[1]Universidad Nacional de Rio Cuarto, Rio Cuarto, Cordoba, Argentina, [2]Instituto de Formacion e Investigacion en Nutricion Animal, Rio Cuarto, Cordoba, Argentina, [3]Universidad Nacional de Villa Maria, Villa Maria, Cordoba, Argentina, [4]IDAS CONICET UNRC, Rio Cuarto, Cordoba, Argentina.*

The goal of this study was to describe the nutritional and management strategies and the productive performance and health events in transitioning cows in commercial dairy farms in Argentina. We surveyed a convenience sample of 29 farms (460 cows on average, range:180–1,200). We registered management factors and milk yield (n = 3,281) and health events (n = 2,640) from cows calved from April to May 2022. We measured BHBA from blood samples of 231 fresh cows (3–14 DIM) to determine subclinical ketosis. Most of the cows were housed in drylots; 97% of the farms had dry and prefresh pens, but only half of them had a fresh pen. Farms had on average a dry period of 59.9 d

Table 1 (Abstr. 1169M). Nutritional composition (average and SD) of transition diets

Pen, n	DM, %	CP, %	NEl, Mcal/kg	NDF, %	fNDF, %	Starch, %	Fat, %	DCAD, meq/kg
Dry, 24	37.2 (11.6)	13.5 (1.8)	1.5 (0.1)	44.2 (6.2)	42.5 (7.4)	12.1 (5.0)	2.7 (0.4)	275.9 (73.5)
Prefresh, 27	46.5 (6.5)	14.6 (2.0)	1.5 (0.1)	43.2 (4.6)	36.4 (6.3)	16.7 (2.9)	3.1 (0.7)	−149.5 (83.9)
Early lactation, 31	47.5 (6.1)	17.1 (1.2)	1.7 (0.1)	30.4 (2.9)	20.3 (3.9)	25.7 (2.6)	4.2 (0.9)	248.7 (81.4)

(range: 46–105 d). Lactating animals produced 33.8 ± 10.6 kg of milk on the first test day (10–30 DIM) and 38.2 ± 10.1 kg at peak (75–105 DIM). First-lactation heifers produced 84% of the milk produced by adult cows at peak. Bulk tank milk had 3.9 ± 0.18% fat and 3.5 ± 0.07% CP on average. All of the farms used anionic diets to feed prefresh cows and monitored urinary pH. Nutritional composition of transition diets is shown in Table 1. Every farm used corn silage, and soybean meal/expeller was the most common protein source at least in one diet. Alfalfa was used in every lactation diet, and wheat straw or millet hay was included in 80% of prefresh diets as a low energy forage source. Most of the farms had nutritional assessment. Mortality and culling during the first 4 mo of lactation were 1.3% and 1.9%, respectively. The average herd incidence of mastitis, metritis, hypocalcemia, ketosis, and displaced abomasum was 2.5% (range: 0–15.7), 6.8% (range: 0–18.6), 2.0% (range: 0–6.2), 7.4% (range: 0–30.0), and 0.2% (range: 0–1.5), respectively. This report is a first approach to describe the transition program in dairy farms in Argentina.

Key Words: management, milk yield, health

1170M Cow factors predictors of culling and death risk among dairy farms from Cordoba, Argentina. C. Vissio[1,2], P. Turiello*[1,3], M. Richardet[1], C. Bonetto[4], and A. Larriestra[1,4], *[1]Universidad Nacional de Rio Cuarto, Rio Cuarto, Cordoba, Argentina, [2]IDAS CONICET–UNRC, Rio Cuarto, Cordoba, Argentina, [3]IFINA, Rio Cuarto, Cordoba, Argentina, [4]Universidad Nacional de Villa Maria, Villa Maria, Cordoba, Argentina.*

The aim of this study was to evaluate the influence of cow factors on the incidence risk (IR) of culling and death in dairy farms of Cordoba, Argentina. A data set of 169 farms and 33,972 cows calved in 2018 provided by the Asociacion Regional Centro, responsible for the official test day, was analyzed. Cow factors predictors included in the analysis were parity, milk yield during the first 5 mo, being inseminated before 100 DIM, being pregnant before 200 DIM and number of tests days with SCC ≥200,000 cells/mL. The association between cow factor predictors and the time at culling or death was assessed fitting a Cox proportional-hazards model including farm as random factor. The IR of culling and death was 20.9% during lactation and 14.7% in the dry period. The median time at culling or death was 156 DIM (IQR: 69–271) for lactating cows and 43 d (IQR: 3–100) in the dry period. At herd level, the average IR was 34.9% (SD: 13.3%). The IR was lowest for cows in first compared with second lactation. Although the IR was greater for non-pregnant cows before 200 DIM (HR: 1.91; 95% CI: 1.72, 2.11) and for non-inseminated cows before 100 DIM (HR: 1.26; 95% CI: 1.14, 1.39), the former had a greater impact. No effect of milk yield on IR was observed. Cows with 4 (HR: 1.52; 95% CI: 1.21, 1.92) or 5 (HR:1.27; 95% CI: 1.06, 1.52) test days with SCC ≥200,000 cells/mL showed a greater IR of culling or death compared with those which had no peaks. There is no previous report about culling and death risk in Cordoba, Argentina. Among the studied cow factors predictors, reproduction was the most relevant, compared with other factors such as udder health, cow age, and milk yield.

Key Words: reproductive performance, somatic cell count peak, mortality

1171M Farm to gate nutrient balance in two dairy farm systems in Uruguay. L. Gil*, G. Ortega, D. Custodio, and P. Chilibroste, *Departamento de Producción animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.*

Table 1 (Abstr. 1171M). Nitrogen (N), phosphorus (P), and potassium (K) monthly inputs sources for high-production (HP) and low-cost (LC) treatments (kg/ha of grazing platform)

	N		P		K	
Source[1]	Mean	SD	Mean	SD	Mean	SD
HP						
Supplements	28.6	14.8	13.4	8.1	24.9	14.2
Fertilizer	14.8	11.1	6.6	12.5	—	—
Bedding	0.6	0.9	0.1	0.2	0.3	0.3
BNF	0.7	0.4	—	—	—	—
N deposition	1.0	0.0	—	—	—	—
LC						
Supplements	10.2	2.6	6.1	3.2	10.1	6.9
Fertilizer	14.2	9.6	8.2	13.1	—	—
BNF	1.0	0.9	—	—	—	—
N deposition	1.0	0.0	—	—	—	—

[1]BNF = biological N fixation.

Milk production in Uruguay has increased due to higher stocking rate and individual milk production. Rise of inputs such as fertilizers and dietary supplements to sustain productivity imply risk to the environment and nutrient balance can be one approach to assess sustainability. The aim of this work was to evaluate preliminarily farm-gate nutrient balance of nitrogen (N), phosphorus (P), and potassium (K), and input or output sources of 2 dairy systems (Table 1). High-productivity treatment (HP) prioritized milk production with 3 milking cow/ha of grazing platform (GP), importing forage reserve (R, from 16 ha) and concentrate (C). Low-cost treatment (LC) prioritized operational simplicity with 1.8 cow/ha GP and producing its own R. Both systems had 20.7 ha GP. Nutrient balance was calculated (Aug. 2021–Jul. 2022) by the farm-gate methodology (Oborn et al., 2003) per hectare of GP as well as per total hectare used (GP + R + dry cow area, RDM). Nutrient surpluses (kg/ha GP) for HP and LC were 363 vs. 197, 136 vs. 79, and 184 vs. 45 of N, P, and K respectively; and for RDM were 172 vs. 157, 91 vs. 67, and 40 vs. 20, respectively. Outputs in milk (kg/ha GP) were 10.2 vs. 6.5, 1.4 vs. 0.9, and 3.6 vs. 2.3 of N, P, and K, respectively. Nitrogen use efficiency (NUE, ha/GP) for HP and LC were 25% and 28%, respectively; and for hectares of RDM were 27% and 29%, respectively. Inputs exceed outputs for N, P, and K in HP and LC, so there was no mining. Supplements were the major input in HP, whereas fertilizers were in LC (kg/ha GP). Nutrient surplus per hectare of RDM were similar for HP and LC due to area increments (inputs structure changes and outputs remain equal), resulting in lower surplus and higher NUE for HP. Since NUE were below 50%, there was potential to improve.

Key Words: nutrient balance, dairy system, sustainability

1172M Canadian dairy farm water footprint: Present and future. S. Binggeli*[1], G. Jégo[2], V. Ouellet[1], A. VanderZaag[3], T. Wright[4], B. Qian[3], and É. Charbonneau[1], *[1]Département des sciences animales, Université Laval, Québec, QC, Canada, [2]Québec research center, Agriculture and Agri-food Canada, Québec, QC, Canada, [3]Ottawa research center, Agriculture and Agri-food Canada, Ottawa, ON, Canada, [4]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada.*

Although Canada has plenty of fresh water, it may become limited in some regions with climate change. The aim of this study was to evaluate water footprint (WF) under climate change of Canadian dairy farms from different regions. To do so, a whole-farm model (Nutrient Cycling:

Table 1 (Abstr. 1172M). Projected water footprint of Canadian dairy farms, liters per kilogram of fat- and protein-corrected milk

Region	Period	Drinking water	Feed production	Energy	Other	Total
East	Reference	4.0	5.3	1.3	3.6	14.2
	Near future 1	4.1	5.0	1.4	3.7	14.1
	Near future 3	4.1	5.1	1.3	3.6	14.2
	Distant future 1	4.0	5.1	1.3	3.6	14.1
	Distant future 2	4.1	5.5	1.3	3.6	14.5
	Distant future 3	4.3	6.0	1.3	3.6	15.3
West	Reference	4.3	245.2	0.8	3.6	253.9
	Near future 1	4.3	15.3	0.8	3.6	24.0
	Near future 3	4.4	16.2	0.8	3.6	25.0
	Distant future 1	4.3	4.9	0.8	3.6	13.6
	Distant future 2	4.2	4.8	0.8	3.6	13.5
	Distant future 3	4.4	5.3	0.9	3.7	14.2

Crops, Livestock, Environmental and Soil) was adjusted and used to maximize profit and calculate WF. The IDF (2017) methodology was used to calculate consumptive WF. Cows performances were adjusted for in-barn temperature and ventilation type. Animal water intakes and diet formulation were calculated using NASEM (2021) equations, and crop yield and nutritive value were projected using STICS crop model. Six different climatic scenarios were used for 3 temporal periods [reference (1981–2015); near future (2040–2069); distant future (2070–2099)] and were then grouped according to the number of days with temperature and humidity index over 58: 1 = least, 2 = intermediate, 3 = highest number of days. Four typical dairy farms were modeled, and results are presented by region: East including Halifax, Québec, and Ottawa; West including Red Deer. For both regions, the 2 main water-consuming sectors were animal drinking water (4.0–4.4 L/kg of fat- and protein-corrected milk; FPCM) and feed production (4.8–245.2 L/kg of FPCM). For the West, it is common to purchase hay from the southern irrigated region, which greatly increased WF. Projections show that future increases in on-farm feed production could decrease this need, reducing WF. Feed production WF included seeds, fertilizer, and purchased feed WF, while other included precooler, manure management, and cleaning water. Although climate is expected to increase animals' water intake, diet composition had overall a greater effect on WF (Table 1).

Key Words: water consumption, dairy cow, climate

1173M Presentation of a simplified method for on-farm greenhouse gas and ammonia emission factor calculations—Illustration based on a European study.

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Estimating the greenhouse gas (GHG) and ammonia (NH_3) emission factors (EF) of cattle farming is challenging in open barn production systems since ventilation rates are very difficult to do in such situations. To overcome this difficulty, we developed a method based on gas measurements and a farm questionnaire. The objective of this "Simplified Method" was to be able to use it in all types of cattle systems and to make it easily usable by people other than scientists, for example farm advisors or agricultural associations. To do so, this method is based on 3 steps: 1) On-farm gas concentration measurements to evaluate the indoor-outdoor gas gradients. 2) Screening protocol based on indicators and used as data quality control to evaluate the reliability level of gas measurements and possible mistakes during data collection. 3) Emission factor calculations. If the gas measurements are not reliable enough, no calculation can be performed. If they are reliable then a 3-tiered calculation approach has been set depending on the results from the questionnaire: Tier 1: no data from the questionnaire are available (only inside and outside gas concentrations), then a generic data set is used (reference data relevant for the cattle farm category). Tier 2: all critical data from the questionnaire are reliable, they are used for the EF calculations. Tier 3: use of more precise input-output data especially the quantities and qualities of feed and manure production. This level of detail can be found with complementary studies or with work done in experimental farms. This method has been applied in an international study including 8 European countries totalizing over 50 dairy farms with seasonal repetitions. Calculations showed that: CH_4 emissions from manure could double those from the enteric fermentation of the animals; the detection level of the N_2O emissions was below 1 g N-N_2O/animal/day; and, in some farms, very few seasonal variations were observed over the year, while for others they were well noticeable. The method and first results of each tier approach will be presented and discussed.

Key Words: simplified method, greenhouse gas, ammonia

1174M The availability of local tech support is the most important factor for dairy farmers when choosing precision dairy technologies.

S. Paudyal*[1], K. Kaniyamattam[1], J. Piñeiro[1,2], J. Spencer[1,3], B. W. Jones[4], and E. Kim[1], [1]*Department of Animal Science, Texas A&M University, College Station, TX,* [2]*Texas A&M Agrilife Extension, Amarillo, TX,* [3]*Texas A&M Agrilife Extension, Stephenville, TX,* [4]*Tarleton State University and Texas A&M AgriLife Research, Stephenville, TX.*

The use of precision technologies on dairy farms is increasing, but producers' experiences with these technologies vary widely. The objective was to understand the adoption experience and the perceived usefulness of precision technologies by dairy farmers in Texas. Dairy farmers were delivered printed copies of a pretested survey instrument to assess technology use, on-farm decisions, and producers' planned behavior related to technology adoption on farms. Fifteen completed surveys were returned and analyzed using descriptive statistics in Microsoft Excel. Most of the respondents used daily milk weights (53%), followed by milk components (40%), rumination sensors (33%), activity monitors (26%), cow sorting gates (20%), automatic feed pushups (13%), and 26% indicated using none of the available technologies. On a scale of 1 to 5 (least useful to very useful), respondents scored the greatest perceived usefulness for estrus detection (mean 4.25; range 1–5), followed by daily milk yield(3.88; 3–5), rumination time (3.71; 3–5), milk components (3.63; 1–5), milk conductivity (3.14; 1–5), BCS (2.85; 1–5), and rumen pH (2.0; 1–4). When choosing a technology, respondents ranked (on a scale of 1 to 5; unimportant to very important) the availability of local tech support as the most important factor (score 4.16; range 3–5), followed by proven performance through research (4.0; 3–5), simplicity of use (3.82; 3–5), benefit to cost ratio (3.66; 2–5), and total cost of investment (3.5;1–5). Other impeding factors stated by respondents include high upkeep costs, technological complexity, and skills of the current labor force. Regarding barriers to investment decisions, 26% ranked lack of access to capital as a primary issue, followed by environmental regulations (12.5%), political environment (12.5%), and sitting regulations (12.5%). Knowing what they know now about the technology, 40% would invest the same way, 6.25% would have invested even quicker, and 12.5% would not have invested. Respondents advise technology

Table 1 (Abstr. 1175M). Effect size for milk, fat and protein yields in 8 herds fed a rumen-protected B-vitamin blend

Item	Mean	Effect size	Minimum	Maximum	CI	*P*-value
Milk, kg/d	42.8	1.25	0.17	1.9	0.81 to 1.70	0.001
Fat, g/d	1,783	42.7	−18.2	81.3	−27.3 to 79.1	0.006
Protein, g/d	1,402	38.1	−9.1	72.7	16.0 to 78.2	0.001

companies to provide a standard price, reduce the cost, and provide a detailed cost analysis when selling a product.

Key Words: dairy, technology

1175M A combined analysis to evaluate effects of a rumen-protected B-vitamin blend in Upper Midwest dairy farms. K. Malinov[1], E. Evans*[2], and V. Brisson[1], *[1]Jefo Nutrition, St. Hyacinthe, QC, Canada, [2]E +E Technical Advisory Services, Bowmanville, ON Canada.*

The objective of this study was to evaluate the impact of a commercially available blend of rumen-protected B vitamins (RPBV, Jefo) on milk production in high-producing Upper Midwest (UMW) dairy herds. Herds were selected based on similarities in production and feed management typical of the region. Ten herds, averaging 649 cows/herd and producing 45 kg of milk/cow/day, were selected to participate in the study. All subscribed to monthly milk monitoring, which served as the source of data for the individual trials. Within herds cows were exposed to treatments via a crossover experimental design. Cows included were between 30 and 400 d in milk (DIM) for the first and last test date, respectively. Cows received their normal diets (corn grain and corn silage based TMR diets), modified only by the inclusion of 3 g/cow/day of the RPBV (pantothenic acid, pyridoxine, biotin, folic acid, and cobalamin) during the treatment periods. Individual trial results were analyzed using a paired T Test (Minitab 16, State College PA). Trial results were used to determine effect size for milk, fat and protein yields. Two herds did not complete the trial due to major feed changes. Statistical analyses of milk, fat and protein yield effect size were analyzed using Meta-Essentials Software (Creative Commons, Mountain View, CA, USA), using treatment as the fixed effect and farms as the random effect, as outlined by Suurmond et al. (2017). Results demonstrated that the inclusion of the RPVB increased milk and protein yield and maintained fat yield (Table 1). These results align with that observed in a previous study (Evans et al., 2021) in which the average milk yield was 35 kg. This demonstrates that similar effects of a rumen-protected B-vitamin blend on production performance can be expected on high-producing herds such those participating in this study.

Key Words: B vitamins, milk production, protein yield

1176M Comparison of deep learning-based models classifying Hanwoo (*Bos taurus coreanae*) and Holstein (*Bos taurus taurus)* individuals trained with whole-face and cropped-muzzle images. T. Lee*[1], Y. Choi[1], Y. Na[2,1], and S. Lee[1], *[1]Department of Animal Science, Konkuk University, Seoul, Republic of Korea, [2]Animal Data Laboratory, Antller Inc., Seoul, Republic of Korea.*

This study was conducted to compare the performance of deep learning algorithm-based models using muzzle pattern to classify cattle individual between model using whole-face and cropped-muzzle images. Total 560 images from 25 Hanwoo (*Bos taurus coreanae*) and 480 images from 25 Holstein (*Bos taurus taurus*) were used. Muzzle area of 50 images were manually cropped and coordinate information were extracted. Muzzle detector was trained with data blocks and coordinate axis using yolo v5 machine learning tool of Roboflow Inc., showing 96.3% accuracy detecting muzzle area. Detected muzzles were automatically cropped for crop model data set. Deep learning was done in python 3.9.12 with PyTorch 1.14.0. Data blocks for each model were randomly split, 80% for training and 20% for validation. Models were trained through data block 10 epochs with convolutional neural network algorithm with 101 layers of ResNet structure. Model trained with cropped images showed worse loss in training and validation process for both Hanwoo and Holstein compared with model trained with whole-face images. But in respect of accuracy, the cropped image model were better for Hanwoo but worse for Holstein (Table 1). This study suggests that the key images for artificial intelligence to classify cattle individuals vary by species.

Key Words: cattle classification, cropped muzzle image, deep learning

1256M Veterinarians' barriers to communication with Ontario dairy producers. G. M. Power*[1], D. L. Renaud[1], C. Miltenburg[2], K. L. Spence[1], B. N. M. Hagen[1], and C. B. Winder[1], *[1]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [2]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada.*

Many dairy producers acknowledge that veterinarians are their primary source of information. The objective of this qualitative study was to understand the barriers veterinarians face when communicating gener-

Table 1 (Abstr. 1176M). Losses from training and validation process and accuracy at start and end of training for model classifying Hanwoo (*Bos taurus coreanae*) and Holstein (*Bos taurus taurus*) with whole-face and cropped images[1]

Hanwoo: Cropped-muzzle image				Hanwoo: Face image			
Epoch	Loss_tr	Loss_val	Accuracy	Epoch	Loss_tr	Loss_val	Accuracy
0	4.392	3.661	0.135	0	3.323	4.262	0.258
10	0.671	0.088	0.981	10	0.139	0.308	0.937
Holstein: Cropped-muzzle image				Holstein: Face image			
Epoch	Loss_tr	Loss_val	Accuracy	Epoch	Loss_tr	Loss_val	Accuracy
0	3.823	2.811	0.239	0	2.581	2.603	0.519
10	0.223	0.042	0.979	10	0.062	0.057	0.990

[1]Tr = training; val = validation. Loss: distance in numeral value from prediction and actual figure. Accuracy: ratio of the correct prediction. Epoch: era of model trains through data set for once.

ally and specifically about biosecurity with their clients to improve knowledge transfer between veterinarians and dairy producers. Recruitment was through snowball sampling and the Ontario Association of Bovine Practitioners listserv. Participants completed a demographic survey using Qualtrics before their semi-structured, one-on-one interview (n = 18). Interviews with veterinarians were conducted between September 2022 and January 2023 through Zoom audio calls. Zoom also recorded and transcribed the conversation. One researcher (GP) edited the transcripts and coded them using NVivo, informed by a constructivist paradigm and grounded theory analysis approach. The participants were mostly female (61%) and between 30 and 39 years old (50%). Additionally, most veterinarians had been in practice between 5 and 9 years (44%), with others practicing for <14 years or >20 years. These veterinarians practiced in all regions of Ontario and did not exclusively work with dairy clients. Many participants attributed life and work experience to learning how to communicate effectively. Common themes surrounding communication barriers were a lack of resources, the perceived low importance of the veterinarian's advisory role, and consistency within and between farms. Specific resources of concern included time and money. Consistency within the farm focused on with whom veterinarians spoke with during calls, whereas between farms was about shifting the conversation based on the producers' mindsets. When specifically discussing preventive intervention, many veterinarians cited that those conversations would occur after the client had an issue. Understanding the barriers between Ontario dairy veterinarians and dairy producers while communicating will lead to practices to minimize the barriers and encourage veterinarians to lead more effective discussions.

Key Words: disease prevention, herd health, knowledge translation and transfer (KTT)

1257M Dairy producers' barriers to biosecurity in Ontario, Canada. G. M. Power*[1], D. L. Renaud[1], C. Miltenburg[2], K. L. Spence[1], B. N. M. Hagen[1], and C. B. Winder[1], *[1]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [2]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada.*

Biosecurity implementation on Ontario's dairy farms is variable. As biosecurity influences many areas, including animal health and welfare, production, and public health, understanding the barriers associated with its use is necessary. The objective of this qualitative study was to identify the barriers to biosecurity implementation for Ontario dairy producers. Recruitment was done through snowball sampling and advertisements. Seventeen Ontario dairy managers and owners completed a demographic survey and semi-structured, individual phone interview between July 2022 and January 2023. Zoom recorded and transcribed the interview and one researcher (GP) edited the transcripts and coded them using NVivo informed by a constructivist paradigm and grounded theory approach. Participants were from Western (53%), Southern (35%) and Eastern (12%) Ontario and were mostly male (71%). They were mostly between 30 and 39 years old (41%) and 50–59 years old (29%). Herd size ranged from 45 to 220 milking cows, using mostly automated milking systems (46%). All the participants had an overall positive association with biosecurity, either through current knowledge or a willingness to learn. Many producers related biosecurity to diseases entering the farm but overlooked other key aspects namely disease spread within or exit from the farm, suggesting some knowledge gaps. Participants stated various reasons for implementing new biosecurity protocols, such as animal health and financial benefit. The main barriers to biosecurity implementation included frustration due to a lack of initiative from other industry members, perceived low risk of disease and lack of resources. Participants cited other dairy producers, veterinarians, salespeople, and additional industry personnel as causes for frustration. Commonly reported resources that were of concern were time, money, and facilities. Some suggested solutions were more digestible information, biosecurity training or peer meetings. Understanding dairy producers' biosecurity practices, knowledge, and barriers to improved implementation is beneficial for veterinarians, researchers, and regulators to allocate resources effectively.

Key Words: disease prevention, management practices, farmer perception

Reproduction 1

1177M Demonstration of an automated system for cattle reproductive management under the Internet of Things framework: e-Synch system and cow responses. Y. Ren[1], D. Duhatschek[2], C. C. Bartolomeu[3], A. L. Kerwin*[2], D. Erickson[1], and J. O. Giordano[2], *[1]Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, [2]Department of Animal Science, Cornell University, Ithaca, NY, [3]Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil.*

Our objective was to demonstrate system functionality and cow responses to the e-Synch, an intravaginal electronically controlled hormone delivery and sensing device with an Internet of Things (IoT) platform. e-Synch includes a case with wings, an antenna, a control board, battery, wireless charging coil, and 2 reservoirs connected to pumps. An accelerometer and temperature sensor are integrated to the circuit board. The IoT platform includes a gateway and a radiofrequency module. Data are displayed in DataStudio. Experiments were done to demonstrate functionality of the IoT platform, hormone release mechanisms, and sensors, and evaluating cow behavioral and vaginal responses to 8 h of exposure to e-Synch. In Experiment (Exp) 1, cows (n = 20) were assigned (n = 5–6 per group) to receive 100 μg of GnRH in 2 mL intramuscular (IM), 100 μg of GnRH in 2 mL with e-Synch, or an empty e-Synch device. In Exp 2, cows (n = 37) were assigned (n = 6–7 per group) to receive 2 mL of GnRH IM, and 100 or 1,000 μg of GnRH in 2 or 10 mL of solution via e-Synch. Before and after device insertion, behavior (signs of discomfort and pain), tail raising (Exp 2), vaginal integrity, and mucus scores (vaginoscopy) were recorded. Impedance shift was used to evaluate signal attenuation by body tissue and analyzed by *t*-test. Despite signal discontinuity due to attenuation by body tissue (before vs. after insertion: −36.9 dBm vs. −87.4 dBm; $P < 0.001$), devices (n = 6) communicated with the IoT platform in 24/27 instances (Exp 2) with sensor data received for at least 1–15 min period during 8 h. Variation in accelerometer data (±8.565 m/s^2) was consistent with cow activity and vaginal temperature (39.1°C; 38.6–39.5°C) demonstrated sensor functionality (Exp 2). Hormone release was confirmed in all use instances except one. Cow behavior, tail raising, vaginal integrity, and mucus scores were mostly unaltered in cows that received e-Synch devices. The e-Synch device integrated with an IoT platform might be used to automate intravaginal hormone delivery and sensing for controlling the estrous cycle of cattle.

Key Words: estrous cycle control, sensors, Internet of Things

1178M Reproductive performance at first artificial insemination of dairy cows assigned to estrous detection or a Double-Ovsynch program. A. Valenza[1] and A. Bach*[2,3], *[1]Ceva Salute Animale, Agrate Brianza, Italy, [2]Marlex Research and Education, Barcelona, Spain, [3]ICREA, Barcelona, Spain.*

At calving, 2,213 Holstein cows from 5 farms were randomly allocated to 2 reproductive schemes to determine their effectiveness in promoting pregnancy at first AI. The 2 schemes were: 1) breeding based on heat detection [HD; 1,141 cows, 34% primiparous (PMC)], or 2) after a synchronization program (SP; 1,072 cows, 35% PMC). Cows were enrolled between July 2021 and November 2022. Cows on HD were bred whenever detected on heat using an accelerometer if DIM >50, and those not showing estrous by 80 DIM (47.9%) were synchronized using either prostaglandin (PG) or an Ovsynch protocol. Cows on SP received a pre-Ovsynch treatment, which consisted of an injection of PG between 52 and 59 DIM followed by a GnRH 3 d later; then 7 d later, the first GnRH of Ovsynch (GnRH on d 0; PG on d 7 and 8; GnRH on d 9, and inseminated 16 h after second GnRH). First service was performed between 73 and 80 DIM. Cows were checked for pregnancy by ultrasound at 32 and 68 d post-breeding. Conception rate (CR), DIM at first breeding, and DIM at pregnancy were calculated for every cow. The proportion of cows pregnant by 150 DIM was calculated. The effect on these parameters of the reproductive scheme, parity, season (hot, June to October; or cold, November to May), and their interactions was assessed using a mixed-effects model accounting for the random effect of herd. Days at first AI were affected ($P < 0.01$) by an interaction between treatment and season. During the cold season, HD and SP cows were first bred at 72.5 ± 1.96 and 76.1 ± 1.96 DIM, respectively; whereas during the hot season, the difference between treatments increased (71.0 vs. 77.9 ± 1.96 DIM, respectively). At first AI, PMC had a greater ($P < 0.001$) CR (44.5 ± 0.03%) than multiparous cows (37.9 ± 0.03%), independently of treatment; but CR tended ($P = 0.05$) to be lower in HD (37.1 ± 0.03%) than SP (41.5 ± 0.03%) cows. The proportion of cows pregnant by 150 DIM did not differ between HD (66.3.2 ± 0.06%) and SP (64.2.2 ± 0.06%) cows. Relying on heat detection may allow for a first AI at lower DIM, but CR tends to decrease when compared with an Ovsynch program, the proportion of cows that reach pregnancy by 150 DIM does not differ, and a large proportion of HD cows not showing estrous needs to be enrolled in a synchronization protocol to become pregnant.

Key Words: conception, fertility, program

1179M Effects of in vitro maturation in the presence of resveratrol, chlorogenic acid, and ellagic acid on in vitro bovine embryo development. K. Giller*[1], D. Schmid[1], I. Serbetci[2], M. Meleán[2], H. Bollwein[2], and C. Herrera[2], *[1]Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland, [2]Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.*

In vitro embryo production (IVP), used for reproduction of dairy cows with high breeding values, promotes the generation of reactive oxygen species that may impair embryo development. Adding antioxidants to the media used throughout the maturation, fertilization, and culture steps may overcome this problem. The aim of this study was to investigate whether a potential "antioxidant priming" of bovine oocytes during in vitro maturation (IVM) allows to refrain from antioxidant supplementation during the following production steps. Slaughterhouse ovaries were used to obtain bovine cumulus-oocyte complexes (n = 1,187) for IVP. The IVM medium was supplemented with antioxidant polyphenols [resveratrol (RES), chlorogenic acid (CHA), or ellagic acid (ELA)] in increasing concentrations of 0.25, 0.5, and 1 μ*M* with 3 replicates per polyphenol treatment. Dimethyl sulfoxide supplemented IVM medium was included as vehicle control. In vitro fertilization and embryo culture media were not supplemented. Cleavage, blastocyst (both as % of initial oocyte number), and hatched blastocyst (% of blastocysts) rates were calculated. Groups of 5 blastocysts derived from oocytes treated with 1 μ*M* per polyphenol were used for measuring the oxygen consumption rate (OCR) in the Seahorse XFp Analyzer. One-way ANOVA was performed including replicate as random variable. Cleavage, blastocyst, and hatched blastocyst rates ranged 81–91%, 39–56%, and 51–88%, respectively, but did not significantly differ between treatments. Likewise, the OCR did not differ ($P > 0.05$) between treatments and equaled, on average, 2.7 ± 0.14 pmol/min (mean ± SEM). In conclusion, the application of RES, CHA, and ELA at concentrations up to 1 μ*M* in bovine IVM medium did not provide a benefit in terms of embryo development. The lack of differences between the experimental groups may have resulted from overall low oxidative stress in the applied IVP system, as reflected by the high in vitro embryo development rates in the control group.

Key Words: polyphenols, blastocyst rate, oxygen consumption

1180M Key performance indicators used by dairy consultants during the evaluation of reproductive performance during routine visits.

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Dairy farms need thorough and efficient control on reproduction. Consultants specialized on reproduction use key performance indicators (KPI) to monitor the reproductive performance of the farms and must be able to decipher between the approach in a first visit, and routine visits. Consultants (n = 49) specialized in dairy reproduction from 21 countries responded to an online survey conducted to determine the most suitable parameters in a routine visit every 2 to 4 weeks. The survey was comprised of 190 questions, 178 of them rated from 0 (irrelevant) to 10 (maximum importance) points. The questions were divided into 5 sections: 1) consultant and farm model, 2) general data of the farm, 3) cow reproduction, 4) postpartum and metabolic disease, and 5) heifer reproduction. The median, interquartile range, minimum and maximum values and 95% CI were determined for each question. A multivariate analysis, using between-group linkage via Ward's hierarchical clustering was conducted to generate clusters of consultants according to their response pattern. A chi-squared test was conducted to assess the association between years of experience of the consultant and farm size within the clusters generated in each section of the questionnaire. The majority of the consultants considered 34 parameters to be highly important (rate 8–10) to analyze during routine visits. Consultants considered that all 5 sections are critical to control. Consultants selected KPI that reflect heat detection, fertility, farming efficiency and information on reproductive efficiency in the near future for cows, such as postpartum and metabolic diseases. Farm size and years of experience of the consultant did not influence the type or number of parameters chosen as KPI during routine visits. The parameters rated with the highest importance (rate 10) that could be considered for an easy, fast, and universal use in routine visits to assess the reproductive status were: First service CR (%), overall PR (%) for cows, and age at 1st calving (d) for heifers.

Key Words: improvement, management, metrics

1181M Milk composition of cows in estrus.

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Efficient estrus detection and correct insemination timing are fundamental to a successful reproductive management program in dairy cows. The objective of this study was to evaluate milk component profiles around estrus using Fourier Transform Infrared Spectroscopy (FTIR). The first experiment involved analysis of milk FTIR data obtained from the Lactanet milk recording program. The breeding records of Holstein cows were used to identify cows in definitive estrus based on the successful insemination leading to parturition and initiation of the next lactation (n = 25,797). Test days (−10 to 0) relative to the successful insemination date (d −10 = test day was 10 d before the insemination date; d 0 = test day and insemination on the same day) were selected to analyze FTIR spectral data. A linear mixed model, which included the random effects of herd nested within the geographical region and the fixed effects of lactation, region, and estrus dates was applied to investigate changes in milk component profiles on days leading to estrus. Milk fat% and fat:protein ratio were higher on d −1 (3.99% and 1.25, respectively, $P < 0.0001$) and d 0 (4.06% and 1.27, respectively, $P < 0.0001$) compared with all other days. The other milk components such as protein, urea, BHBA and somatic cell count did not show a trend among the 10 d before estrus. The second experiment was carried out in lactating Holstein cows (n = 9), in which estrus was synchronized using the Ovsynch protocol beginning at d 42 in milk. The onset of estrus began 12 h after the second GnRH treatment. The ultrasound examination revealed that the dominant follicle developed from about 6 d before and ovulated within 24 h after the onset of estrus. Milk fat % increased from the second GnRH administration (4.48%) to the onset of estrus (6.6%, $P < 0.05$) followed by a decline until 2 d after estrus (4.7%). The other milk parameters did not show a trend. The results of both experiments suggest the potential exploration of milk fat profiles around estrus as a tool for on-farm estrus detection procedures which could aid in precision dairy farming leading to sustainable animal agriculture. However, further analyses are needed to explore the potential of milk composition especially fat profiles to discriminate between cows approaching versus not approaching estrus.

Key Words: dairy cow, estrus, milk fat %

1182M Effect of delaying the additional $PGF_{2\alpha}$ on luteolysis in a modified 6-day Co-Synch protocol without progesterone implant for dairy heifers.

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Our objective was to determine the effect of delaying the additional $PGF_{2\alpha}$ treatment at the end of a modified 6-d Co-Synch protocol in dairy heifers on luteolysis dynamics. Holstein heifers (n = 258) were pre-synchronized with $PGF_{2\alpha}$ (Pre-PGF) 2 d before the first GnRH (100 μg) of the program (d 0). On d 6, all heifers received a $PGF_{2\alpha}$ (PGF1) and were randomly assigned to 2 treatments, P6&7 or P6&8. The additional $PGF_{2\alpha}$ was administered on d 7 in P6&7 heifers and on d 8 in P6&8. On d 8, all heifers received a second GnRH (G2). Each $PGF_{2\alpha}$ injection consisted of 0.5 mg of cloprostenol. Ovarian ultrasonographic examinations (US) were performed on d 0, 2, 6, 8, and 10. Blood samples were collected on d 6, 7, 8, and 9 in a subset of heifers (n = 100) to determine serum progesterone (P4) concentrations. Binary and continuous variables were analyzed by logistic and linear regression, respectively. All response variables analyzed ≤d 7 were similar for heifers in both treatments. For instance, the proportion of heifers with a visible CL by US (P6&7: 92.8% vs. P6&8: 96.2%, $P = 0.24$), with more than 1 CL (P6&7: 11.2% vs. P6&8: 11.1%, $P = 0.98$), and with P4 >1 ng/mL at d 6 (P6&7: 93.9% vs. P6&8: 96.1%, $P = 0.62$) did not differ between treatments. In addition, mean serum P4 at d 6 (P6&7: 4.07 ± 0.33 vs. P6&8: 3.45 ± 0.21; $P = 0.17$) and d 7 (P6&7: 0.67 ± 0.09 vs. P6&8: 0.54 ± 0.07; $P = 0.18$) were similar between treatments. A greater proportion ($P = 0.02$) of heifers in P6&7 had P4 <0.40 ng/mL (89.1%) than P6&8 (67.3%) on d 8, but no difference was observed on d 9 (P6&7: 97.8% vs. P6&8: 95.9%; $P = 0.98$). Only 6/256 heifers ovulated from d 6 to 8 (before G2; P6&7: 4.0% vs. P6&8: 0.8%, $P = 0.13$). Treatment did not affect ovulatory response to G2 (P6&7: 92.0% vs. P6&8: 94.7%; $P = 0.40$) and ovulatory follicle size (P6&7: 14.14 ± 0.15 vs. P6&8: 14.13 ± 0.14; $P = 0.76$). In summary, delaying the additional $PGF_{2\alpha}$ to the day of G2 decreased the proportion of heifers with P4 <0.4 ng/mL on that

Table 1 (Abstr. 1184M). Effects of supplemental rumen-protected arginine (RPA) on reproduction in Holstein cows

Item	CON (n = 50)	RPA (n = 52)	SEM	*P*-value
HR days to first ovulation (95% CI)	1.0	0.69 (0.44–1.08)	—	0.11
Cyclic by 39 DIM, %	89.5	79.1	6.8	0.19
Conceptus				
DIM at AI	57.8	58.3	0.4	0.38
Progesterone d 6 to 16, ng/mL	3.48	3.57	0.18	0.72
Pregnant d 16, %	66.7	72.8	8.3	0.59
Conceptus length, cm	10.8	11.5	1.3	0.70
IFNτ, ng/mL	2.18	3.09	0.75	0.38
21-d cycle AI rate, %	68.7	78.1	4.3	0.07
Pregnancy per AI, %	21.8	34.3	5.1	0.03
21-d cycle pregnancy rate	15.0	26.9	4.5	0.02
HR of days to pregnancy (95% CI)	1.0	1.99 (1.23–3.21)	—	0.01

day, but it did not affect the proportion of heifers with P4 <0.40 ng/mL on d 9 and ovulatory response to G2 compared with the P6&7 group.

Key Words: heifers, timed AI, luteolysis

1183M Prevalence of anatomical defects and pathological conditions involving the cervical os and cranial vagina in lactating dairy cows evaluated using a digital vaginoscope.

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Early detection of reproductive tract abnormalities can guide treatment and culling decisions and, therefore, have the potential to improve reproductive and economic outcomes. Objectives were to characterize the prevalence of anatomical defects and pathological conditions involving the cervical os and cranial vagina. Lactating dairy cows (n = 5,042; 1,601 primiparous, 3,441 multiparous) from 3 farms were enrolled in a cross-sectional study. Cervical os and cranial vagina were examined using a digital vaginoscope (AlphaVision v2, IMV Technologies) during weekly herd visits [1 (n = 3,864), 2 (n = 972), 3 (n = 181), 4 (n = 22), or 5 (n = 3) exams performed per cow]. Anatomical abnormalities, delayed cervical involution postpartum, inflammation, trauma, and cervical prolapse were evaluated cow-side (live image) and using recorded still images. Vaginal discharge (VD) was scored (0 = no discharge, 1 = clear mucus, 2 = flecks of pus, 3 = mucopurulent $<50\%$ pus, 4 = mucopurulent $\geq 50\%$ pus, 5 = watery brownish/reddish liquid). Metritis was defined as VD 5 within 21 d in milk (DIM) and purulent vaginal discharge (PVD) as VD ≥ 3 after 21 DIM. Prolapse of the first cervical ring was classified as full or partial. Metritis prevalence was 5.1% (0–7 DIM) and 7.4% (8–21 DIM; live image) or 2.9% (0–7 DIM) and 2.6% (8–21 DIM; live image). Prevalence of PVD was 10.0% (22–35 DIM), 7.8% (36–50 DIM), 5.5% (51–70 DIM), 1.3% (71–100 DIM), 0.6% (101–150 DIM), and 1.5% (>150 DIM). Proportion of cows with well-defined cervical folds were 23.8% (0–7 DIM), 53.0% (8–21 DIM), 84.8% (22–70 DIM), and 95.9% (>70 DIM). Proportion of cows with bruising was 72.4% (0–7 DIM), 37.3% (8–21 DIM), 12.1% (22–35 DIM), and 2.7% (>35 DIM). Prevalence of full prolapse decreased after 21 DIM, whereas that of partial prolapses remained constant across DIM. Prevalence of adhesions (0.5%), double cervical os (1.7%), masses (1.1%), and scars (0.9%) was small. The variability in cervical recovery postpartum depicted in this study is expected to partially explain subsequent reproductive efficiency.

Key Words: reproduction, cervix, uterine disease

1184M Effects of rumen-protected arginine (RPA) on conceptus development and reproduction in dairy cows.

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The objectives were to determine the effects of supplying 30 g/d of metabolizable arginine (Arg) as rumen-protected Arg (RPA) from 250 d of gestation to 21 d in milk (DIM) on d-16 conceptus development and reproduction in dairy cows. A total of 102 Holstein cows were blocked by parity and predicted transmitting ability of energy-corrected milk (ECM) in nulliparous or 305-d ECM yield in parous cows. Within block, cows were randomly assigned to control (CON), 30 g/d of metabolizable protein (MP) from heat-treated soybean meal, or RPA, 30 g/d of metabolizable Arg (Ajinomoto Co. Inc.), which increased the dietary Arg from 5.7 to 7.5% of the MP (NASEM Dairy, 2021). Ovaries were scanned by ultrasonography weekly from 11 to 39 DIM. The estrous cycle was synchronized for insemination (AI) and blood was assayed for progesterone from d 6 to 16 after AI. The uterus was flushed on d 16 after AI and the conceptus was evaluated and interferon-tau (IFNτ) concentration measured in the fluid. The estrous cycle was re-synchronized, and reproduction evaluated for the first 300 DIM. Data were analyzed by generalized mixed-effects models fitting a binomial distribution using the GLIMMIX procedure of SAS. Days to first ovulation and to pregnancy were analyzed by the Cox's proportional hazard regression model in SAS and the hazard ratios (HR) and respective confidence intervals (CI) calculated. Results are presented in Table 1. Supplementing RPA did not affect conceptus development, but it improved pregnancy per AI, which increased the rate of pregnancy.

Key Words: arginine, conceptus, reproduction

Ruminant Nutrition: Calves and Heifers 1

1185M Effect of starter protein content and milk replacer feeding level on calf performance during preweaning. G. Frossasco[1,2], A. Echeverria[1,3], and P. Turiello*[4,5], [1]*Universidad Nacional de Villa Maria, Villa Maria, Cordoba, Argentina,* [2]*INTA, Rafaela, Santa Fe, Argentina,* [3]*INTA, Manfredi, Cordoba, Argentina,* [4]*Universidad Nacional de Rio Cuarto, Rio Cuarto, Cordoba, Argentina,* [5]*Instituto de Formación e Investigación en Nutricion Animal (IFINA A), Rio Cuarto, Cordoba, Argentina.*

In milk-fed calves, starter protein is key for microbial crude protein synthesis and ruminal development (RD). The aim of the study was to evaluate the effects of the level of CP of the starter combined with different levels of milk replacer (MR) on performance and blood metabolites of young calves. The concentration of CP of the starter was 18 or 26% and the MR (21% CP and 12% fat) was offered at 500 or 750 g/d from trial entry (2–3 d of life) to weaning (60 d of trial). Male Holstein dairy calves (40.8 ± 9.05 kg body weight) were randomly assigned to 1 of 3 treatments (n = 6): high (H; 750 g/d MR + 26% CP starter), medium (M; 500 g/d MR + 26% CP starter), or low (L; 500 g/d MR + 18% CP starter). Starters were iso-energetic (2.8 Mcal ME/kg DM) and with similar ingredients except for milk powder (12.5% in DM) in 26% CP starter. The performance variables were measured once a week and analyzed as a completely randomized design with repeated measures. Blood metabolites were measured at the beginning and end of the trial and were analyzed using ANOVA. The treatment differences were compared using the LSD Fisher test ($P < 0.05$). All performance variables increased significantly with calf age ($P < 0.01$) and there was no effect of treatment by week ($P > 0.10$). Although no statistical difference was observed in starter or total dry matter intake ($P > 0.05$), there was a positive response on average daily gain (ADG) with increasing starter protein and MR intake (L = 558, M = 662 and H = 761 ± 31.0 g/d; $P = 0.04$). At weaning, BHB concentration, an indicator of RD in pre-ruminant calves, was greater in calves consuming low levels of MR but fed with a high-CP starter (M = 0.32 vs. 0.22 and 0.22 ± 0.02 mmol/L in L and H; $P = 0.01$). The glucose concentration was greater in M and H (101.3 and 104.7 vs 75.8 ± 5.06 mg% in L; $P = 0.01$), likely because of the greater intake of digestible nutrients through MR or high-CP starter. In conclusion, under low or moderate rates of MR feeding, using a higher-CP starter allows for greater calf growth, although RD should be monitored in the latter case.

Key Words: rumen development, growth, intake

1186M Effects of different tropical forages sources in the diet of dairy calves. A. F. Toledo*[1], A. P. Silva[1], C. R. Tomaluski[1], R. D. F. Barboza[1], I. C. R. Oliveira[1], N. I. Carvalho[1], E. D. Marino[1], J. G. Dantas[1], A. M. Teixeira[2], and C. M. M. Bittar[1], [1]*Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil,* [2]*Methodist University of Piracicaba, Piracicaba, SP, Brazil.*

The study aimed to investigate the effects of different tropical climate forage sources in the TMR of dairy calves on performance and rumination time. A total of 48 Holstein calves were used in a randomized block design, with the blocks defined by sex, birth date, and BW at 28 d of age, when the total mixed ratio (TMR) supply started. Four experimental solid diets based on ground corn, soybean meal, and wheat meal were evaluated: 1) Starter concentrate (SC: 16.9% no-forage NDF); 2) TMR with 7.5% high-quality chopped hay (HQ: 20.9% NDF); 3) TMR with 7.5% low-quality chopped hay (LQ: 21.5% NDF); 4) TMR with 10% corn silage (CS: 20.1% NDF). The calves were evaluated from 28 d to 56 d of age. Calves were equally managed during the first 28 d, fed with 6 L/d of whole milk and a commercial pelleted starter concentrate ad libitum. After that, the experimental solid diets were fed ad libitum every morning, and calves were gradually weaned from 52 d to 56 of age. Dry matter intake (DMI) was measured daily while average daily gain (ADG) was evaluated weekly, and a behavioral analysis was performed on the seventh week. There was an interaction between age and diets for solid diet DMI ($P < 0.04$), with an increase at wk 7 until weaning for calves fed with LQ compared with those fed SC. The intake of HQ and CS diets did not differ compared with the other diets. The same result was observed for solid diet intake as % BW ($P < 0.02$). On the other hand, diets had no effect ($P > 0.05$) on ADG (645.7 g/d, SEM = 31.71), efficiency (0.54, SEM = 0.027) or final weight (64.8 kg, SEM = 2.58). Rumination time during weaning process was higher for calves fed HQ and LQ as compared with SC, with no differences for SC ($P < 0.03$). Including 7.5% chopped hay–low quality in the TMR maximized the feed intake around weaning, and both qualities of hay on the TMR diets anticipated the rumination cycles during the weaning. Corn silage did not negatively impact calves' intake and performance, indicating a potential alternative source of fiber in the diets preweaning. Data suggest that the presence of fiber from forage, regardless of quality, is essential for the preweaning diets.

Key Words: effective fiber, hay, rumination

1187M Effects of forage supplementation on growth and development of pre-weaning Holstein calves. K. M. Krause* and E. E. Felton, *West Virginia University, Morgantown, WV.*

The objective of this study was to assess the effects of offering chopped grass hay in the first 10 weeks of life on feeding behavior, rumen pH, intake, and growth of calves. Thirty neonatal Holstein calves were individually housed and randomly assigned to treatment (either ad libitum access to chopped grass hay or no forage; n = 15 calves per treatment, 13 bulls and 2 heifers). All calves were provided ad libitum access to water and starter throughout the study. All calves were offered 4 L of milk replacer per day from d 1 to 7, 6 L/d from d 8 to 47, and 3 L/d from d 48 until weaning at d 56. Feeding behavior was observed for a 2-h period during wk 2, 4, 6, and 8. Body weights (BW) were collected weekly. Rumen fluid samples were collected during wk 9, and blood samples were collected wk 5, 7, and 9. At the end of the study, 5 calves from each treatment were slaughtered to measure rumen development parameters. Overall starter intake did not differ between the 2 treatments, but total dry matter (DM) intake from solid feed (starter plus hay) was higher during wk 8 (2,659 ± 66 g/d vs. 2,392 ± 66 g/d; $P = 0.005$) and wk 9 (3,452 ± 66 g/d vs. 3,268 ± 66 g/d; $P = 0.05$) for calves offered hay. Weekly BW and average daily gain did not differ between treatments. Calves offered hay spent more time ruminating than calves not offered hay (17.4 ± 1.84 vs. 11.8 ± 1.84 min/2-h period; $P = 0.04$) and tended to spend more time chewing (21.8 ± 2.02 vs. 16.5 ± 2.02 min/2-h period; $P = 0.07$). Rumen pH was higher in calves fed hay compared with those fed no forage (6.02 ± 0.15 vs. 5.39 ± 0.16; $P = 0.01$). Blood β-hydroxybutyrate did not differ between treatments. No differences were found in the weight of full and empty digestive tracts as a percentage of BW between treatments. In conclusion, offering chopped hay to pre-weaning calves can promote total DM intake and increase time spent ruminating, resulting in increased rumen pH, without affecting BW gain.

Key Words: calves, forage, growth

1188M Impact of starter starch content on pre-weaning performance of beef × dairy calves.

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The objective of this study was to investigate the impact of starter starch levels on pre-weaned beef × dairy calves. Angus × Holstein bull calves (n = 39) were alternatingly assigned at enrollment to a high starch (HS: 26% starch, 20% protein, 7% fiber) or low starch (LS: 13% starch, 20% protein, 11% fiber) starter. Calves were sourced from the Iowa State University Dairy Research and Teaching Unit (ISU; n = 7) and a local dairy (LOC; n = 32) for an 18-d enrollment, and initial body weights (BW) were recorded. Calves (≤24 h old) from LOC were delivered 1×/d. Blood samples were collected at 24–48 h of age, and serum proteins were estimated using a Brix refractometer. Starter was offered free choice beginning d 1; intake was measured daily. Calves were weaned at d 57. Body weights were collected for all calves on Tuesdays and Fridays. Frame size measurements [body length (BL), heart girth (HG), withers height (WH), hip height (HH), hip width (HW)] were taken once before leaving the research farm; all calves aged between 63 and 81 d. Average daily gain (ADG) until weaning, weaning weight (WW), and frame size measurements were analyzed using PROC GLM (SAS 9.4) with the effects of source, starter, initial BW, serum protein level, age, and starter × age. Starter intake and BW were analyzed in PROC MIXED with the addition of repeated measures by calf. Source impacted initial BW (ISU: 38.2 ± 2.65 kg vs. LOC: 45.1 ± 0.87 kg; $P < 0.01$) and ADG (ISU: 0.85 ± 0.038 vs. LOC: 0.74 ± 0.024 kg/d; $P < 0.01$). Starter type did not influence daily intake (HS: 0.49 ± 0.059 vs. LS: 0.56 ± 0.060 kg/d; $P = 0.98$), ADG (HS: 0.77 ± 0.034 vs LS: 0.79 ± 0.034 kg/d; $P = 0.68$), BW (HS: 55.6 ± 0.66 vs. LS: 56.0 ± 0.66 kg/d; $P = 0.18$), or WW (HS: 85.4 ± 1.81 vs. LS: 86.6 ± 1.82 kg; $P = 0.68$). For frame size, HG (HS: 111.3 ± 1.13 vs. LS: 112.4 ± 1.19 cm), WH (HS: 89.6 ± 0.55 vs. LS: 89.3 ± 0.58 cm), and HH (HS: 93.5 ± 0.64 vs. LS: 93.8 ± 0.67 cm) were influenced by starter ($P \leq 0.05$). Although all calves were >7.0 on Brix scale, serum protein affected ADG, starter intake, WW, BL, WH, and HH ($P \leq 0.05$). Overall, pre-weaning calves showed limited response to starter starch content in this trial.

Key Words: beef × dairy, starch, weaning

1189M Impact of trace mineral source supplied in milk replacer on performance of calves fed via an automatic calf feeder.

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The objective was to evaluate amino acid-complexed trace minerals (TM) in milk replacer (MR) fed to calves via automatic calf feeder (ACF) on calf performance. Forty Holstein calves (n = 10/treatment) were assigned to 1 of 4 treatments in a CRBD: 1) backgrounded in individual pens for 3 d before introduction to an ACF; fed 100% of their trace mineral in MR from sulfate sources (3INO), 2) backgrounded for 10 d and fed the same treatment as 3INO (10INO), 3) backgrounded for 3 d and fed 100% of trace minerals in MR from amino acid complexed sources (ProPath, Zinpro Corp.; 3ORG), or 4) backgrounded for 10 d and fed the same treatment as 3ORG (10ORG). Calves on all treatments were fed trace minerals at the same rate in MR (50, 50, 10, 100 ppm of Zn, Mn, Cu, and Fe, respectively) with 100% of TM supplementation coming from either inorganic (INO) or amino acid-complexed (ORG). Calves were fed either INO or ORG MR from first feeding after colostrum, and starter was offered from d1. Starter TM source was not different. Following backgrounding, calves were introduced to an ACF and allowed ad libitum intake of MR for 40 d followed by a 16-d weaning period. Feed intake was measured daily and BW and frame measures were recorded weekly. Data were analyzed using Proc GLM, MIXED, and FREQ in SAS 9.4 (SAS Institute Inc.). Least squares means were separated using orthogonal contrast statements and, here, the main effects of TM source are discussed. Calves fed ORG consumed more MR (6.23 vs. 6.10 L/d; $P < 0.01$) compared with calves fed INO. Calves fed INO consumed more starter compared with calves fed ORG ($P < 0.01$). Due to increased starter intake, overall DMI was greater for calves fed INO ($P < 0.01$). However, body weight and ADG were not different; thus, calves fed ORG experienced improved FE compared with INO calves (0.58 vs. 0.36 gain:feed; $P = 0.02$). Additionally, calves fed ORG accumulated more body length (5.1 cm; $P = 0.05$) and tended to accumulate more hip width (1.5 cm; $P = 0.13$) over the trial. Results agree with previous work in calves fed amino-acid complexed trace minerals by way of improved feed efficiency.

Key Words: whole milk, trace minerals, group feeding

1190M The impact of feeding a whole milk fortifier to calves on a commercial dairy farm on calf growth.

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The recently updated NASEM provides a trace mineral (TM) requirement of 65, 60, 5, and 85 ppm for Zn, Mn, Cu, and Fe, respectively, in the liquid diet of the young calf. Most milk replacers are formulated to provide these levels of TM; however, whole milk (WM) does not provide the same suggested levels to the calf. Additionally, WM does not provide the recommended levels of Vitamins D, E, B_1, B_3, B_6, B_9, or B_{12}. The objective of this study was to assess the impact of adding a WM fortifier containing the above-described nutrients on calf growth in calves fed WM. This study was conducted on a commercial dairy in California. A total of 364 calves were fed 1 of 2 treatments: 1) A control WM diet fed at a rate of 3.8 L/d (CON), or 2) CON with the additional 5 g/d of a WM fortifier designed to meet NASEM levels of Zn, Mn, Cu, Fe (Zn, Mn, Cu, and Fe provided from amino acid complexed sources; Zinpro), I, vitamins D and E, thiamine, niacin, folic acid, and vitamins B_6 and B_{12} (FORT). Calves began receiving their treatments at the first WM feeding following colostrum feeding and received their treatments until weaning (d 70). Calves were observed until d 90 of age, and body weights were recorded at d 0, 30, 60, and 90 and ADG calculated. Calves were housed individually and had ad libitum access to grain and water. Data were analyzed using Proc MIXED in SAS 9.4. Average daily gain did not differ from 0 to 30 d of age (441 vs. 459 g/d for FORT vs. CON; $P > 0.10$). However, ADG was improved for calves fed FORT from d 0 to 60 (532 vs. 377 g/d for FORT vs. CON; $P < 0.05$) and overall (d 0 to 90; 918 vs. 818 g/d for FORT vs. CON; $P < 0.05$). The greatest magnitude of response occurred between d 30 and 60 where calves fed FORT grew at 227 g/d greater rate compared with CON calves ($P < 0.01$). A tendency existed for calves fed FORT to grow at a faster rate from d 60 to 90 (1,782 vs. 1,750 g/d for FORT vs. CON; $P < 0.10$), which may be indicative of a potential carryover effect of the FORT treatment post-weaning. Overall results indicate that providing additional TM and vitamins to calves fed WM show promise to improve growth performance.

Key Words: whole milk, trace minerals, vitamins

1191M Effect of milk replacer nutrition on performance and health of beef × dairy calves. M. Scott[1], M. Klejeski[2], V. Dahle[2], B. Hansen[1], and I. Salfer*[3], *[1]Milk Specialties Global, Eden Prairie, MN, [2]University of Minnesota Southern Research and Outreach Center, Waseca, MN, [3]University of Minnesota, Saint Paul, MN.*

Dairy producers are seeking to add value to their dairy operation by marketing crossbred calves produced by breeding their lower genetic merit dairy cows to beef breed sires. Currently, limited research has been conducted on the impact of milk replacer nutrition on the growth of beef × dairy calves. The objective of this study was to examine the effects of increased protein and fat concentration of milk replacer (MR) on growth, feed intake, feed efficiency, and health of beef × dairy calves during pre- (d 1 to 42) and post-weaning (d 43 to 56) periods. Angus-Holstein cross bull calves (n = 40; 2 to 5 d old) were randomly assigned to 1 of 2 treatments in a completely randomized design. Treatments included either a 20% protein, 20% fat MR (MR20) or a 24% protein, 24% fat MR (MR24). Body weight (BW) was measured at d 0, 14, 28, 42, 49 and 56. Fecal scores were measured weekly. Feed intake was measured daily and calculated bi-weekly. Calves were housed in individual outdoor hutches and the experiment was conducted from Jul. to Dec. 2022. Data were analyzed tested using the MIXED procedure of SAS 9.4 with the fixed effect of treatment. Initial body weight was included as a covariate for body weight, average daily gain, and feed intake. Average daily gain (d 1 to 56) was increased by 30% in calves fed MR24 compared with MR20 (0.70 vs. 0.54 kg/d respectively; $P = 0.007$), resulting in greater BW (81.2 vs. 71.4 kg respectively; $P = 0.008$) and hip height (87.9 vs. 85.3 cm respectively; $P = 0.03$) on d 56. Milk replacer and starter intake were not affected by treatment ($P > 0.28$). As a result, feed efficiency during the pre-weaning period and the entire 56-d experiment was greater for calves receiving MR24. Finally, calves fed MR24 tended to have lower incidence of diarrhea during the pre-weaning period ($P = 0.09$), and lower therapeutic costs ($P = 0.02$) compared with MR20. Results indicate a higher plane of milk replacer nutrition improves growth rate, feed efficiency, and health of beef × dairy calves.

Key Words: beef × dairy, milk replacer, calf nutrition

1192M Effects of lysophospholipids on growth performance and nutrient digestibility in dairy calves. H. Baraz, H. Jahani-Azizabadi*, and O. Azizi, *University of Kurdistan, Sanandaj, Kurdistan, Iran.*

There are evidences that at least for the first 3 weeks of age, newborn calves have limitations in intestinal nutrient digestion. According to studies with monogastric animals, lysophospholipids can improve gut permeability to macromolecules, modifying membranous proteins channels, upregulating various genes in the intestinal epithelium, and immunomodulatory actions. Therefore, in the present study, 48 newborn female Holstein calves [37.5 ± 2.9 (SD) kg of body weight (BW)] were used in a completely randomized design to investigate the effects of a mixture of lysophospholipids (Lipidol, LPL, Easy Bio System Inc.) incorporated into calf starter on growth performance and nutrient digestibility (CP, EE, OM, and DM) of pre-weaning dairy calves. We hypothesized that the LPL might improve nutrient digestion, and gastrointestinal tract health and consequently, enhanced ADG in dairy calves. Calves had free access to calf starter and clean water from d 3 to weaning time. The treatments were the control (CON, without additive) and a commercial blend of LPL at 0.5, 1, and 1.5 g/kg of starter feed. The CONTRAST statement of SAS was used to test the linear, quadratic, and orthogonal contrasts (CON × LPL) of LPL supplementation. The threshold of significance was set at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$. The results of the present study showed that overall ADG (1–63 d) was significantly affected by LPL supplementation compared with the CON group (0.520 vs. 0.468 g/d, respectively; $P = 0.027$). The LPL supplementation did not affect the apparent digestibility of DM and OM. However, the CP digestibility linearly improved ($P = 0.046$) by increasing the LPL levels in the calf starter. Our observation showed a 7% increase in total-tract CP apparent digestibility with LPL supplementation, relative to the CON group ($P = 0.059$). In this study, a trend for increased EE digestibility was observed with LPL supplementation compared with the CON (86.7 vs. 83.4, $P = 0.1$). In conclusion, supplementation of LPL in starter feed improved ADG and CP digestibility in pre-weaning dairy calves.

Key Words: lysophospholipids, dairy calves, digestibility

1193M Evaluation of branched-chain amino acid inclusion in milk replacers on growth of Holstein calves. S. Y. Morrison*[1] and J. Ono[2], *[1]The William H. Miner Agricultural Research Institute, Chazy, NY, [2]ZEN-NOH National Federation of Agricultural Cooperative Associations, Tokyo, Japan.*

Branched-chain amino acids (BCAA; Leu, Ile, and Val) are required in the diet. Previous work showed no change in growth but decreased serum Met when BCAA was supplemented in milk replacer (MR). The objective of this study was to evaluate the effect of including BCAA at

Table 1 (Abstr. 1193M). Effect of a control milk replacer (CMR) vs. supplemented amino acid milk replacer (SMR) on the growth of Holstein calves

	Treatment			*P*-value		
Item	CMR	SMR	SE	Treatment	Time	Treatment × Time
Milk replacer, g/d	1,005	1,034	19	0.30	<0.001	0.85
Starter, g/d	468	458	57	0.90	<0.001	0.98
Hay, g/d	83	87	2	0.28	0.05	0.25
Weight (wk 8), kg	87.9	88.9	1.0	0.49	—	—
Weight (wk 10), kg	103.8	103.3	1.2	0.78	—	—
Average daily gain (wk 1–10), g/d	874	882	18	0.49	<0.001	0.66
Gain:feed (wk 1–9), kg/kg	0.65	0.66	0.01	0.56	<0.001	0.41
Serum Lys, mg/mL	39.04	43.14	0.91	0.006	<0.001	0.005
Serum Met, mg/mL	9.39	11.46	0.23	<0.001	<0.001	<0.001
Serum Ile, mg/mL	20.69	23.13	0.45	0.001	0.002	0.004
Serum Leu, mg/mL	32.54	35.56	0.74	0.08	<0.001	0.006
Serum Val, mg/mL	36.86	48.52	0.86	<0.001	<0.001	<0.001

similar levels to transition milk (TM), while maintaining Lys and Met ratios in calf MR on calf growth through 10 wk of age. Female Holstein calves (n = 50) were blocked by birth body weight (BW) and assigned to one of 2 milk protein-based MR in a complete block design. Treatments were a control MR [CMR; 26.0% crude protein (CP), 2.40% Lys, 0.72% Met, 2.66% Leu, 1.53% Ile, and 1.48% Val] or a MR with additional Lys, Met, and BCAA included at concentrations observed in TM (SMR; 26.8% CP, 2.60% Lys, 0.81% Met, 2.88% Leu, 1.73% Ile, and 1.94% Val). Intake of MR (wk 1–8), starter (wk 1–9), and hay (wk 7–9) were recorded daily through wk 9 of age. Body weight and stature measurements were recorded weekly through 10 wk of age. Blood was sampled at wk 2, 6, and 8 of age. Data were analyzed using the MIXED and GLIMMIX procedures in SAS. The model included the fixed effect of treatment, time, and block. Initial measures were used as a covariate when appropriate. Initial body weight (41.6 ± 0.7 kg) and serum total protein (6.7 ± 0.2 g/dL) were not different between treatments. No difference in intake, weight and body stature measurements were observed between treatments (Table 1). At current feeding levels and formulation of AA there may not be additional benefit to supplementation of BCAA in calf milk replacers.

Key Words: branched-chain amino acid, milk replacer, calf

1194M Replacing milk ingredients in calf milk replacer with Dried Cheese MR.

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Dried Cheese MR is a unique ingredient containing 31% dairy protein (casein) and 41% milk fat. The objective for this study was to determine performance of calves fed increasing level of dried cheese MR (0, 14, 28, and 42%) of the milk replacer formula. Dried cheese MR was formulated into the milk replacer (24% CP and 18% fat) at the expense of whey protein and dry fat. A total of 133 Holstein bull calves were randomly assigned to 1 of 4 milk replacer treatments in a completely randomized experiment. Calves were raised in individual pens bedded with wheat straw and constructed of wire hog panels (0.81 m × 1.07 m). Each pen had its own water bucket, feed bucket and bottle holder. Milk replacers and water were mixed at 60°C for 5 min and fed at 45°C in 1.8-L bottles with snap-on nipples at a concentration of 189 g/L. Calves were fed twice per day through 28 d of age, then once per day through d 35 and weaned on d 36. Calves were moved from the nursery to the grower barn on d 42 where they were housed in groups of 10 calves per pen. On d 1, calves had ad libitum access to water and calf starter feed which consisted of whole shelled corn, supplement pellets, oats, and liquid molasses. Starter contained 21% crude protein on DM basis and contained 82.8 g lasalocid per 1,000 kg on an as-fed basis. Calves were weighed on arrival, d 28, 42, and 56. Data were analyzed using repeated measures AOV and Tukey means separation. Differences were declared significant with $P < 0.05$. Live weights were not different among calves fed milk replacer containing 0, 14, 28, or 42% dried cheese MR; however, live weights increased with age (Table 1). Mortality and morbidity were not different among treatments. Dried Cheese MR can effectively replace whey protein and dry fat in calf milk replacer formulas with no loss of performance.

Table (Abstr. 1194M). Effects of Dried Cheese MR on Holstein bull calf BW (kg)

	Concentration of Dried Cheese MR, % (n calves)				
Day	0 (32)	14 (30)	28 (28)	42 (30)	SE
1	39.8^{ab}	39.3^{a}	39.1^{a}	39.5^{ab}	0.62
28	46.1^{c}	44.6^{c}	44.2^{cd}	45.4^{c}	0.69
42	51.1^{d}	51.2^{d}	52.0^{d}	51.6^{d}	0.43
56	64.5^{e}	64.6^{e}	64.6^{e}	66.4^{e}	0.77

[a–e]Means with different letters differ significantly ($P < 0.05$).

Key Words: ingredients, calves, replacer

1195M Evaluation of high hydrophilic-lipophilic balance emulsifier in calf milk replacer and grain.

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Bile salts are high in hydrophilic-lipophilic balance (HLB) value, and thus adding additional high HLB value emulsifiers to calf diets might improve feed utilization. The study's objective was to investigate the effects of different methods of high HLB emulsifier (Solumul HE 100) administration on the health and growth of calves. Farm and auction-sourced male calves (n = 160) arrived at a research facility and were randomly assigned to 1 of 3 treatments: (1) Solumul HE 100 at a rate of 500 ppm in milk replacer (SMR); (2) Solumul HE 100 at a rate of 500 ppm in milk replacer and grain (SMG); (3) control (CON). Four calves were removed due to death within 36 h of arriving or mobility concerns, leaving 135 Holstein and 21 crossbred calves enrolled in the trial. They were fed 50:50 skim:WPC-based 24% CP, 22% fat CMR void of additives for 8 wk in a 2×/d strategy (39 kg) and offered access to starter (texturized, 20% CP with 4% inclusion of straw). At weaning, calves were transitioned to corn and pellet with 4% straw (18.1% CP). Calves were individually weighed weekly through d 77, with milk refusals, fecal scores, respiratory scores (UC Davis system), and medical treatments recorded 2×/d. Blood was collected at arrival to determine serum total protein (STP). All statistical analyses were conducted in Stata 17 with linear regression and Cox proportional hazard models. No differences in arrival STP or body weight were found between groups. No differences were found in mortality, treatment for diarrhea or respiratory disease, ADG, or feed conversion pre-weaning; however, calves in the SMR and SMG groups had improved feed conversion in the post-weaning (d 56–77) period compared with the control group (2.57, 2.16, and 2.04 for CON, SMR, and SMG, respectively; $P = 0.004$) and tended to have a greater 11-wk body weight [SMR (122.6 kg) vs. CON (120.4 kg) $P = 0.09$; SMG (123.3 kg) vs. CON $P = 0.06$]. In this study, high HLB emulsifier improved feed conversion. High HLB emulsifiers should be explored in further calf research.

Key Words: calf, emulsifier, digestibility

1196M Effects of supplementing the direct-fed microbial *Enterococcus faecium* 669 on performance of pre-weaning dairy calves.

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The pre-weaning period is critical for the dairy production sector, as it influences the dairy cow's productive life and, therefore, the entire profitability of the operation. This trial was conducted to evaluate the effects of supplementing the direct-fed microbial (DFM) *Enterococcus faecium* 669 on growth performance of pre-weaning dairy calves. Twenty Holstein calves (10 males and 10 females) were ranked by birth body weight (BW; 42.4 kg ± 2.20 kg) and sex in 1 of 2 treatments: 1) no DFM supplementation (CON; n = 10) or 2) daily supplementation of *E. faecium* 669 at a daily dose of 2.0×10^9 cfu/kg of milk replacer

(DFM; n = 10; Chr. Hansen A/S, Hørsholm, Denmark). Throughout the experimental period (42 d), the DFM was mixed directly into the milk replacer. A soybean-based starter supplement was offered daily (87.0% dry matter; 21.1% crude protein; 2.1% ether extract; 5.5% fiber). Individual BW measurements were taken every 14 d and average daily gain (ADG) was determined from d 0–14, 14–28, and 28–42 (weaning) of the experiment. Milk replacer and starter supplement intakes were measured once a day and the resulting data used for gain to feed ratio determination. All data were analyzed using SAS (version 9.4; SAS Institute Inc.). No 2- or 3-way interactions or main effects of sex were observed herein ($P \geq 0.22$). A treatment × day interaction was observed on BW of the calves ($P = 0.02$), as DFM-supplemented calves tended to be heavier on d 28 ($P = 0.10$) and were significantly heavier at weaning on d 42 vs. CON ($P = 0.03$). Moreover, mean ADG was greater for DFM vs. CON calves (590 vs. 506 g/d; SEM = 2.7; $P = 0.04$). For total feed intake, a treatment × day interaction was observed ($P < 0.001$), as CON calves had a greater feed intake from d 14–28 ($P = 0.04$), but opposite results were observed from d 28–42 ($P < 0.0001$). No further main effects or interactions were observed for gain to feed ratio ($P \geq 0.21$). In summary, supplementing the direct-fed microbial *E. faecium* 669 improved mean ADG over a 42-d period.

Key Words: direct-fed microbial, *Enterococcus faecium* 669, performance

Ruminant Nutrition: Carbohydrates and Lipids 1

1197M Effect of palmitic and stearic acids on plasma ceramide concentrations in lactating dairy cows. S. L. Burtnett* and K. J. Harvatine, *Pennsylvania State University, State College, PA.*

Ceramides are bioactive lipids and some are associated with insulin resistance and have been reported to increase with palmitic acid supplementation. Our objective was to investigate the effect of palmitic (PA) and stearic acid (SA) on plasma ceramide profile. We hypothesized that total ceramide concentrations and long-chain ceramides would be influenced by PA more than SA supplementation. Blood samples from a previous study stored at −80C were used for the project. The study used 12 multiparous cows in a 4 × 4 Latin square design. Treatments were 1) control with no supplemental fat (CON), 2) high PA (HP; 91% C16:0), 3) high SA (HS; 92.5% C18:0), and 4) PA and SA blend (MIX; 45.3% C16:0 and 49.1% C18:0). Fatty acid supplements were fed at 1.95% of DM intake. Plasma samples were analyzed for ceramide concentrations via LC-MS/MS. Data were analyzed using the MIXED procedure of SAS. The model included the random effects of cow and period and the fixed effect of treatment. A total of 14 different ceramides were identified. The predominant ceramides for all treatments were C16:0 and C24:0 (averaging 36.1% and 34.2% respectively), in agreement with previous work from other labs. Total ceramide concentration increased with increasing PA supplementation; ceramide was highest ($P < 0.01$) in HP (601.5 ng/mL) followed by MIX (456.7 ng/mL) compared with HS and CON (399.3 and 382.1 ng/mL), which were not different from each other. A similar pattern occurred in C16:0 ceramide concentration. The C24:0 ceramide was also highest ($P < 0.01$) in HP (240.0 ng/mL) and MIX (174.4 ng/mL) versus CON (146.2 ng/mL) while HS (152.0 ng/mL) tended to be greater than CON ($P = 0.08$). In conclusion, ceramide concentration increased with PA supplementation while high SA supplementation did not increase ceramides. Differences in nutrient partitioning, milk fat yield, and body weight gain with PA supplementation may be through changes in ceramide signaling.

Key Words: ceramides

1198M Supplemental palmitic acid and chromium propionate impact milk fatty acid yield and content during the immediate postpartum in multiparous dairy cows. J. E. Parales-Giron* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of supplemental palmitic acid (C16:0) and chromium propionate (Cr) on milk fatty acid (FA) yield and content of early-lactation cows. Forty-eight multiparous cows were used in a randomized complete block design and assigned to one of 4 treatments in a 2 × 2 factorial arrangement of treatments. Treatment diets were fed from 1 to 24 DIM and contained 0% or 1.5% diet DM C16:0 (NP and PA) and 0 or 0.45 ppm Cr/kg diet DM (NC and CR). The FA supplement was an 85% C16:0-enriched supplement and replaced soyhulls in NP. The diets contained (% DM) 22.5 forage NDF, 26.3 starch, and 17.3 CP. The statistical model included the random effect of block, cow within block and treatment, and Julian date, and the fixed effects of C16:0 supplementation, Cr supplementation, time, and their interactions. Sources of FA were classified as de novo (<16 carbons), mixed (16 carbons), and preformed (>16 carbons). Results are presented in the following order: NPNC, PANC, NPCR, and PACR. Overall, C16:0 supplementation increased the content (g/100 g FA) of mixed FA (31.8, 35.4, 31.0, 34.6; $P < 0.01$) and tended to reduce de novo FA (23.0, 21.2, 22.1, 20.7; $P = 0.01$) and preformed FA (45.2, 43.4, 46.9, 44.7; $P < 0.01$). Chromium supplementation tended to reduce mixed FA content ($P = 0.10$). Regarding FA milk yields, C16:0 supplementation increased the yields of mixed FA (642, 777, 646, 813 g/d; $P = 0.05$), C4:0 (69.6, 74.9, 73.2, 84.8 g/d; $P = 0.05$) and C16:0 (593, 725, 595, 749 g/d; $P < 0.01$), and had no effect on de novo and preformed FA yields. Chromium supplementation tended to increase the yields of C18:3 n3 (8.7, 8.7, 9.2, 10.0 g/d; $P = 0.07$), C18:2 9c, 11t (5.2, 5.3, 5.5 5.9 g/d; $P = 0.09$) and had no effect on de novo, mixed, and preformed FA yields. There were no interactions between C16:0 and Cr supplementation on milk FA yields and contents. In conclusion, C16:0 supplementation increased the yields of C4:0 and mixed FA. Chromium supplementation did not alter milk FA yield by sources.

Key Words: chromium, early lactation, palmitic acid

1199M Effect of seasonality on production responses and milk fatty acid profile of lactating dairy cows when C16:0-enriched supplements are included in diets: A meta-analysis. M. A. Karpyn Esqueda*, J. M. dos Santos Neto, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We performed a meta-analysis to evaluate the effects of seasonality on milk production responses and milk fatty acid (FA) profile of dairy cows fed non-FA-supplemented control diets (CON) compared with diets supplemented with C16:0-enriched supplements (PA). The data set included 366 individual observations from multiparous Holstein cows averaging 187 ± 51 DIM from 8 studies across 4 seasons (spring = 147; summer = 122; fall = 47; winter = 50). Treatments were: CON, n = 180; and PA, n = 186. The C16:0-enriched supplements were included at 1.45 ± 0.08% diet DM. On average, across the seasons, CON diets contained (%DM mean ± SD) 30.9 ± 2.27 NDF, 20.1 ± 2.14 forage NDF, 27.5 ± 1.73 starch, 16.7 ± 0.62 CP, and 2.8 ± 0.42 FA. The PA diets contained (%DM) 30.2 ± 2.23 NDF, 20.1 ± 2.16 forage NDF, 27.5 ± 1.77 starch, 16.6 ± 0.61 CP, and 4.74 ± 0.31 FA. The statistical analyses included the fixed effects of treatment, season, and the interaction between treatment and season, and the random effects of study and cow nested within study and period. Period refers to periods from change-over designs and time points from continuous designs. Our model also used DIM and previous lactation 305-d mature-equivalent yield as covariates. Overall, compared with CON, PA increased the yields of milk (1.18 kg/d), fat (0.08 kg/d), FCM (1.82 kg/d), and ECM (1.47 kg/d), and milk fat content (0.10%; all $P < 0.01$). We observed interactions between season and treatment for the yields of milk, fat, FCM, and ECM ($P \leq 0.07$). Compared with CON, during spring and summer PA improved the yields of milk (0.99 and 3.02 kg/d), FCM (1.54 and 3.63 kg/d), and ECM (1.24 and 3.17 kg/d; all $P < 0.01$), respectively. Moreover, feeding PA tended to increase FCM during fall 1.05 kg/d and winter 1.07 kg/d ($P \leq 0.07$). Compared with CON, PA increased fat yield across all seasons ($P < 0.01$) with varying magnitudes (spring 0.07, summer 0.13, fall 0.08, and winter 0.06 kg/d). In conclusion, feeding C16:0-enriched supplements improved production response of lactating dairy cows throughout the year but responses were better during warmer seasons of the year.

Key Words: meta-analysis, seasonality, palmitic acid

1200M Different ratios between palmitic and oleic acids in calcium salts of fatty acids form greatly influenced the digestibility and production performance of dairy cows. J. Shpirer*[1,2], L. Lifshitz[1], H. Kamer[1], Y. Portnik[1], and U. Moallem[1], [1]*Department of*

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The form of fat supplements, degree of saturation, and the fatty acids (FA) profile influence the cow's productive response. The objective was to examine the effects of supplemental fats in the form of calcium salts of fatty acids (CSFA) in different ratios between palmitic (PA) and oleic (OA) acids on nutrient digestibility and cows' performance. Forty-two dairy cows were assigned into 3 groups and fed for 13 wk rations containing 2.2% of CSFA (on DM basis) consisting of 1) CS45:35: 45% PA and 35% OA; 2) CS60:30: 60% PA and 30% OA; and 3) CS70:20: 70% PA and 20% OA. Rumen and fecal samples were taken for VFA and digestibility measurements, respectively. Production data were analyzed with PROC MIXED, and rumen and digestibility data with GLM models of SAS. Milk yields were the highest in the CS45:35 (52.0 kg/d), intermediate in the CS60:30 (51.1 kg/d), and lowest in the CS70:20 cows (47.3 kg/d; $P = 0.002$). Milk fat content was ~0.35 percentage units lower in the CS45:35 cows than in the other 2 groups (3.55, 3.94, and 3.87% in the CS45:35, CS60:30, and CS70:20 groups, respectively; $P = 0.001$), and fat yields were higher in the CS60:30 than in other groups ($P = 0001$). The FCM 4% and ECM yields were higher in the CS60:30 than in other groups. Feed intake was highest in the CS60:30 group (33.5 kg/d) and lowest in the CS70:20 group (31.3 kg/d; $P = 0.001$). The milk-to-DMI ratio was the highest in the CS45:35($P = 0.001$), with no differences in the efficiency for 4% FCM or ECM production. In conclusion, increasing the PA proportion in the fat supplements greatly increased the milk-fat content, and a high OA ratio increased the milk yields. The digestibility of most nutrients was lower in the CS70:20 than in other groups; however, the total fat digestibility was similar between all groups, indicating that the form more than the FA profile influences the fat digestibility. The different impacts of the PA-to-OA ratio in the fat supplements indicates that in the future the FA profile of the provided supplement will be determined according to the defined goal: milk or milk-fat.

Key Words: calcium salts of fatty acids, palmitic acid, oleic acid

1201M Feeding a fat supplement containing palmitic and oleic acids interacts with parity in peak-lactation dairy cows during summer in Michigan.

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Heat-stressed dairy cows eat less, produce less milk, and are less profitable. Fatty acid (FA) supplements sometimes mitigate heat stress. We hypothesized that a FA supplement of palmitic and oleic acids would promote milk production during warm weather. Cows (12 primiparous and 28 multiparous) in peak lactation were blocked by parity, days in milk (DIM), and milk energy/body weight and randomly assigned to a control diet (CON; corn silage-based containing 30% NDF, 17% CP, and 29% starch) or a high FA diet (HiFA; same as CON but with an extra 1.3% palmitic and 0.4% oleic acids from a Ca-salt, slightly more CP, and slightly less NDF. Cows were fed CON for 2 wk starting in mid-July and then CON or HiFA for 6 wk. Milk yield and DM intake were measured daily. Body weights were measured 3 d/wk and milk was sampled for component analysis 2 d/wk. Barn temperatures were measured continuously; daily highs ranged from 22 to 31°C and lows ranged from 14 to 23°C. Data were analyzed as repeated measures with block and cow nested within block as random effects and diet, parity, time, and their interactions as fixed effects. On average, cows ate 25 kg/d, produced 39 kg milk with 30 Mcal energy per day, and gained 0.4 kg BW per day with no overall effect of treatment, but treatment interacted with parity for intake and milk ($P \leq 0.09$). For multiparous cows, HiFA did not alter DMI, but increased milk yield 5% ($P = 0.02$) and milk energy output 5% ($P = 0.05$) compared with CON. In primiparous cows, HiFA decreased DMI (7%, $P = 0.04$) but did not alter milk yield or energy ($P = 0.4$). We found few significant correlations of treatment responses with the minimum or maximum daily temperature or temperature humidity index (THI), but max daily THI was only 76 ± 3.1 during the study. Compared with cows fed CON, HiFA increased energy-corrected milk/DMI (1.69 kg/kg vs. 1.62 kg/kg, $P = 0.01$) but did not alter milk energy per unit feed NEL. In conclusion, supplementing cows with a blend of palmitic and oleic acids increased milk yield in multiparous but not primiparous cows in summer.

Key Words: palmitic acid, oleic acid, heat stress

1202M Effect of increasing dietary inclusion of whole cottonseed on nutrient digestibility of high-producing dairy cows.

A. M. Bales* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of increasing dietary inclusion of whole cottonseed (WCS) on nutrient digestibility of high-producing dairy cows. Twenty-four multiparous Holstein cows (52.7 ± 2.63 kg/d of milk; 104 ± 23 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were increasing doses of WCS at 0, 8, 16, and 24% DM. Whole cottonseed replaced soybean meal and hulls to maintain diet nutrient composition (%DM) of 32.5% NDF, 17.1% forage NDF, 26.9% starch, and 17.5% CP. Total FA content of each treatment was 1.70, 2.96, 4.20, and 5.40%DM, respectively. The statistical model included the random effects of period and cow within square, and the fixed effect of treatment. Pre-planned contrasts included the linear (L), quadratic (Q), and cubic (C) effects of increasing WCS. Results in the text are presented in the following order: 0%, 8%, 16%, and 24% WCS. There was an effect of increasing WCS for intakes (kg/d; both $P < 0.001$) of DM (34.4, 35.2, 34.6, 32.5; Q) and NDF (11.2, 11.4, 11.2, 10.4; Q) and intakes (g/d; all $P < 0.001$) of 16-carbon (84.9, 195, 291, 364; L), 18-carbon (494, 857, 1167, 1400; L), and total FA (593, 1075, 1490, 1802; L). Increasing WCS affected digestibility of DM (65.6, 63.7, 65.1, 66.8%; Q, $P < 0.001$) and NDF (45.2, 41.9, 43.5, 45.8%; Q, $P < 0.01$), and increased digestibility of 16-carbon FA (71.6, 75.7, 75.9, 77.8%; C, $P = 0.01$) and decreased digestibility of 18-carbon (80.1, 76.2, 71.6, 71.2%; L, $P < 0.001$) and total FA (77.8, 75.6, 72.3, 72.5%; C, $P = 0.03$). There was an increase in the amount of absorbed (g/d; all $P < 0.001$) 16-carbon (60.4, 147, 221, 284; L), 18-carbon (386, 653, 835, 1001; L), and total FA (451, 812, 1075, 1313; L). In summary, increasing dietary inclusion of WCS from 0 to 24% DM inclusion increased intake and absorbed 16-carbon, 18-carbon, and total FA. Furthermore, WCS inclusion at 24% decreased DMI and NDF intake. Despite the decrease in total FA digestibility with increasing dietary WCS inclusion, absorbed total FA increased by 361, 624, and 862 g/d, respectively.

Key Words: oilseeds, whole cottonseed, nutrient digestibility

1203M Effect of increasing dietary inclusion of whole cottonseed on milk production of high-producing dairy cows.

A. M. Bales*, J. M. dos Santos Neto, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of increasing dietary inclusion of whole cottonseed (WCS) on production responses of high-producing dairy cows. Twenty-four multiparous Holstein cows (52.7 ± 2.63 kg/d of milk; 104 ± 23 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were increasing doses of WCS at 0, 8, 16, and 24% DM. Whole cottonseed replaced soybean meal and hulls to maintain similar diet nutrient composition (%DM) of 32.5% NDF, 17.1% forage NDF, 26.9% starch, and 17.5% CP. Total FA content of each treatment was 1.70, 2.96, 4.20, and 5.40%DM, respectively. The statistical model included the random effects of period and cow within square and the fixed effect of treatment. Pre-planned contrasts included the linear (L), quadratic (Q), and cubic (C) effects of increasing WCS. Results in the text are presented in the following order: 0%, 8%, 16%, and 24% WCS. Increasing WCS affected DMI (34.4, 35.2, 34.6, 32.5 kg/d; Q, $P < 0.001$) and yields (kg/d; all $P < 0.001$) of milk (50.9, 52.9, 52.5, 50.4; Q), 3.5% FCM (52.3, 55.0, 54.8, 50.4; Q), ECM (52.6, 54.8, 54.6, 51.7; Q), milk fat (1.87, 1.98, 1.97, 1.85; Q), and milk protein (1.65, 1.67, 1.65, 1.55; Q). Increasing WCS increased BW change (0.26, 0.25, 0.50, 0.54 kg/d; L, $P = 0.02$), did not affect BCS change ($P = 0.26$), and decreased plasma insulin (0.96, 0.90, 0.92, 0.87 ug/mL; L, $P < 0.01$). Increasing WCS decreased yields (g/d; all $P < 0.001$) of de novo FA (503, 500, 457, 386; L) and mixed FA (674, 664, 632, 576; L), but increased preformed FA (565, 689, 761, 784; L). There was an increase in the FA content (g/100g) of *trans*-10 C18:1 in milk fat (0.46, 0.47, 0.54, 0.56; L, $P < 0.001$), suggesting altered biohydrogenation pathways. Increasing WCS in treatments increased gossypol intake (0.00, 18.6, 36.8, 52.0 g/d); thus there was an increase in plasma gossypol content (0.91, 2.46, 4.09, 6.15 ug/mL; L, $P < 0.001$). In summary, increasing dietary inclusion of WCS up to 16% DM increased production responses in high-producing cows. The 24% WCS diet reduced DMI, milk production yields, and plasma insulin.

Key Words: oilseed, cottonseed, milk fat yield

1204M Impact of abomasal infusion of linoleic and linolenic acids on the incorporation of n-6 and n-3 fatty acids into milk fat of lactating cows.

J. M. dos Santos Neto, L. C. Worden, M. Miller*, J. E. Parales-Giron, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of abomasal infusions of linoleic (18:2n-6) and linolenic acids (18:3n-3) on the incorporation of n-6 and n-3 fatty acids (FA) into milk fat using 6 rumen-fistulated Holstein cows (252 ± 33 DIM; 44 ± 6 kg milk/d) in a completely randomized design (3 cows per treatment). Treatments were abomasal infusions of 43 g/d 18:2n-6 and 8 g/d of 18:3n-3 (N6); or 43 g/d 18:3n-3 and 8 g/d 18:2n-6 (N3). Infusions were at 6-h intervals for 20 d. Cows received the same diet containing (%DM) 29.5% NDF, 17.9% CP, 28% starch, and 3.25% FA. Milk FA were evaluated throughout the treatment period (d 1 to 20) and during a 16-d carryover period (d 21 to 36). The model included the fixed effects of treatment, time (day), and their interactions ($P \leq 0.05$ for main effects; $P \leq 0.10$ for interactions). We used repeated measures. During the treatment period, we observed interactions between treatment and time for the yields of some milk FA ($P \leq 0.06$). Compared with N6, N3 increased the yields of total n-3 FA (16.7 g/d), 18:3n-3 (15.8 g/d) and 20:5n-3 (0.44 g/d) from d 4 to 20 ($P < 0.01$), decreased total n-6 FA from d 8 to 20 (16.4 g/d, $P \leq 0.01$), 18:2n-6 from d 8 to 16 (18.7 g/d, $P \leq 0.02$), and tended to decrease 20:4n-6 at d 12 and 16 ($P \leq 0.08$). Overall, N3 increased 22:6n-3 yield compared with N6 (0.2 g/d, $P = 0.05$). A similar pattern was observed for milk FA content. During the carryover period, we observed an interaction between treatment and time for the yield of total n-3 FA ($P = 0.10$), where N3 increased or tended to increase it from d 22 to 28 (3.02 g/d, $P \leq 0.08$) compared with N6. Overall, N3 increased 20:5n-3 yield compared with N6 (0.32 g/d, $P = 0.03$). A similar pattern was observed for milk FA content, but with an additional interaction between treatment and time ($P < 0.01$); N3 increased 18:3n-3 from d 22 to 24 (0.16 g/100 g, $P \leq 0.03$) compared with N6. We did not observe any effect of N6 ($P \geq 0.20$) or detect 22:6n-3 during the carryover period. In conclusion, abomasal infusions of 18:2n-6 and 18:3n-3 increased the yields and contents of n-6 and n-3 FA in milk fat, respectively, although increases in n-3 were more consistent than n-6 FA. Furthermore, carryover effects were observed for n-3 but not for n-6 FA.

Key Words: milk, PUFA, milk fat

1205M Mammary gland responses to altering the dietary supply of de novo and preformed fatty acids: Effects on milk fatty acid composition and yield.

A. C. Benoit* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effect of supplementing sodium acetate, palmitic acid, and whole cottonseed (WCS) on the yields of de novo and preformed FA. Thirty-two multiparous cows were used in a Latin square split plot design, and cows were assigned to a main plot receiving a basal diet (n = 16) with no supplemental C16:0 (Low PA) or a basal diet (n = 16) with 1.5% DM C16:0-enriched supplement (85% C16:0; High PA). The following treatments were fed in 4 × 4 Latin squares: 1) control (CON), 2) 12% DM WSC (CS), 3) 3% DM sodium acetate (AC), and 4) 12% DM WSC and 3% DM sodium acetate (CS+AC). The C16:0 supplement and sodium acetate replaced soyhulls and WCS replaced cottonseed hulls and meal. Treatment periods were 14 d with sampling the last 4 d of each period. The statistical model included the random effect of cow within basal diet and fixed effect of basal diet, treatment, period, and their interactions. Sources of FA were classified as de novo (<16 carbons), mixed (16 carbons), and performed (>16 carbons). Treatment by basal diet interactions were observed for yields of mixed FA and C4:0 ($P = 0.01$) where the magnitude of increase was greater in High PA vs. Low PA diets. Overall, compared with Low PA, High PA increased the yield (g/d) of mixed FA (640 vs. 749; $P < 0.01$) and tended to increase C4:0 (48.9 vs. 54.2; $P = 0.08$). Treatment results are presented as: CON, CS, AC, and CS+AC. Compared with the other treatments, AC increased yields of de novo (453, 405, 473, 443; $P < 0.01$) and mixed FA (678, 635, 746, 721; $P < 0.01$). The CS+AC treatment increased preformed yield (565, 659, 596, 686; $P < 0.01$) compared with the other treatments, while CS and AC increased preformed yield compared with CON. Compared with CON, AC and CS+AC increased C4:0 yield (48.5, 48.8, 54.3, 54.6; $P < 0.01$), and AC increased C14:0 (207, 184, 215, 204; $P < 0.01$) and C16:0 yield (646, 608, 713, 693; $P < 0.01$). Additionally, CS+AC increased C18:0 yield (131, 186, 145, 195; $P < 0.01$) compared with the other treatments, and CS+AC and CS increased C18:1 yield (258, 292, 260, 296; $P < 0.01$) compared with CON and AC. Balancing the supply of de novo and preformed FA to the mammary gland is important for optimizing milk fat synthesis and yield.

Key Words: mammary gland, fatty acid, milk fat

Ruminant Nutrition: General 1

1206M Feed preference in lactating dairy cows of different pellet formulations. A. L. Carroll*, G. M. Fincham, and P. J. Kononoff, *University of Nebraska-Lincoln, Lincoln, NE.*

Pelleted feeds are offered within automated milking systems (AMS) to provide nutrients and incentivize cows to enter. Understanding pellet preference could provide information to improve feeding strategies to increase visits. The objective was to compare 4 different pellet formulation strategies on feed preference. A taste preference experiment was conducted with 8 multiparous lactating Jersey cattle (100 ± 7.1 DIM, 30.5 ± 4 0.06 kg milk yield, 18.8 ± 2.52 kg DMI). Four 6.35-mm pellets were manufactured; 1) an equal proportion (45.7%) of alfalfa meal and corn grain and 8.57% wheat middlings (ALFC), 2) 72.3% corn grain and 18.5% wheat middlings (ENG), 3) a pellet containing dehydrated alfalfa meal (DALF), and 4) a pellet containing a mixture of concentrate ingredients (GMIX; 43.1% corn grain, 26.3% dried distillers grains and solubles, and 13.8% wheat middlings). Cows were offered 0.50 kg of pellets in a randomized arrangement within the feed bunk at 930 h before feeding for 1 h or until the feed was fully consumed. All 4 pellets were offered in individual 30.5 cm × 40.6 cm plastic tubs for the first 4 d, then the most preferred feed for each cow was removed, and the remaining 3 feeds were offered for 3 d. The process was repeated for the last 2 d. Feed preference was ranked from 1 to 4 with 1 being the most preferred and 4 the least. The resulting preference rankings were averaged resulting in a ranking of ALFC (1.38 ± 0.518), ENG (2.13 ± 1.126), GMIX (2.88 ± 0.835), and DALF (3.13 ± 0.835). Data were examined utilizing the Plackett-Luce analysis to estimate the probability animals would choose a pellet first. The probabilities of first choice were 70.6 ± 0.55% ALFC, 16.5 ± 0.46% ENG, 5.50 ± 0.475% DALF and 7.48 ± 0.455% GMIX. A Z-test was conducted to determine the percentage a treatment would be chosen first differed from the value of no preference at 25%, ALFC and DALF differed ($P \leq 0.04$) from the mean value while no difference ($P \geq 0.13$) was observed for ENG and GMIX. Mixtures of corn grain and dehydrated alfalfa meal bound by wheat middlings may serve as a feeding strategy that is preferred and may serve a valuable reward to cows entering an AMS.

Key Words: automated milking systems, pellets, palatability

1207M Preliminary study into methane emissions in mid-lactation primiparous dairy cows. K. M. Kennedy*, S. J. Johnson, S. J. Kendall, M. M. Caputo, F. Peñagaricano, K. A. Weigel, and H. M. White, *University of Wisconsin–Madison, Madison, WI.*

Identification of metabolic and digestive sources of variance in residual feed intake (RFI) will allow for co-selection of efficiency and environmental sustainability. Our objective was to determine GreenFeed (C-Lock Inc.) usage patterns and baseline methane relationships in a high-throughput RFI quantification system. Primiparous dairy cows (n = 12; 111 ± 36.8 DIM) were housed in freestalls equipped with Calan gates for 6 wk. Milk yield and DMI were recorded daily. Milk samples were collected from 4 milkings weekly for milk component analysis, and BW and BCS were measured during wk 1, 3, and 6. Residual feed intake was determined as DMI regressed on DIM, milk energy, metabolic BW, and ΔBW. Cows had ad libitum access to a GreenFeed unit for 1 wk during wk 5 or 6, and alfalfa pellet bait was included in daily DMI. Data were analyzed for 6 cows per pen although all 16 cows per pen had access to the GreenFeed unit. Average production measurements and corresponding GreenFeed data for the week was used. Methane results were analyzed as CH_4 yield (L), CH_4/ECM (L/kg), and CH_4/DMI (L/kg). Spearman's rank correlations (SAS 9.4) were calculated for either CH_4 (553 ± 71.9 L), CH_4/ECM (12.1 ± 1.48 L/kg), or CH_4/DMI (22.1 ± 3.08 L/kg) against milk yield (41.2 ± 4.76 kg), DMI (25.2 ± 2.14 kg), ECM (45.8 ± 4.36 kg), gross feed efficiency (ECM/DMI; 1.82 ± 0.08), RFI (0.00 ± 0.48), BW (641 ± 46.8 kg), and BCS (3.23 ± 0.18). Statistical significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Each cow visited the GreenFeed unit 3.16 ± 1.54 visits/d (range: 0 to 19 visits/d) and ate 0.50 ± 0.17 kg DM alfalfa pellets/d. There was a positive correlation between CH_4 and ECM/DMI ($r = 0.65$; $P = 0.02$) and a tendency for a negative correlation between CH_4/ECM and DMI ($r = -0.57$; $P = 0.055$). Although limited in size, this study provides baseline data on visit patterns that is consistent with past indications that CH_4 is partially driven by DMI. Our results support that introducing a GreenFeed into an existing high-throughput RFI quantification system yields sufficient data for most cows and can be used to determine the contribution of methane emissions to individual cow variance in RFI.

Key Words: methane, production, dairy cows

1208M Determination of the milk urea and milk urea nitrogen content of fluid milk by spectrophotometric enzymatic analysis: Collaborative study. M. Portnoy* and D. M. Barbano, *Cornell University, Northeast Dairy Foods Research Center, Ithaca, NY.*

Urea, or milk urea nitrogen (MUN), is a minor component in milk that is of growing interest for the dairy industry. MUN can be measured by milk mid-infrared (MIR) instruments that are used throughout the dairy industry for payment testing and herd monitoring, as they provide fast/reliable milk composition results. However, milk MIR analysis is a secondary method that requires calibration samples for MUN with reference values from a chemical method. Our objective was to conduct a collaborative study, based on guidelines by the Association of Official Analytical Chemists (AOAC), to determine the within and between laboratory method performance of an enzymatic/spectrophotometric chemical method for determination of the MUN/urea content of bovine milk. The method's principle is (1) milk sample preparation to obtain a protein-free, fat-free filtrate, and (2) enzymatic assay with addition of 2 enzymes (urease and glutamate dehydrogenase) to hydrolyze urea into ammonia and carbon dioxide and, in presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH), form L-glutamic acid and NADP$^+$. The amount of NADPH consumed is measured by the absorbance decrease at 340 nm and is stochiometric with urea content of milk. Gravimetric reagent additions were recorded and MUN content was reported on weight/weight basis. Thirty milk samples (MUN content between 4.89 to 23.26 mg MUN/100 g milk; urea content between 10.5 to 49.9 mg urea/100 g milk) were distributed to a group of 10 laboratories for MUN determination using the proposed method. Statistical performance based on the method's repeatability (r) and reproducibility (R) in units of milligrams of MUN and urea/100 g milk, were: mean = 13.94 mg N/100 g milk and 29.88 mg urea/100 g milk, $s_r = 0.063$ and 0.135, $s_R = 0.092$ and 0.197, $RSD_r = 0.453\%$ and 0.453%, $RSD_R = 0.661\%$ and 0.661%, r-value = 0.177 and 0.379, and R-value = 0.258 and 0.553, respectively, when using a gravimetric method in conducting the assay.

Key Words: urea, MUN, reference method

1209M Effect of steam pressure toasting times on truly digestible protein supply of whole faba bean seeds to dairy cows evaluated with the DVE/OEB dairy nutrition system. M. E. Rodríguez Espinosa, V. H. Guevara Oquendo, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada.*

The objectives of this study were to determine the effect of steam pressure toasting times on the true protein supply of faba bean seeds based on the Dutch dairy system. The faba bean samples (cv. Snowbird) were processed by steam pressure toasting at 121°C for 0 (control), 30, 60, 90, and 120 min. Total truly digestible protein supply (DVE value) and degraded protein balance (OEB value) were determined based on the DVE/OEB dairy nutrition system. Total DVE value consisted of truly digestible rumen undegraded protein (ARUP), truly digestible rumen microbial protein (AMCP) and correction for endogenous nitrogen loss (ENDP). The degraded protein balance is a balance between microbial protein synthesis from rumen-degradable CP and that from the energy extracted during anaerobic fermentation in the rumen. The experimental design was a RCBD with processing time as a fixed effect and processing batch and animals as random block effects. The data were analyzed using the Mixed model procedure in SAS 9.4 with the analysis RCBD model. The orthogonal polynomial contrast was used to detect linear and quadratic relationship between steam pressure toasting time and true protein supply to dairy cows. The results showed that there was quadratic effect ($P < 0.01$) between steam pressure toasting time and AMCP, ARUP, DVE, and OEB values. With increasing steam pressure time, the ARUP was increased from 68 (control) to 244 g/kg DM. The AMCP was decreased from 72 (control) to 40 g/kg DM. Total DVE was increased from 138 (control) to 282 g/kg DM. The degraded protein balance was decreased from 103 (control) to −21 g/kg DM (from positive OEB value to negative). In conclusion, total truly digestible protein supply to dairy cow was increased with increasing steam pressure toasting time. However, the degraded protein balance was decreased from positive to negative OEB value.

Key Words: steam pressure time, truly digestible protein supply, degraded protein balance

1210M Effects of a specific blend of essential oil compounds on in vitro ruminal fermentation and nutrient degradation. A. Jili, S. Yang, Y. Huang, Y. Lin, and H. Shi*, *Southwest Minzu University, Chengdu, Sichuan, China.*

The demand for more natural and safer alternative feed additives that can be used to improve animal production rapidly increased. Numerous studies have been conducted to evaluate essential oils and their active compounds as natural feed additives in ruminant nutrition. This study aimed to investigate the effects of a specific blend of essential oil compounds (BEO) including eugenol, thymol, and cinnamic aldehyde on in vitro ruminal fermentation characteristics. The standard in vitro procedures with minor modifications was employed. The substrate was composed of forage and concentrate with a ratio of 50:50 on a dry matter (DM) basis. The BEO was added at 0, 100, 200, 400, and 600 mg/L of culture fluid. The degradability of DM, crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined after 48 h of incubation. The concentrations of microbial protein (MCP), ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) were also tested. Data were analyzed by one-way ANOVA with the general linear model (GLM) procedure in SAS 9.4, and the linear and quadratic effects of BEO were determined by orthogonal contrasts. Significance level was set at $P < 0.05$. The in vitro ruminal fermentation parameters were affected ($P < 0.01$) by BEO, and most of them changed quadratically ($P < 0.01$) with increasing BEO levels. The greatest nutrient degradability was observed in the 100 mg/L BEO treatment, and the degradability for CP, NDF, and ADF was increased by 7.6%, 8.2%, and 12.3%, respectively, when compared with the control group. Moreover, the 100 mg/L BEO treatment increased ($P < 0.01$) the MCP concentration by 12.5% and decreased ($P < 0.01$) the level of NH_3-N by 14.5%. With increasing BEO levels, the concentrations of total VFA, acetic acid and propionic acid decreased linearly ($P < 0.01$), whereas the concentration of butyric acid and the ratio of acetic acid/propionic acid increased linearly ($P < 0.01$). Suppressed fermentation was observed in the 400-mg/L and 600-mg/L BEO groups, and the nutrient degradability was decreased ($P < 0.01$). In conclusion, the BEO used in the study could manipulate in vitro ruminal fermentation and more research is needed to confirm its potential application in ruminant production.

Key Words: essential oils, ruminal fermentation, nutrient degradation

1211M Performance of lactating Holstein cows fed a diet containing a blend of essential oils. H. Tucker*[1], M. Swango[2], and J. Boerman[2], *[1]Novus International, St. Charles, MO, [2]Purdue University, West Lafayette, IN.*

A lactation trial was conducted to validate if a blend of cinnamaldehyde and garlic oil enhances lactation performance. The experiment was conducted as a randomized complete block design with 2 treatments (n = 20 cows/treatment) with 14 d of adaptation to the basal diet followed by 56 d of feeding treatments. Treatments consisted of 1) CON: basal diet with no essential oils and 2) NECGO: basal diet supplemented with 710 mg/d of a blend of cinnamaldehyde and garlic oil (Next Enhance CGO feed additive, Novus International Inc.). Amount of feed offered and refused was recorded daily and adjusted by dry matter to determine dry matter intake (DMI) while milk yield was electronically collected at all milkings. Data were reduced to a weekly mean for statistical analysis. Individual samples of milk were collected from all milkings on one day for compositional analysis each week. Data were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Institute Inc.) and repeated measures where appropriate. Cow served as the experimental unit, appropriate covariate measures included, and parity was included in the model. Results demonstrated that DMI of primiparous cows supplemented NECGO decreased (24.97 ± 0.20 kg/d; $P < 0.01$) compared with CON counterparts (25.64 ± 0.18 kg/d). No effect ($P = 0.16$) on DMI of NECGO was observed for multiparous cows (26.72 ± 0.17 versus 26.19 ± 0.18 kg/d, NECGO versus CON). Milk yield of multiparous cows supplemented NECGO increased (30.00 ± 0.25 kg/d; $P < 0.01$) compared with CON counterparts (28.68 ± 0.29 kg/d). No effect ($P = 0.16$) of NECGO on milk yield was observed for primiparous cows (28.26 ± 0.30 versus 29.12 ± 0.28 kg/d, NECGO versus CON). No changes to milk component yield were observed ($0.11 < P \leq 0.98$). Greater ($P = 0.06$) body weight was observed with NECGO (620 ± 1.8 versus 615 ± 1.7 kg, NECGO versus CON). The interaction of treatment and time was not significant ($0.14 < P \leq 0.99$) for most measures except protein ($P = 0.01$) and lactose ($P = 0.03$) yield. Together these data suggest a beneficial mode of action of NECGO to improve milk yield or to reduce DMI depending on lactation number.

Key Words: cinnamaldehyde, garlic oil, lactation performance

1212M Health, reproduction, and lactational performance of dairy cows supplemented with a rumen-protected blend of B vitamins under a grazing system. R. Balogun*[1], A. Henry[1], and O. AlZahal[2], *[1]Jefo Australia Pty Ltd., Toowoomba, Qld, Australia, [2]AlZahal Innovation and Nutrition, Kitchener, Ontario, Canada.*

Table 1 (Abstr. 1212M). Parameters measured in the experiment

Item	Control	RPBV[1]	SE	*P*-value
Energy-corrected milk, L/d	21.6	23.2	0.38	0.02
Fat, %	3.66	3.90	0.08	0.03
Fat yield, kg/d	0.83	0.91	0.02	0.01
Protein, %	3.37	3.42	0.02	0.07
Protein yield, kg/d	0.76	0.80	0.02	0.04
Blood BHBA				
Average, m*M*	1.02	0.82	0.03	<0.001
Proportion of cows >1.2 m*M*, %	20.1	6.0		<0.001
6-wk in-calf rate, %				
Primiparous	48.6	61.3		0.30
Multiparous	48.5	69.7		0.01

[1] Rumen-protected blend of B vitamins.

The objective of this study was to evaluate the effect of supplementation with a rumen-protected blend of B vitamins (RPBV) on health, reproduction, and lactation performance of dairy cows under a grazing system. A total of 274 multiparous and 88 primiparous dairy cows on a commercial farm in Southeast Australia were blocked by expected calving date, previous milk production, and lactation number. Cows were then randomly assigned to a close-up diet, fed from 21 d before calving until calving, either without (Control) or with 100 g/head per day of RPBV for transition (B_2, B_9, and B_{12}) plus choline. Post-calving, cows remained in their respective groups and received a lactation diet from calving until dry-off, either without, or with 3 g/head per day of RPBV for lactation (B_5, B_6, B_8, B_9, and B_{12}). Parameters measured included blood β-hydroxybutyrate (BHBA) at 3 and 10 DIM, milk yield, fat and protein content obtained from herd testing, and the proportion of cows diagnosed pregnant within 6 weeks from the start of mating (6-week in-calf rate). Statistical analyses of BHBA and milk production parameters were performed using the Mixed procedure of SAS with repeated measures and fixed effects of treatment, milk test, parity, and their interactions. The proportion of cows with BHBA concentration >1.2 m*M* and the 6-week in-calf rate were analyzed using the chi-squared test. The results from this study (Table 1) showed that RPBV supplementation reduced blood BHBA concentration and improved the reproduction and lactation performance of dairy cows under a pasture-based system.

Key Words: B vitamins, pasture, dairy cows

1213M Effect of red clover extract hormone level, immune trait, and on plasma biochemical parameters in lactating dairy cows. S. Q. Zhang, X. Y. Zhang, Z. B. Xiong, K. X. Li, Y. Gao, Y. Bu, N. Zheng, S. G. Zhao*, and J. Q. Wang, [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality and Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Red clover is rich in isoflavones, which play an estrogenic, anti-inflammatory, and immune-enhancing role. The study aimed to demonstrate the effect of red clover isoflavones on the health indicated by immune status, and blood biochemistry in dairy cows. Sixty-eight healthy Holstein lactating cows were randomly divided into 4 treatment groups (n = 17 per treatment) from 5 blocks according to milk yield using a randomized complete block design. No initial differences in parity (2.13 ± 1.21; $P = 0.618$), DIM (165 ± 21 d; $P = 0.586$), and MY (33.93 ± 3.81 kg/d; $P = 0.618$) between groups. Cows were fed with the same basal diet supplemented with 0, 2, 4, or 8 g/kg of red clover extract (RCE) in TMR (DM based). All cows received the same basal diet and were fed the diet ad libitum with a target refusal rate of 10%. Both DMI and MY were collected at 0, 4, 8, and 12 weeks. Blood was collected on the last day of 4, 8 and 12 weeks. All data analyses were conducted by the MIXED program of SAS. Data were analyzed using the following model: $Y_{ijkl} = \mu + Block_j + RCE_k + Cow_{i:k} + Week_l + RCE \times Week_{kl} + e_{ijkl}$. Orthogonal polynomial comparison coefficients determined the linear and quadratic effects of the elevated dietary RCE levels on different parameters. The DIM was significantly decreased in the 8-g/kg group. The MY and feed conversion efficiency were significantly higher in all RCE-treated groups ($P < 0.05$), showing a quadratic change with increasing RCE levels (quadratic, $P < 0.05$), with the highest in the 2-g/kg group. Estradiol and prolactin showed quadratic changes with increasing RCE levels (quadratic, $P < 0.05$), with the highest in the 4-g/kg group. Tumor necrosis factor-α, IL-1β, and IL-6 decreased linearly with increasing RCE dose (linear, $P < 0.05$). Diamine oxidase, D-lactate, and immunoglobulin A varied quadratically with increasing RCE levels (quadratic, $P < 0.05$), with the highest in the 4-g/kg group. Liver function and kidney function indicators were similar in all groups. Dietary supplementation with 2–4 g/kg of red clover extract does not modify feed intake, liver activity marker, and kidney activity marker levels. There was an increased immunity, anti-inflammatory capacity, and improved lactation performance endocrine hormone levels as well as improved intestinal barrier function to increase milk production in dairy cows.

Key Words: dairy cow, hormonal, immunity

1214M Estimation of the nutrient variation in feed delivery and impacts on milk production and measures of fertility. A. L. Carroll*[1], P. J. Kononoff[1], K. J. Hanford[1], and C. Abney-Schulte[2], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*Cargill, Minneapolis, MN.*

Diets formulated for dairy cattle are designed to supply nutrients, but rations delivered to the animal may deviate from original formulations resulting in implications on milk production, composition, fertility, and overall health. The objective of this study is to evaluate retrospective feed mixing records collected from 8 commercial dairy farms over 52 weeks. Farms averaged 8,586 ± 5,735.3 animals, producing 31.8 ± 5.93 kg milk with 4.14 ± 0.461% fat and 3.26 ± 0.218% protein. Data collected included daily TMR nutrient deviation; calculated as diet nutrient content minus the observed divided by the formulated concentrations of CP, fat, NDF, and starch. Data were analyzed with fixed effects of time (modeled as 4-week periods) and days of positive nutrient deviation in fat, CP, NDF and starch alone and in combination with other nutrients, random effects of herd, and repeated measure of time within herd. The nutrient deviation by farm averaged (±SD) 1.68 ± 2.655, 2.28 ± 4.473, 1.75 ± 2.621, and 1.47 ± 2.147% for CP, fat, NDF, and starch, and days of positive nutrient deviation per period averaged (±SD) 25.5 ± 3.47, 25.5 ± 3.72, 25.6 ± 3.49, and 25.9 ± 2.61. Dry matter intake (DMI;17.2 ± 1.78 kg/d) decreased with increasing positive deviation days in starch (−0.0483 ± 0.01265; $P < 0.01$) and increased with increasing positive deviation days in CP (0.0211 ± 0.00942; $P = 0.03$). Milk yield (31.4 ± 2.50 kg/d) increased with positive deviation days in starch (0.0486 ± 0.02110; $P = 0.02$) and decreased with increased positive deviation days in NDF (−0.0298 ± 0.02202; $P = 0.18$). Finally, pregnancy rate (21.7 ± 4.34) increased with increasing positive deviation days in fat (0.385 ± 0.1635; $P = 0.02$) and decreased with increasing positive deviation days in CP (−0.420 ± 0.1879; $P = 0.03$). For these models, period significantly affected milk yield ($P < 0.01$) and but was not observed to affect pregnancy rate and dry matter intake ($P \geq 0.12$). Results suggest that farm and nutrient type were significant sources of variation, and that overall farms overfeed nutrients CP, fat, NDF, and starch 92% of the days of a period.

Key Words: nutrient variation, milk production

1216M Evaluation of a rumen-protected B-vitamin blend in a California Jersey dairy.

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The objective of this field study was to evaluate the effect of supplementing close-up cows with a commercially-available blend of rumen-protected choline plus B vitamins (riboflavin, folic acid, cobalamin; RPBV, Jefo) on early-lactation performance. Multiparous dry cows (545) were blocked by calving date and randomly allocated in a staggered manner into treatment pens over a period of 75 d. All cows received the same close-up, fresh, and lactation TMR. The close-up TMR was composed of hay (9.6%), close-up premix (68.6%) and corn silage (21.8%) (DM basis). The lactation TMR was composed of hay (5%), premix (71%), sorghum silage (4%) and corn silage (20%) (DM basis). Eighty (80) g/cow per day of RPBV was included in a ground-corn carrier and delivered as a topdress immediately after TMR delivery based on the number of cows/pen for 21 d before calving. The control diet (CON) did not include RPBV. Test day milk and components, mastitis, metritis, and pregnancy data were recorded. Four test day milk records up to 120 DIM were included for each cow (4 DIM blocks). Data were analyzed using the Mixed procedure of SAS with repeated measures. The model included all possible interactions of treatment (CON vs. RPBV), parity (2 vs. 3+), and DIM blocking (1–4). Because the number of control cows was greater than that of the treatment cows, the Survey select procedure of SAS was used to randomly select several control cows equivalent to treatment cows. Chi-squared analysis was used for health and pregnancy data. The supplementation of RPBV increased milk and milk component yields (Table 1). On average, ECM yield was increased by 1.6 ± 0.7 kg/d. The treatment increased the proportion of cows confirmed pregnant by 120 DIM from 34 to 47% ($P = 0.09$). In conclusion, the supplementation of the RPBV blend to close-up Jersey cows has the potential to optimize their milk production and reproductive performance during early lactation.

Key Words: B vitamins, milk production, Jersey cows

Table 1 (Abstr. 1216M). Least squares means for milk parameters (CON = control, RPVB = rumen-protected choline plus B vitamins)

Item	CON	RPVB	Pooled SE	*P*-value
Milk yield, kg/d	40.1	41.4	0.35	0.01
Milk fat, kg/d	1.86	1.94	0.03	0.03
Milk fat, %	4.63	4.68	0.05	0.49
Milk protein, kg/d	1.14	1.46	0.03	0.05
Milk protein, %	3.55	3.53	0.02	0.53

1217M Supplementation strategy and level of replacement of corn with cassava root silage in dairy grazing system.

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The objective was to evaluate the effects of supplementation and replacement of corn with cassava root (*Manihot esculenta*) silage (CRS) for primiparous grazing dairy cows on nutrient intake, apparent digestibility, milk yield, and composition. Ten Girolando cows (body weight of 373 ± 63 kg and DIM 76 ± 27 d) were distributed in two 5 × 5 Latin squares according to DIM. Cows were kept on a rotational grazing system of *Megathyrsus maximus* cv. Mombaça and were randomly assigned to 1 of 5 treatments, which consisted of non-supplemented (NS) or supplemented with 5 kg of corn and soybean meal-based concentrate, where 0, 33, 66, or 100% of the corn was replaced by CRS. The experiment was divided into 5 periods, each with 14 d of adaptation and 6 d of collection. To depict the treatment effect, orthogonal contrast was used to compare NS vs. supplemented animals and the level of supplementation (linear and quadratic). Intake of dry matter (DM; 8.25 vs. 9.25 kg/day) and crude protein (CP; 0.86 vs. 1.27 kg/day) were lower for the NS compared with the supplemented animals. In contrast, pasture DM intake (8.25 vs. 4.19 kg/day) and neutral detergent fiber (NDF; 5.11 vs. 3.27 kg/day) were greater for the NS compared with the supplemented groups. The digestibility of DM (58.5 vs. 50.3%) and CP (67.8 vs. 54.1%) were greater for the supplemented animals compared with NS. Digestibility of NDF (51.5 vs. 60.6%) was lower for supplemented animals when compared with NS. Supplementation enabled greater milk yield (MY; 13.2 vs. 9.4 kg/day) and fat-corrected milk (12.5 vs. 9.2 kg/day) compared with the NS. There was no difference on milk fat concentration (3.77%) and total solids (12.2%) concentration between NS and supplemented animals. There were no linear or quadratic effects for the level of CRS replacement on the evaluated parameters. Therefore, supplementation at pasture improves performance by increasing DM and CP digestibility and consequently milk yield and composition in Girolando dairy cows. In addition, CRS can be used as an alternative feed enabling a similar nutrient digestibility and milk production when compared with corn supplementation.

Key Words: alternative feed, grazing supplementation, milk production

1218M Associations of diets fed and their nutrient content with milk and component yield on Canadian dairy farms utilizing automated milking systems.

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The objective of this study was to determine what dietary components and nutrients were associated with milk yield and composition on Canadian dairy farms with automated milking systems (AMS). Formulated rations, including ingredients and nutrient content, were collected from 01/04/2019 until 30/09/2020 on 161 AMS farms (Atlantic CAN = 8, ON = 76, QC = 23, and Western CAN = 54). For each farm, milk recording data were collected and summarized by farm to calculate average yield of milk and components. Univariable models were used to screen independent variables and covariates (breed, parity, DIM, region) in mixed-effect linear regression models and variables with $P < 0.20$ were tested in multivariable models. Milk yield (37.0 ± 0.3 kg/d) was associated with the PMR ether extract concentration (PMREE; $P = 0.003$) and tended to be associated with the major forage source on farm ($P = 0.07$). Every percentage point (p.p.) increase in PMREE (4.43 ± 0.09%) was associated with an increased milk yield of +0.7 ± 0.2 kg/d. Farms that fed barley silage (n = 16) or corn silage (n = 95) as primary forage tended to produce +2.0 ± 0.95 kg/d or +1.1 ± 0.57 kg/d more milk than farms that fed haylage (n = 42), respectively. Milk fat yield (1.51 ± 0.011 kg/d) was associated with the PMREE ($P < 0.01$), total diet CP (CP of AMS concentrate and PMR; tCP; $P < 0.01$), and total diet DM (DM of AMS concentrate and PMR; tDM; $P = 0.01$). Each p.p. increase in tDM (49.7 ± 0.4%), PMREE, or tCP (16.5 ± 0.05%) was associated with changes in milk fat yields of +0.01 ± 0.002, +0.05 ± 0.009, and −0.04 ± 0.02 kg/d, respectively. Increased milk protein yield (1.25 ± 0.01 kg/d) was also associated with tDM ($P < 0.01$), forage % of PMR ($P = 0.04$), and with PMREE ($P = 0.02$). For every 10-p.p. increase in tDM and 1 p.p. in PMREE, milk protein yield increased by +0.04 ± 0.02 and +0.02 ± 0.01 kg/d, respectively. Each 1-p.p. increase in PMR forage % (70.3 ± 7.8%) resulted in −0.003 ± 0.001 kg/d less milk protein. These data give insight to the ingredients and nutrient levels of diets fed on AMS dairy farms across Canada and the association of those levels with milk and component yield.

Key Words: robotic milking, concentrate, partial mixed ration

1219M Effects of lysophospholipid supplementation on performance and some reproduction parameters of early-lactating Holstein dairy cows. F. Mirzaei, H. Jahani-Azizabadi*, and O. Azizi, *University of Kurdistan, Sanandaj, Kurdistan, Iran.*

In the first weeks of lactation, dairy cows often do not consume enough DMI to meet their nutrient requirement, which leads to a negative energy balance (NEB). The NEB is the reason for the most infectious diseases and metabolic disorders during the early lactation. Lysophospholipids (LPL) have been identified as novel highly potent feed additives. There is some evidence that LPL can increase dietary nutrient absorption and has anti-inflammatory properties. The objectives of this study were to investigate the effects of LPL on the performance and some reproduction parameters of Holstein early lactation dairy cows. Therefore, 18 Holstein early lactation dairy cows (DIM = 25 ± 5) were used in a repeated measurement design (n = 9). Treatments were; control (CON, no additive), LPL (Lipidol Ultra, Easy Bio Inc.) at 15 g/d/cow. This experiment consisted of 4 periods that lasted 21 d (14-d diet adaptation and7-d sampling). For evaluation of the effects of treatments on the number and diameters of mature follicles of ovaries, cows' estrous cycles were synchronized at the beginning of the experiment. Then, the number of mature follicles (15 mm < 0.05) in the milk yield (52.6 vs. 45.1 kg/d), FCM 3.5% (49.7 vs. 43.9 kg/d), and ECM (32.8 vs. 29.4 kg/d), relative to the control group. Lipidol supplementation at 15 g/d/head in the condition of the present study increased the number of mature follicles (1.7 vs. 1.2), mature follicles diameters (19.3 vs. 16.9, mm), and serum estradiol (155.7 vs. 75.9, ng/mL) concentration. The present study demonstrated that LPL supplementation could improve the performance of early-lactation dairy cows.

Key Words: follicles diameters, milk production, lysophospholipids

1220M Production and composition of milk from Holstein cows supplemented with polyphenol-based sugarcane extract. E. D. Marino[1], L. Castelani[2], L. C. Roma Jr.[2], R. Marchetto[2], W. V. B. Soares[2], and C. M. M. Bittar*[1], [1]*Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil,* [2]*Animal Science Institute, Secretary of Agriculture and Supply of the State of São Paulo, Nova Odessa, ão Paulo, Brazil.*

Some polyphenols have been used as manipulators of ruminal fermentation to reduce methane emissions from dairy cows. However, more information about the effect of this supplement in milk composition and quality is needed. This completely randomized block design study aimed to evaluate the influence of the supplementation of a polyphenol-based sugarcane extract on the milk production and composition, body weight (BW) and body condition score (BCS) of dairy cows. Sixteen multiparous Holstein cows (8 per treatment) were blocked according to average production (20 kg/d ± 4.1), 120 DIM and BW (547 kg ± 17.4) and were fed a TMR twice a day. The polyphenol-based supplement (TPM, Australia) or distilled water (control) were orally administered to each cow individually for 49 d in a dose of 50 mL. Milk samples for composition and somatic cell count (SCC) analysis were collected 3 times a week. Data of individual milk production were collected daily. Cows' BW and BCS were recorded weekly. Data were analyzed as repeated measures using PROC MIXED of SAS. There were no effects of the polyphenol-based sugarcane extract on milk production, BW, BCS or milk composition. However, there was a significant interaction effect between treatments and week for the SCC score, but this did not occur between treatments within a specific week. Results show that the

Table 1 (Abstr. 1220M). Body weight, body condition score, milk production and composition of cows supplemented with polyphenol-based sugarcane extract for 49 days

	Treatment			*P*-value[1]		
Item	Control	Polyphenol	SEM	T	W	T × W
Milk production, kg/d	20.4	22.3	1.75	0.4406	<0.0001	0.3175
Body weight, kg	528.6	558.9	24.17	0.3897	0.0855	0.8197
BCS	2.52	2.3	0.13	0.2576	0.5202	0.1530
Milk composition						
Solids non-fat, %	8.66	8.76	0.096	0.4919	0.0202	0.7329
Fat, %	3.76	3.59	0.238	0.6092	<0.0001	0.9373
Protein, %	3.20	3.17	0.091	0.8124	0.0901	0.7830
Casein, %	2.56	2.56	0.072	0.9775	0.0065	0.6355
Lactose, %	4.41	4.56	0.079	0.2028	0.0013	0.1767
MUN, mg/dL	15.59	14.99	1.030	0.6886	<0.0001	0.8748
ESCC[2]	1.83	1.52	0.149	0.1537	0.2671	0.0241

[1] T = treatment effect; W = week effect; T × W = interaction effect of treatments and week.

[2] ESCC = $\log_2$ (SCC/100) + 3 (Shook, 1982).

supplementation of dairy cows with polyphenol-based sugarcane extract did not change the composition, production and quality of milk (Table 1).

Key Words: milk composition, somatic cell count

1221M 25-Hydroxyvitamin D$_3$ supplementation increased productive performance during lactation in dairy cows. J. M. Albuquerque[1], C. Cortinhas[2], T. Acedo[2], A. S. Silva[1], K. M. Borges[1], J. Diavão[1], M. H. Ferreira[3], F. C. F. Lopes[1], M. M. Campos[1], D. S. C. Paciullo[1], C. A. M. Gomide[1], M. J. F. Morenz[1], and P. Gott*[4], *[1]Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil, [2]DSM Produtos Nutricionais, São Paulo, São Paulo, Brazil, [3]Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil, [4]DSM Nutritional Products, Parsippany, NJ.*

Vitamin D$_3$, which plays an important role in modulating bone, mineral and energy metabolism. Dietary supplementation of 25-hydroxyvitamin D$_3$ is more effective at increasing the concentrations of 25-hydroxyvitamin D$_3$ in blood. The aim of this study was to evaluate the effects of 25-hydroxyvitamin D$_3$ [25(OH)D$_3$] on productive performance, dry matter intake (DMI), feed efficiency, body weight (BW), body condition score (BCS), and serum and plasma metabolites of grazing lactating dairy cows. Twenty-two Holstein × Gyr cows [18 ± 3.5 kg/d milk yield and 94 ± 21.9 d in milk (DIM)] were blocked according to milk yield and allocated to 1 of 2 treatments in a completely randomized block design. Treatments were: (1) no addition of 25(OH)D$_3$ (CTRL) or (2) 25(OH)D$_3$ at 1.0 mg/cow/d (Rovimix HyD, DSM Produtos Nutricionais). Treatments were mixed into 10 g of a finely ground corn and top-dressed to the concentrate (3 kg/cow) offered during the morning and afternoon milking. Diets were formulated according to NRC (2001). Pasture was composed by *Megathyrsus maximus* cv. Mombaça. Cows were adapted to the experimental diets for 14 d and the measurements lasted 56 d. Titanium dioxide was used to estimate forage intake. Blood samples were collected 2 h after the morning feeding on d 21 and 71. Data were analyzed using SAS and statistical significance was defined at $P \leq 0.10$. No treatment effects were observed on pasture or total DMI. Cows fed 25(OH)D$_3$ had higher fat (CTRL = 15.3 vs. 25(OH)D$_3$ = 16.8 kg/d; $P = 0.09$) and energy-corrected milk ($P = 0.09$), but no effects on milk composition, milk urea nitrogen, and feed efficiency were observed. Also, 25(OH)D$_3$ increased BCS compared with CTRL cows ($P = 0.07$), without difference in BW. Plasma concentration of 25(OH)D$_3$ was increased on d 71 (CTRL = 32.1 vs. 25(OH)D$_3$ = 70.8 ng/mL; $P = 0.01$) for 25(OH)D$_3$ cows. Plasma nonesterified fatty acids was 34% greater for 25(OH)D$_3$ than CTRL ($P = 0.03$), but no differences were observed on plasma Ca and Mg. In conclusion, dietary supplementation of 25(OH)D$_3$ brings benefits on productive performance of lactating grazing dairy cows.

Key Words: milk yield, tropical grass pasture

1222M Changes of rumen microbes related to hormonal response in the rumen of lactation dairy cows under heat stress. Z. Guo, S. Gao, L. Ma, and D. Bu*, *State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Microorganisms could perceive the neurohormones secreted by animals under stress to regulate their own growth activities, and the harmful metabolites released are also regulated by the neurohormones secreted by the host animals. However, the regulation mechanism of ruminal hormones on rumen microbes is still unclear in lactation dairy cows. The purpose of the current study was to investigate the effect of heat stress on hormonal response in rumen as well as its regulation to rumen microbes. Eight multiparous Holstein dairy cows with rumen cannula were randomly equally allocated to 2 replicates (n = 4), with each replicate being subjected to heat stress (HS) or thermal neutrality and pair-feeding (PFTN) in 4 environmental chambers. Samples of blood, rumen fluid and saliva were collected every other day in the trial period of each replicate for the analysis of growth hormone, insulin, cortisol and inflammatory factors. Meanwhile, the change of ruminal pH as well as the frequency of rumen contraction was recorded every hour on the last day of the trial period. The results showed that heat stress significantly reduced the pH value of rumen fluid (6.34 vs. 6.71, $P = 0.034$), but had no significant effect on rumen motility frequency (25.6 vs. 25.0 times/s, $P = 0.782$), suggesting the decrease in rumen pH is more likely due to the decrease in the saliva swallowed in the rumen instead of the changes of rumen peristalsis. Compared with the PFTN group, heat stress decreased the concentration of cortisol hormone in blood (6.32 vs. 9.57 ng/mL, $P = 0.035$) and growth hormone in rumen fluid (4.41 vs. 8.63 ng/mL, $P = 0.007$) of dairy cows, but increased the concentration of insulin in rumen fluid (262.19 vs. 107.31 μIU/mL, $P = 0.011$). In addition, the total amount of TNF-a (55.95 vs. 35.52 pg/mL, $P = 0.044$) in the blood during heat stress increased significantly compared with the PFTN group. Overall, the consistent trend changes of insulin and growth hormone in rumen fluid and blood may inform the potential hormonal interaction between the rumen microbes and the host dairy cows.

Key Words: rumen microbes, heat stress, hormone

1223M Complete genome sequence of *Corynebacterium* sp. SCR221107, a potential probiotic strain, isolated from the rumen fluid of Holstein dairy cows. S. H. Na[1], K. S. Baik[1], S. H. Kim[1], A.-R. Son[1], M. J. Ku[2], and S. S. Lee*[1], *[1]Ruminant Nutrition and Anaerobe Laboratory, College of Bio-Industry Science, Sunchon National University, Suncheon, Republic of Korea, [2]Livestock Research Institute, Jeonnam Agricultural Research and Extension Services, Gangjin, Republic of Korea.*

The *Corynebacterium* sp.SCR221107 was isolated from the rumen fluid of Holstein dairy cows collected from local farm in Republic of Korea. The strain was incubated in an anaerobic atmosphere (5% carbon dioxide, 5% hydrogen, and 90% nitrogen) at 37°C for 48 h on DeMan, Rogosa and Sharpe (MRS) media. *Corynebacterium* sp.SCR221107 is the functional probiotic candidate with an ability to vitamin B$_{12}$ production. The whole genome of *Corynebacterium* sp.SCR221107 was sequenced using the PacBio RS II platform and Illumina HiSeq platform, and assembled de novo. The complete genome of the *Corynebacterium* sp.SCR221107 contains one circular chromosome (3,043,024 bp) with a guanine + cytosine (GC) content of 60.1%. Annotation analyses revealed 2,639 protein-coding sequences, 15 rRNA genes, and 57 tRNA genes. Based on genome analysis, we found that *Corynebacterium* sp.SCR221107 possessed various genes associated with vitamin B$_{12}$ synthesis and transport. The genome information adds to the comprehensive understanding of *Corynebacterium* sp.SCR221107 and suggest that the isolate might have a potential probiotic application.

Key Words: *Corynebacterium* sp., Holstein dairy cow, vitamin B$_{12}$

1224M Effects of feeding silage inoculated with microbial inoculants on lactational performance, and rumen fermentation of transition dairy cows. C. Niño-de-Guzman*[1], J. Portuguez[2], R. Trumpp[2], D. Vassolo[1], C. Cornejo[1], S. Paladugu[1], L. Lima[1], F. Amaro[1], K. Arriola[1], and D. Vyas[1], *[1]Department of Animal Sciences, University*

of Florida, Gainesville, FL, [2]Department of Agronomy, University of Florida, Gainesville, FL.

This study evaluated the effect of feeding inoculated corn and ryegrass silage on the lactational performance and rumen fermentation parameters of transition dairy cows. Eighty-two multiparous dry cows were blocked by the previous 305-d ECM and fed either 1) CON: TMR composed of uninoculated corn and ryegrass silage, or 2) INO: TMR composed of inoculated corn and ryegrass silage. The experiment was a randomized complete block design, and the duration was 21 d prepartum and 42 d postpartum. Animals were fed ad libitum once daily at 0700 h, and dry matter intake was recorded. Cows were milked twice daily at 1000 and 2200 h, and milk yields were recorded. Milk samples were taken from 4 consecutive milkings and analyzed for fat, lactose, protein, SCS, and MUN. Data were analyzed using PROC GLIMMIX of SAS, and significance was declared when $P \leq 0.05$ and tendency when $0.05 < P \leq 0.10$. No treatment effects were observed on DMI (19.1 vs. 20.3 kg/d) and milk yield (41.0 vs. 43.2 kg/d). However, 3.5% FCM (42.0 vs. 44.9 kg/d) and ECM (41.9 vs. 44.5 kg/d) increased for cows fed INO. Similarly, milk fat yield was greater (1.50 vs. 1.62 kg/d) for cows fed INO while milk fat concentrations did not differ. Milk lactose and milk protein concentrations and yields of milk protein and milk lactose were not different between treatments. No treatment effects were observed on MUN and SCS. Ammonia-N concentrations in the rumen were greater (5.47 vs. 4.18 m*M*) for cows fed INO during the prepartum phase. There was no treatment effect on ammonia-N during the postpartum phase. Ruminal pH and volatile fatty acids did not differ between treatments during the pre and postpartum phases. In conclusion, feeding inoculated silage did not affect DMI and MY along with concentrations and yields of milk protein and lactose. However, feeding inoculated corn and ryegrass silage increased 3.5% FCM, ECM, and milk fat yield. In addition, it increased ruminal ammonia-N concentration during the prepartum phase, but it did not affect ruminal pH and VFA during the pre- and postpartum phases.

Key Words: inoculated silage, fat-corrected milk, energy-corrected milk

1225M Effect of starch and forage NDF concentrations on intake, milk yield, and composition of dairy cows receiving rations containing OneTrak.

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OneTrak is a high-energy, high-feeding-rate corn co-product with 64% DM, 27% CP, and 37% NDF. This study determined dairy cow performance responses to varying dietary starch and forage NDF (fNDF) on OneTrak-based diets. Ninety-six multiparous cows (90 ± 26 DIM) were group-housed (12 pens, 8 cows/pen) and used in a 4 × 4 Latin square design with factorial (2 × 2) arrangements of treatments. Treatment main effects were starch level [22 (LS) and 26% (HS)] and fNDF level [17 (LF) and 20% (HF)], which resulted in 4 treatments: 1) LSLF, 2) LSHF, 3) HSLF, and 4) HSHF. OneTrak was incorporated into the diets at a constant amount (40% DM basis), whereas amounts of corn silage, alfalfa silage, and ground corn were altered to achieve starch and fNDF levels. Cows were fed a TMR once daily and milked thrice daily. In addition to intake and milk composition, serum was collected during the last week of each period. Data from the last week of each period were analyzed using the MIXED procedure of SAS with diet and period as fixed effects and pen as a random effect. Dry matter intake was lower for high fNDF and was less for LSHF (28.4 kg/d) compared with HSHF (28.9 kg/d) resulting in a starch × fNDF interaction ($P < 0.05$), whereas DMI did not differ for LSLF versus HSLF (30.0 kg/d). Milk yield (49.8 vs. 48.0 kg/d; $P < 0.01$), ECM (52.0 vs. 49.9 kg/d; $P < 0.01$), and 4% FCM (49.1 vs. 47.3 kg/d; $P < 0.01$) were greater for HS compared with LS. Forage NDF did not affect milk yield, ECM, or FCM. Feed efficiency was higher for HS compared with LS and HF vs. LF. Starch level did not affect concentrations of milk fat, lactose, or mixed fatty acids. Compared with LS, HS resulted in greater yields of milk fat, milk protein, lactose, de novo fatty acids, and mixed fatty acids. Forage NDF level did not affect milk parameters other than protein yield, which was greater for LF compared with HF. Serum glucose was unaffected by starch level but greater for HF versus LF. Under conditions of this experiment, cows fed OneTrak in rations with HS had greater DMI, ECM, and FE compared with LS.

Key Words: starch, forage NDF, co-product

1226M Effect of abomasal starch and hindgut buffers on lymphocyte kinome profile.

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High-starch diets fed to cows to support milk production can lead to acidotic conditions in the rumen and large intestine that drive systemic inflammation. The objective of this study was to determine the efficacy of 2 products fed to buffer hindgut pH, and their effects on phosphorylation of lymphocyte peptides as an indicator of cow immunometabolism. Nine multiparous, ruminally cannulated Holstein cows in late-lactation were fed a 30% starch diet (on a DM basis). The experiment was conducted as a 5 × 5 Latin square with 14-d experimental periods followed by 1-wk washout periods. Cows received either no dietary buffer (CON, IS), 150 g/d (T1) or 300 g/d (T2) prototype 1, or 150 g/d prototype 2 (T3). During d 5–14 of each experimental period, cows were abomasally infused with either 6 L/d of tap water (CON) or 4 g/kg bodyweight cornstarch mixed with 6 L/d of tap water (IS, T1, T2, T3). Peripheral blood mononuclear cells were isolated on d 14 of each treatment period, flash-frozen in liquid nitrogen, and stored at −80°C for kinome peptide array analysis. Cells were later thawed, lysed, and centrifuged before activation with ATP and application to PepStar Peptide Microarrays. After incubation, array fluorescence was imaged and statistically analyzed with PIIKA2. Pathway analyses were performed with STRING, KEGG, and PhosphoSitePlus. Treatment effects were evaluated relative to IS. Pathway analysis showed fewer numbers of statistically significant changes when comparing CON and T3 to IS, and increased changes when comparing T1 and T2 to IS. Relative to IS, phosphorylation patterns in CON suggested reduced glycogen and starch metabolism, proliferation of cells, presentation of antigens, and MAPK immune activity. Both T1 and T2 exhibited increased protein synthesis and cell differentiation, and possible increased T-cell activity. We found that T1 increased NF-kB activity and T2 increased MAPK activity. However, T3 reduced metabolic activity in comparison to IS, with decreased production of cytokines from both the NF-kB and MAPK pathways. Both CON and T3 reduced immunometabolic activity compared with IS, while T1 and T2 increased protein synthesis and immune activity. Finally, T3 may reduce negative effects of high-starch diets.

Key Words: buffers

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1227M Three new genera of bacteria that carry out biohydrogenation of unsaturated fatty acids. T. Hackmann*, H. De Groot, and P. Vahmani, *University of California, Davis, Davis, CA.*

Bacteria in the rumen convert unsaturated fatty acids to saturated ones through biohydrogenation. This process is important because some consumers of dairy products view saturated fat as unhealthy. Further, some intermediates of biohydrogenation cause milk fat depression in dairy cows. Despite this importance, we know of few bacteria capable of this metabolism. Our objective was to identify more bacteria using genomics and biochemical assays. We first searched genomes of 142,000 strains for a gene for linoleate isomerase, which catalyzes the first step of biohydrogenation of linoleic acid. We then biochemically tested 3 of these strains (*Tractidigestivibacter scatoligenes* SK9K4, *Sporanaerobacter acetigenes* Lup33, *Parafannyhessea umbonata* A2) to determine if they could carry out biohydrogenation of linoleic acid in growing cultures. The first 2 bacteria (*T. scatoligenes*, *S. acetigenes*) were positive for forming conjugated linoleic acid, the first intermediate of linoleate biohydrogenation, when we used a spectrophotometric assay. The rate of formation was fastest for *T. scatoligenes*, reaching 57.6% (8.8 SEM) of the original dose of linoleic acid by 24 h. The third bacterium (*P. umbonata* A2) was negative when using the spectrophotometric assay, but it was positive for forming *trans*-10 18:1, a downstream intermediate, when using gas chromatography. Our experiments have identified bacteria from 3 genera capable of biohydrogenation. Our experiments establish proof of concept and can be readily extended to identify more bacteria. Bacteria identified in the future could be targets for manipulating the fatty acid profile of dairy products and reducing milk fat depression.

Key Words: rumen, bacteria, biohydrogenation

1228M Comparing rumen headspace sampling devices in ruminal cannulated cows. E. M. V. Hvas*, M. R. Weisbjerg, T. N. Jakobsen, and M. Larsen, *Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Aarhus University, Tjele, Denmark.*

The aim was to test 2 devices for sampling rumen headspace gas in ruminal cannulated cows for obtaining samples with low atmospheric air contamination. Four ruminal cannulated Danish Holstein cows were used in a Latin square design and the test was performed in the last period. Dry matter intake and milk yield of cows were averaged (±SD) 22.7 ± 1.12 kg/d, and 22.5 ± 3.87 kg/d, respectively. Rumen headspace gas was sampled 3 times a day for 3 d using both sampling devices. The first sampling device (D1) consisted of a silicone tube (Codan) terminating in a suction head attached to the internal cannula collar top. The silicone tube was threaded through a hole in the cannula tightened with silicone. On the outside end of the tube, a 3-way valve was attached. The second sampling device (D2) consisted of a 30-cm suction strainer (Bar Diamond) with a suction head attached to the rumen headspace end and a 3-way valve attached to the outside end. The suction strainer was bent in a "U" shape and inserted into the headspace of the rumen through a screw top stopper in the rumen cannula. A sponge was immediately fitted around the suction strainer to minimize rumen contamination of atmospheric air. With each device, 10 mL of gas was sampled (first 10 mL was discarded) using a syringe and transferred to Exetainers with a needle. The D1 samples were always drawn before D2. Data were analyzed using linear mixed models with device, day, time, and device × time interaction as fixed effects, cow as random effect, and considering time as repeated measure. The CH_4 and CO_2 concentrations were greater ($P < 0.01$) when using D1 compared with D2 (mean ± SEM; 17.4 vs. 7.37 ± 1.28%, and 58.1 vs. 30.7 ± 5.12%, respectively). The O_2 and N_2 concentrations were lower ($P < 0.01$) when using D1 compared with D2 (6.09 vs. 11.5 ± 1.12%, and 24.6 vs. 47.4 ± 3.92%, respectively). Hydrogen concentrations did not differ between sampling devices ($P = 0.08$; 0.45 ± 0.02%). In conclusion, using the D1 sampling device is preferred as it appears to give the least contamination with atmospheric air (lowest O_2 and N_2) of rumen headspace gas sampled from ruminal cannulated cows.

Key Words: methane, oxygen, sampling methods

1229M Characterization of microbial population changes in continuous culture during the adaptation phase. B. A. Wenner*[1], G. Praisler[1], and T. Park[2], [1]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [2]*Department of Animal Science and Technology, Chung-Ang University, Anseong-si, Gyeonggi-do, Korea.*

Despite resurgence in dual-flow continuous culture (DFCC) as a research tool, few data sets exist on the difference between inoculum, fermenter content, and effluent microbiomes. Our objective was to assess adaptation of the rumen microbiome in DFCC relative to effluent typically sampled from DFCC. Previous inoculum (IN), adapted fermenter contents (from d7, AD), and effluent (EF) samples taken from DFCC (n = 2) across 4 experimental periods were extracted for metagenomic DNA, sequenced using a universal 16s rRNA gene primer, and matched to taxonomy. Treatments were control (60:40 concentrate:orchardgrass pellet, 17.1% CP, 36.4% NDF, 22.3% ADF, and 22.6% starch) fed twice daily, totaling 80 g/d DM, or control + an essential oil dosed at 3 mg/d. Analysis of microbial features was conducted using MaAsLin2 and the adonis2 function in R for d0-d7, while a mixed model approach included fixed effect of treatment and sample type, and random effect of period. Significance was declared at $Q < 0.05$ and $P < 0.05$ for MaAsLin2 and mixed model analyses, respectively. Regardless of index, α-diversity decreased ($Q \leq 0.05$) over time, characterized by decreases in *Bacteroidetes* and *Firmicutes*, and increased *Proteobacteria*, while overall microbial communities were not different ($Q > 0.05$). Many bacteria shifted relative abundance over time including increases ($Q < 0.05$) by *Succinivibrionaceae* UCG-002, *Succinivibrio*, and *Bacteroidales* F082, and decreases ($Q < 0.05$) including *Butyrivibrio*. Compared with IN, AD increased ($P < 0.01$) *Pseudobutyrivibrio* and *Succinivibrionaceae* UCG-002 by more than 10-fold, and *Treponema* and *Bacteroidales* F082 by more than 1.5-fold ($P \leq 0.02$). Uncultured *Prevotellaceae* and *Ruminococcaceae*, and *Oscillospiraceae* NK4A214 decreased from IN to AD ($P < 0.01$) by more than 70%. Relative abundance of *Succinivibrionaceae* UCG-002 was 1.8-fold greater ($P < 0.01$) for EF compared with AD while uncultured *Muribaculaceae* and *Lachnospiraceae* decreased by 30% ($P \leq 0.01$). Sequences from DFCC samples indicate a shift in primary genera versus in vivo; a deeper look into predicted metabolic pathways may clarify the impact of such trade-offs within DFCC.

Key Words: continuous culture, in vitro methods

1230M Treatment of continuous culture fermenters with an organic essential oil product minimally influenced bacterial relative abundance. T. Park[1], G. Praisler*[2], and B. A. Wenner[2], [1]*Department of Animal Science and Technology, Chung-Ang University,* *Anseong-si, Gyeonggi-do, Korea, [2]Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Previous research reported an essential oil (EO) product decreasing methane (CH_4) production by dual-flow continuous culture (DFCC); this product could assist organic dairy producers in decreasing emissions. Our objective was to assess the effect of this EO product on the microbial populations within DFCC. We hypothesized the EO either decreased protozoal population or induced shifts in the bacterial abundance to decrease CH_4 production. Previous effluent samples taken from DFCC (n = 2) across 4 experimental periods were extracted for metagenomic DNA and sequenced using a universal primer for 16S rRNA gene, matching taxonomy through SILVA v138; protozoa were also enumerated from effluent samples. Treatments were either a control (CON, 60:40 concentrate:orchardgrass pellet mix, 17.1% CP, 36.4% NDF, 22.3% ADF, and 22.6% starch) fed twice daily for a total of 80 g/d DM, or CON + EO at 3 mg/d. The statistical model included fixed effects of treatment and fermenter, and random effect of period, using either MaAsLin2 or the adonis2 function in the vegan package of R for microbial features, or a mixed model for protozoal counts. Significance was declared at $Q < 0.05$ and $P < 0.05$ for MaAsLin2 and other analyses, respectively. Protozoal populations averaged 1.45×10^4 cells/mL, but there were no treatment effects ($P > 0.10$) on counts or cell outflow. Addition of EO increased relative abundance of *Methanobacteriaceae* and decreased *Methanomethylophilaceae* ($P < 0.05$) but there was no effect of EO on archaeal α- or β-diversity ($P > 0.05$). While there was no effect ($P > 0.05$) of EO on β-diversity measured in bacterial communities, EO decreased ($Q < 0.05$) α-diversity indices including Chao1, Pielou’s evenness, Shannon, and Simpson. Supplemental EO decreased ($Q < 0.01$) several genera including *Clostridia* UCG-014, *Rikenellaceae* RC9 Gut Group, and *Christensenellaceae* R7 Group, and increased ($Q < 0.01$) others including *Treponema*, *Succinivibrionaceae* UCG-002, and *Ruminococcus*. Offsetting shifts in relative abundance of fiber-degrading bacteria deserve further investigation including predicted metabolic pathways impacted by population changes.

Key Words: methane, rumen microbiome

1231M Use of appropriate molecular biological techniques to identify true microbial community in low microbial biomass samples. R. Nakandalage*[1,2], P. J. Griebel[3], L. L. Guan[1], and N. Malmuthuge[2], *[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, [2]Lethbridge Research and Development Center, Agriculture Agri-Food Canada, Lethbridge, Alberta, Canada, [3]Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Amplicon sequencing (16S rRNA gene sequencing) is widely used to profile host-associated microbial communities. However, profiling low microbial biomass samples using amplicon sequencing is challenging due to contaminations that lead to artificial microbial sequences. This study compared amplicon sequencing-based microbial profiles generated by different genetic materials (DNA vs. RNA) and primer pairs targeting 2 hypervariable regions of the 16S rRNA gene (V1V3 vs. V3V4) to optimize the profiling of microbial communities from low microbial biomass samples. Fecal swabs (n = 40) were collected from 20 newborn calves and used to extract DNA and RNA. Both DNA- and RNA-based sequencing was performed by targeting the V3V4 region of the 16s rRNA gene. In addition, only DNA-based sequencing was performed by targeting both V1V3 and V3V4 regions. All sequence runs included no template controls and positive controls (*Clostridium butyricum*). Data were analyzed using the QIIME2 platform with customized quality filtering steps to remove environmental contaminations and to filter out low frequent and low abundant amplicon sequencing variants (ASV). The Kruskal-Wallis test was used to compare the microbial diversity data. Sequencing analysis revealed that no template controls contained diverse ASV profiles irrespective of the genetic materials and target regions, suggesting that amplicon sequencing introduces contaminations to host-associated microbial communities. When comparing the impact of the target region on the microbial communities, the V1V3 region generated highly diverse microbial profiles compared with that of the V3V4 region. Besides, DNA-based sequencing increased the diversity of microbial profiles more than those RNA-based sequencing. In conclusion, microbial profiles generated from the low microbial biomass samples were dependent on the genetic materials used and the target hypervariable region of the 16S rRNA gene. The use of no template controls is crucial to identify potential contaminations when using low microbial biomass samples.

Key Words: amplicon sequencing, low biomass samples, 16S rRNA gene

1232M Insight into the ruminal microbiota of dairy cows using the culturomics and amplicon-based approaches. X. Duan[1,2], R. Ma[3], S. Vigors[2], L. Ma[1], J. Gu[3], and D. Bu*[1], *[1]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland, [3]Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China.*

The rumen ecosystem is complex and dynamic, and is closely related with the feed utilization efficiency of cattle. In this study, we aimed to reveal the rumen microbiome of dairy cows by using the culturomics and amplicon-based metagenomic approaches. The ruminal fluid of 6 cows were sampled via a stomach tube with a rumen vacuum sampler. The samples were mixed, one portion of which were added with 60% contained glycerol and stored for amplicon sequencing, the other portion for culturomics. Eleven media involving 11 recipes and 2 gas conditions were employed to isolate aerobic, facultative anaerobic and strict anaerobic bacterial strains. We combined MALDI-TOF MS and 16S rRNA gene sequencing to identify specific strains. A total of 234 prokaryotic strains representing 1047 isolates were assigned to 3 phyla (*Firmicutes*, *Proteobacteria* and *Actinobacteria*), 3 classes, 7 orders, 12 families, 18 genera, and 38 species, including 7 potential new species. At the genus level, *Bacillus*, *Brevibacillus*, *Cronobacter*, *Cutibacterium*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Kurthia*, *Lacticaseibacillus*, *Ligilactobacillus*, *Limosilactobacillus*, *Microbacterium*, *Pediococcus*, *Pseudomonas*, *Rothia*, *Staphylococcus*, *Streptococcus* and *Weissella* were identified, and *Escherichia*, *Limosilactobacillus* and *Ligilactobacillus* were predominant in the ruminal culturome. Wilkins-Chalgren broth was the most efficient medium to isolate ruminal bacteria under aerobic and anaerobic conditions, and 179 strains were collected under facultative anaerobic conditions. The culturomics results covered 9% of the OTU found in the rumen microbiota. This study indicates the key role of culturomics in the exploration of complex microbiota in the rumen.

Key Words: rumen, culturomics, amplicon

1233M Characterization of proteins involved in the absorption of fatty acids in the gastrointestinal tract of cattle. F. Hentz, B. Barreta, and F. Batistel*, *University of Florida, Gainesville, FL.*

Fatty acid absorption is directly linked with the efficiency of fat utilization in ruminant diets. Previous studies in non-ruminant models demonstrate that the absorption of long-chain fatty acids is a transporter-mediated process. However, proteins involved in this process have been scarcely evaluated in cattle. The objective of this research is to investigate the presence and distribution of proteins involved in the absorption of long-chain fatty acid in the gastrointestinal tract of cattle. Samples of epithelial tissue from the reticulum, all rumen sacs, duodenum, proximal, medial, and distal jejunum, and ileum were collected from 8 adult cattle. Protein expression was analyzed via capillary immunoelectrophoresis using an Abby system from ProteinSimple. The results were normalized to a housekeeping protein (β-actin). The expression of solute carrier family 27 member 4 (SLC27A4), fatty acid binding protein 3, and acyl-CoA synthetase long-chain family member 1 was upregulated in the proximal and distal jejunum compared with the other gastrointestinal sections. The expression of fatty acid binding proteins 1 and 2, and microsomal triglyceride transfer protein were higher in the duodenum, proximal jejunum, and ileum compared with the other gastrointestinal sections. Acyl-CoA-diacylglycerol acyltransferase 1 (DGAT1) and acetyl-CoA acetyltransferase 2 (ACAT2) were upregulated in the distal jejunum and ileum compared with the other gastrointestinal sites. Apolipoprotein A1 was exclusively expressed in the reticulum and rumen. Interestingly, SLC27A4 and ACT2 were expressed in the reticulum and the rumen. Our preliminary results indicate a distinct pattern of long-chain fatty acid transport proteins across the gastrointestinal tract of cattle. Although the jejunum and ileum are the main sites of fatty acid absorption, the rumen and reticulum also seem to contribute to this process.

Key Words: gut physiology, nutrient absorption

1234M Effect of acetate on dry matter intake may not be related to circulating GLP-1 and PYY plasma concentration.

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Acetate infusions can suppress intake when infused as sodium acetate at high doses into the abomasum or rumen or at lower doses if infused as acetic acid. The mechanism of the anorexigenic effect of acetate in ruminants is unknown. The objective of this experiment was to determine if anorexigenic infusions of acetate are associate with an increase in glucagon-like peptide 1 (GLP-1) and peptide YY (PYY). The study was replicated twice with each block having 10 ruminally cannulated Holstein cows arranged in a 4 × 4 Latin square with a 2 × 2 factorial treatment design. Experimental periods consisted of an 8-h infusion period starting at feeding (0900 h) followed by a 16-h observation period and a 24-h washout. Treatment factors were infusion (sodium acetate or sodium chloride) and site of infusion (abomasal or ruminal) each with 2 levels. A 6 *M* sodium acetate solution was infused in either the rumen or abomasum to provide 0.75 mol/h for 8 continuous hours at start of feeding. Isomolar and isovolumetric infusions of sodium chloride were used as a control. Feeding behavior was observed during the infusion and observation period using feed tubs hanging on load monitors. Blood samples were taken from a tail vessel at −10, 0, 15, 30, 60, 120, and 480 min relative to start of infusion during the second replicate to minimize the disruption to the feeding behavior. There was an effect of infusion on intake during the infusion period ($P = 0.002$) but no interaction with infusion site ($P = 0.81$). Infusions of acetate decreased intake by 12% compared with control ($P = 0.02$). There was an infusion by replicate interaction for the 24-h cumulative intake ($P = 0.03$), as cows had a compensatory increase in intake in block 2 and were not different from control ($P = 0.55$). In Block 1 acetate decreased daily intake by 8% ($P = 0.009$). There was no effect of infusion, infusion site, or interaction between them for plasma GLP-1 ($P = 0.80$, 0.61, 0.15, respectively) and PYY ($P = 0.34$, 0.39, and 0.20, respectively). In conclusion, decreased intake during sodium acetate infusion does not appear to be due to changes in GLP-1 or PYY concentration.

Key Words: anorexigenic, gut peptides, feeding behavior

1235M Gut microbiome is linked to functions of peripheral immune cells in transition cows during excessive lipolysis.

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This study was aimed to investigate the potential links between the gut microbiome and postpartum immunosuppression in transition dairy cows with excessive lipolysis. Cows with plasma NEFA levels >750 μmol/L on d 7 postpartum were defined as high/excessive lipolysis (HNF, n = 9), whereas those with plasma NEFA levels <600 μmol/L were defined as low/normal lipolysis (LNF, n = 9). The peripheral immune cells were collected to perform single-cell RNA sequencing (scRNA-seq), the fecal samples were conducted the analysis of 16S amplicon sequencing, metagenomics, and targeted metabolomics, the plasma were collected to measure the blood parameters and targeted metabolomics. We identified 10 different immune cell types and revealed a downregulation of immune functions in HNF compared with LNF ($P < 0.001$). Beta diversity analysis revealed a clustering of fecal bacterial communities based on lipolysis grouping ($P = 0.001$). Totally, 9 differentially bacterial genera and 21 species were identified between 2 groups. Functional analysis showed that secondary bile acids (SBA) biosynthesis was significantly higher in HNF cows than LNF cows ($P = 0.047$), and the total SBA with higher tendency ($P = 0.10$) in HNF. Furthermore, the plasma glycolithocholic acid (GLCA, $P = 0.02$) and taurolithocholic acid (TLCA, $P = 0.03$) content was lower in HNF than LNF. Additionally, the results showed that MON exhibited a uniquely higher BA receptor expressing score. The GPBAR1 expression was lower ($P = 0.007$) in $CD14^+$MON in HNF cows. Enrichment analysis revealed that immune functions were significantly downregulated in $CD14^+$MON ($P < 0.001$) isolated from HNF cow and significantly correlated with BA metabolism ($P < 0.001$). In summary, our results showed that the gut microbial alteration and reduced plasma GLCA and TLCA could contribute to the immunosuppression of $CD14^+$MON during excessive lipolysis by decreasing the expression of GPBAR1 in transition dairy cows, which provides a novel insight for the healthy feeding of transition dairy cows.

Key Words: bile acids, gut microbiome, immunosuppression

1236M Effects of early lactation milking frequency in an automatic milking system on rumen fermentation characteristics.

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Our study objective was to investigate the effects of early lactation milking frequency on cows milked via automated milking system (AMS) on rumen fermentation. Multiparous Holstein cows blocked by parity and due date were randomly assigned to fetch schedule treatments

(TRT, n = 8 per TRT): 3 (3×) or 6 times (6×) per day. All cows were milked 3 times per day (MPD) from 0 to 3 d in milk (DIM). From 4 to 29 DIM, 3× cows were set to 3 MPD while 6× cows were allowed 6 MPD. Rumen fluid was collected via esophageal sampling, preserved, and measured for concentrations (CONC) of volatile fatty acids (VFA), ammonia, and total AA (TAA). Samples were collected at 3, 13, and 23 ± 1 DIM. Data were analyzed using generalized linear mixed models (SAS 9.4, GLIMMIX). Fixed effects were TRT, lactation group (LG; 2 vs 3+ parity), DIM, and their interactions. Random effects were cow, and a repeated measure of cow across DIM. The 6× TRT had more ($P < 0.01$) MPD compared with 3× TRT (4.9 ± 0.2 vs 3.1 ± 0.1) Butyrate CONC and molar proportions (PRP) decreased across DIM ($P = 0.04$; $P < 0.01$). Cows milked 6× at later DIM had lower butyrate compared with 3× ($P < 0.08$). The PRP of valerate decreased ($P = 0.09$) as DIM increased. Rumen CONC and PRP of branched-chain VFA (isobutyrate, 2- and 3-methylbutyrate) decreased ($P < 0.01$) at later DIM irrespective of TRT ($P = 0.98$). The PRP of isobutyrate was greater in LG 2 ($P = 0.02$). Lactate was greater in LG 2 ($P = 0.06$). Rumen ammonia CONC did not differ by TRT ($P = 0.66$), and TAA increased ($P = 0.08$) by DIM. Rumination measured on the AMS software did not detect differences between TRT ($P = 0.29$) or DIM ($P = 0.19$). Overall, results indicate early lactation MPD has minimal impact on rumen fermentation. Shifts in VFA in early lactation are likely due to ingredient and nutrient shifts changing from the prepartum to postpartum partial mixed ration diet irrespective of MPD. Lower butyrate in 6× TRT at later DIM may suggest greater ruminal clearance to support increased energy requirements at a higher milking frequency. Increases in TAA by DIM are consistent with increases in dry matter intake as lactation progresses.

Key Words: robotic milking, rumen fermentation, transition period

1237M ***Bacillus*-based direct-fed microbial alters fecal parameters during feed restriction in mid-lactation Holstein cows.**

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Objectives were to evaluate the effects of a *Bacillus subtilis* and *Bacillus pumilus*-based direct-fed microbial (DFM; Strateris ECL; United Animal Health) on fecal pH (fpH), dry matter (fDM), starch (fS) and volatile fatty acids (fVFA) during feed restriction (FR) in mid-lactation Holstein cows. Thirty-six cows were randomly assigned to 1 of 3 dietary treatments (TRT): 1) control (CON; 7.5 g/d rice hulls; n = 12), 2) DFM10 (10 g/d *Bacillus* DFM, 4.9×10^9 cfu/d; n = 12) or 3) DFM15 (15 g/d *Bacillus* DFM, 7.4×10^9 cfu/d; n = 12). Cows were fed CON, DFM10, or DFM15 for a 32-d pre-trial phase, and continued to receive their respective TRT for the following 3 trial periods (P): P1 (5 d) an ad libitum baseline for P2 (FR, 5 d), during which all cows received 40% of P1 feed intake, and P3 (5 d), where cows were fed ad libitum to recover. Fresh fecal samples were obtained during P1, P2 and P3. Following sampling, fpH was assessed with a portable pH meter. Frozen samples were sent to a commercial laboratory (Dairyland, WI) for fS, fDM and fVFA. Data were analyzed using PROC MIXED with repeated measures in SAS 9.4. The main effects of TRT, time, TRT × time, replicate, and parity were evaluated, with time as repeated and cow as random. During P1, DFM10 cows had decreased fpH and fDM relative to CON (0.19 units and 8%; $P < 0.03$). Fecal lactic acid (fLA) was increased (1.1-fold; $P < 0.01$) in DFM15 cows compared with CON and DFM10. During FR, from d 3 to 5, fLA increased in CON cows (42%) and decreased in DFM10 and DFM15 (27 and 41%; $P = 0.05$). Fecal ethanol tended to be higher in DFM10 cows relative to CON (52%; $P = 0.09$). Overall fpH increased ($P < 0.01$) whereas fDM, fS, acetic, propionic, butyric, fLA, and ethanol decreased ($P < 0.01$) with FR. During P3, relative to P2, fDM, acetic, propionic, ethanol, and fLA increased (4, 97, 83, 50 and 83%, respectively; $P < 0.01$). We found that DFM cows had increased fLA in recovery compared with CON (18%; $P = 0.02$), and fecal propionic acid tended to linearly increase with DFM ($P = 0.09$). Altered fecal parameters of DFM cows before and after FR might be a result of anaerobic metabolism of *Bacillus* in response to substrate availability.

Key Words: probiotic, short-chain fatty acid

1238M **Microbial inoculum alters the rumen epithelial transcriptome and associated meta-transcriptome in calves.**

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Early-life alteration of the rumen microbial ecosystem may affect ruminal fermentation and enhance the productive performance of dairy cows. We evaluated the effects of dosing 3 different types of microbial inoculum on the rumen epithelial tissue (RET) transcriptome and its associated meta-transcriptome in dairy calves. Rumen fluid was collected from 4 adult cows and processed for 3 inocula: ARF (autoclaved; control), BE (bacteria-enriched), or PE (protozoa-enriched). Fifteen Holstein bull calves were enrolled at birth and randomly assigned to 1 of the 3 inocula dosed once weekly from 3 to 6 wk of age. Calves were euthanized at 9 wk of age, and RET tissues were collected for transcriptome and microbial meta-transcriptome analyses. We used Cufflinks for host differentially expressed gene analysis (DEG), DEseq2 for microbial taxa abundance analysis, and CoDiNa for Co-expression Differential Network analysis. The rarefied read count table was used to calculate the microbial α-diversity. Compared with ARF, 36 DEG were identified ($P < 0.01$) in BE and 107 in PE. Between BE and PE, 127 DEG were identified. Microbial α-diversity was reduced in ARF (observed genera and Chao1 [$P < 0.05$]). Nine microbial genera showed significant abundance changes among the treatments ($P < 0.05$) with several genera associated with expression changes in RET genes. They included: *Roseburia* (26 genes), *Entamoeba* (2 genes), *Anaerosinus* (1 gene), *Lachnospira* and *Succiniclasticum* (1 gene each). Co-expression Differential Network Analysis indicated that both BE and PE had an impact on the abundance of KEGG modules related to acyl-CoA synthesis, type VI secretion, and methanogenesis, while PE had a significant impact on KEGG related to ectoine biosynthesis and D-xylose transport. Our study provides evidence that different microbial dosing regimens elicited changes in the host RET transcriptome. Furthermore, it suggests that differential dosing in early life not only altered the microbial community composition, but also the associated microbial functions in the RET.

Key Words: transcriptome, microbial community, early inoculation

1239M **Immune and antioxidant cellular pathways and ruminal microbiota are altered during a gastrointestinal challenge in Angus steers.**

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Cattle often experience feed restriction (FR) when facing stressors such as parturition, transportation, and dietary transitions. We aimed to identify alterations in immune and antioxidant pathways and ruminal

Table 1 (Abstr. 1240M). Effects of a diet without essential oils (CON) or with an essential oil blend (NECGO) on Holstein cow performance (Trt = treatment)

	Treatment			*P*-value		
Item	CON	NECGO	SEM	Trt	Time	Trt × Time
DMI, kg/d	26.4	26.0	0.2	0.09	<0.01	0.12
DMI, % of BW	3.96	3.89	0.03	0.05	<0.01	0.14
Fat, kg/d	1.74	1.74	0.02	0.97	<0.01	0.54
True protein, kg/d	1.30	1.30	0.01	0.96	<0.01	0.37
Milk, kg/d	44.7	44.8	0.5	0.94	<0.01	0.47
Milk/DMI, kg/kg	1.69	1.73	0.01	0.04	<0.01	<0.01
ECM, kg/d	47.0	47.1	0.4	0.90	<0.01	0.51
ECM/DMI, kg/kg	1.78	1.81	0.01	0.10	<0.01	<0.01
BW, kg	670	670	2	0.94	<0.01	0.02
BCS	2.98	3.00	0.01	0.26	<0.01	0.87

microbiota due to moderate FR. Eight cannulated Angus steers (663 ± 73 BW) fed a corn silage and dry rolled corn diet were used. In the first 15 d of the pre-FR phase (PRE), steers had ad libitum access to feed. This was followed by an FR phase (FRP) during which steers were fed 25% of the PRE phase for 3 d. An additional 15 d were used as a post-FR phase (POST) during which steers had ad libitum access to feed. Ruminal fluid, plasma, and ruminal tissue were collected at the end of each phase. The bacteria abundance was measured by RT-qPCR. The RNA and protein abundance were detected by RT-qPCR and Western blotting. Cystathionine-β-synthase (CBS) activity in ruminal tissue was detected by ^{14}C radio-labeling assays. Data were analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc.) with fixed effect of period and random effect of steer. Tukey's multiple comparison test was used to determine differences between phases. Plasma fatty acids were higher ($P < 0.01$) in FRP than PRE and POST, and plasma IL1-β followed a similar trend ($P = 0.09$). The mRNA abundance of *TNF*, *TLR2*, and *TLR4* was higher ($P < 0.05$), and *XBP1spliced* tended to be higher ($P = 0.07$) in FRP than PRE and POST. The protein abundance of the inflammation regulator pMAPK was higher ($P < 0.01$) in FRP than PRE and POST ($P < 0.05$). The activity of CBS was higher ($P = 0.06$) in FRP and POST than PRE. Among antioxidant proteins studied, protein abundance of NFE2L2 and its targets GPX1 and GPX3 was higher ($P < 0.01$) in FRP than PRE and POST. These responses were also observed at the mRNA level for *GPX1* and *GPX3*. The abundance of *Bifidobacteria spp. (N124)*, *Eubacterium ruminantium*, *Selenomonas ruminantium*, and *Succinimonas amylolytica* were higher ($P < 0.05$) during FRP than PRE and POST. Overall, preliminary results indicated that an FR challenge altered the ruminal microbiota, potentially triggering signals that led to inflammation within ruminal tissue. In spite of that, ruminal tissue possesses antioxidant mechanisms that help counteract stressors produced during FR.

Key Words: rumen microbiome, immunity, stress

1240M Lactation performance of Holstein cows fed a diet containing an essential oil blend.

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Holstein cows (n = 46; 90 ± 16 d in milk; 1.8 ± 0.7 lactations) were enrolled in a randomized complete block design study with 2-wk covariate and 8-wk treatment periods. Treatments were: 1) a diet without essential oils (CON), and 2) a diet with an essential oil blend (NECGO, Next Enhance CGO feed additive, Novus International Inc.). Treatments were included into diets as premixes (1.54% of DM). The CON premix was ground corn. The NECGO premix was ground corn and blend of cinnamaldehyde and garlic oil (710 mg/cow/d). Cows were housed in freestalls, fed individually 1×/d, milked 3×/d, and milk sampled 6×/wk. Feed intake, body weight (BW), body condition, and lactational performance were assessed weekly. Rumen volatile fatty acid (VFA; n = 12/trt) concentration was assessed during wk 4 and 8 of treatment period 4 h after feeding. Data from 45 cows were analyzed using PROC MIXED in SAS (v. 9.4). The models included a covariate, fixed effects of block, treatment, week, including interaction, and random effect of cow. The model for rumen VFA did not include effect of time. Cows fed NECGO tended to have less DM intake (DMI) than cows fed CON and significantly less DMI as a percentage of BW (Table 1). There was no effect of treatment on milk yield or energy-corrected milk yield (ECM). As a result of lower intake and no change in milk yield, there was a significant effect of treatment by time on feed efficiency, with cows fed NECGO having higher feed efficiency compared with cows fed CON. In wk 8, cows fed CON compared with NECGO had higher concentrations of total VFA (122.9 vs 111.5 m*M*), butyrate (12.3 vs 11.6% of total VFA), and valerate (1.7 vs 1.5% of total VFA). The NECGO appears to be promising for use in high-producing cows when improved feed efficiency is desired.

Key Words: essential oils, feed additive, efficiency

1241M Effects of heat stress on rumen fermentation characteristics, blood parameters, and rumen microbiota in Holstein dairy cows during late lactation.

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This study aimed to determine the effects of heat stress on rumen fermentation characteristics, blood parameters, and rumen microbial communities of Holstein dairy cows during late lactation. Eight lactating Holstein cows, 55.54 ± 9.47 mo old; parity = 3.5 ± 0.65; 201 to 305 DIM, were used in the study. All dairy cows were fed total mixed ration (TMR). Rumen fluid and blood samples were taken from the dairy cows during heat stress (THI: 81.16) and recovery period (THI: 59.4). Rumen fermentation characteristics including pH, ammonia-nitrogen, and volatile fatty acids were analyzed. Complete blood count, blood chemistry, and heat shock proteins (HSP27, HSP70, and HSP90) were also analyzed. Taxonomic characterization of the rumen microbial community of dairy cows was analyzed using Illumina MiSeq technology. Results showed that propionate concentration was significantly low ($P < 0.05$) while acetate-to-propionate (A/P) ratio was high ($P < 0.05$) during the heat stress period. Blood glucose was significantly lower

($P < 0.05$) and magnesium was higher ($P < 0.05$) in dairy cows under heat stress conditions than in cows during the recovery period. The HSP27 tended to be lower in dairy cows during the heat stress period ($P = 0.060$), whereas HSP70 was higher in dairy cows under heat stress conditions than those during the recovery period. Taxonomic classification showed that *Bacteroidetes* and *Firmicutes* dominated the bacterial community. Linear discriminant analysis (LDA) effect size (LEfSe) analysis showed that relative abundances of *Butyricicoccus pullicecorum*, *Muribaculum intestinale*, *Parabacteroidetes goldsteinii*, *Oscillibacter ruminantium*, *Roseburia faecis*, *Ethanoligenens harbinense*, and *Sporobacter termitidis* significantly decreased ($P < 0.05$) during the heat stress period. Overall, dairy cows under heat stress conditions showed decreased propionate concentration, increased A/P ratio, and high concentrations of serum HSP70. In addition, heat stress induces shifts in the rumen microbial community composition in Holstein dairy cows during late lactation.

Key Words: heat stress, microbiome, rumen

1242M Impacts of acute in vitro heat stress on preruminant jejunal integrity. C. L. M. Parsons*[1], L. J. Banda[2,1], M. D. Ellett[1], and K. M. Daniels[1], *[1]Virginia Tech, Blacksburg, VA, [2]Lilongwe University of Agriculture and Natural Resources, Lilongwe, Malawi.*

Dairy animals typically experience a ~2°C increase in core body temperature during heat stress (HS). To study the impact of such temperature elevation on intestinal integrity, a series of Ussing chamber experiments were undertaken. Jejunal segments (~10 cm) from a total of 4 healthy calves (41 ± 6 kg body weight, mean ± standard deviation) were excised and kept in aerated ice-cold Krebs-Henseleit buffer (KHB; pH 7.4) until use; calves were processed on separate days. Jejunal segments were stripped of serosa, opened longitudinally, and inserted between acrylic chambers with an exposed mucosal-serosal tissue surface area of 0.71 cm^2. Within each calf, tissue pieces were assigned to one of 2 treatments: thermoneutral (TN; $n = 4$), or HS ($n = 4$). Each reservoir (mucosal, serosal) was filled with 4 mL of KHB, 10 m*M* D-glucose, and aerated with carbogen. Desired temperatures (TN, 38.5°C ± 0.4°C; HS, 40.0°C ± 0.5°C) were maintained via 2 circulating water baths. After approximately 20 min of equilibration, individual segments were clamped at 0 mV (time 0) and intestinal permeability was measured by transepithelial electrical resistance (TER) every 15 min until 105 min, resulting in 8 time points. The permeability marker fluorescein isothiocyanate lipopolysaccharide (FITC-LPS; 80 μg) was administered to mucosal chambers at time zero and flux was measured in serosal chambers at 105 min by fluorimetry. ANOVA included the fixed effect of temperature and time and temperature × time, where applicable. Calf within temperature tested temperature; the residual tested time effects. TER increased over time ($P = 0.01$; 68.50 ± 5.77 to 95.39 ± 5.77 Ω·cm^2) but was not affected by temperature ($P = 0.535$). Flux of FITC-LPS was not affected by temperature and averaged 173 ± 18 ng/cm^2·h^{-1} for TN and 157 ± 18 ng/cm^2·h^{-1} for HS ($P = 0.541$). Under conditions of this experiment elevated tissue temperature, such as occurs during in vivo HS, did not affect intestinal permeability. This was contrary to expectations and could be due to tissue source, relative short duration of the experiments, or both.

Key Words: heat stress, leaky gut, Ussing chamber

1243M Live or autolyzed yeast supplementation: effects on performance, feed sorting, and nutrient digestibility in dairy cows. C. S. Takiya[1], G. Poletti[1], A. C. de Freitas[1], O. P. Sbaralho[1], D. J. C. Vieira[1], R. G. Chesini[1], P. C. Vittorazzi Jr[1], N. P. Martins[1], N. T. S. Grigoletto[1], C. V. de Almeida[1], T. S. Acedo[2], C. Cortinhas[2], and F. P. Rennó*[1], *[1]University of São Paulo, Pirassununga, SP, Brazil, [2]DSM Produtos Nutricionais Brasil S.A., São Paulo SP, Brazil.*

Autolysis of yeast cell content provides functional components (RNA, cell wall carbohydrates, and peptides in a pre-digestive form) claimed to enhance gut health. This study was conducted to evaluate yeast (*Saccharomyces cerevisiae*) products on performance and nutrient digestibility. Eight Holstein cows (28.4 ± 3.96 kg/d milk yield and 216 ± 30 DIM) were blocked according to DIM and milk yield and enrolled into a 4 × 4 Latin square (LS) experiment with 21 d-periods. Cows were randomly assigned to treatment (TRT) sequences with: Control (CON); live yeast (LY), 0.125 g/kg DM of Vistacell (AB Vista); autolyzed yeast (AY) 1 (AY1) 0.625 g/kg of Levabon Rumen E (DSM Animal Nutrition); or AY at 0.834 g/kg (AY2). Treatments were provided mixed with minerals. Samples of TMR and orts were analyzed for particle size distribution and chemical composition. Fecal samples were collected for 3 consecutive days of each period at 9 h-intervals. Fecal excretion was estimated based on indigestible NDF. Cows were milked 2× daily and samples were collected during 6 consecutive milkings. Data were analyzed using the MIXED procedure of SAS modeling the fixed effects of TRT, period, LS, and their interaction. Animal within LS was considered a random effect. Differences between TRT were analyzed by orthogonal contrasts: CON vs yeast products, LY vs AY (AY1 + AY2), and AY1 vs AY2. Neither nutrient intake nor apparent digestibility (DM, NDF, and CP) were affected by treatments. Feed sorting for particles >19 mm was greater ($P = 0.02$) for AY than LY, but no differences were observed in ruminal pH. Fat-corrected milk was greater ($P = 0.04$) in cows fed yeast products (29.4, 31.2, 31.0, and 31.7 for CON, LY, AY1, and AY2, respectively). Fat yield was greater ($P = 0.03$) in cows fed yeast supplements than CON. No differences in milk composition were observed when comparing cows fed different yeast products. Milk yield and feed efficiency (FCM ÷ DMI) tended to be greater ($P = 0.07$) in cows fed yeast than CON. Feeding yeast can improve milk and fat yield without altering nutrient digestibility in cows.

Key Words: additive, *Saccharomyces cerevisiae*

1244M Red sorghum powder modifies rumen fermentation characteristics in vitro. R. Yi[1,2], S. Vigors[2], J. Xu[1], D. Bu[1], and L. Ma*[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China, [2]School of Agriculture and Food Science, University College Dublin, Belfield, Ireland.*

The incorporation of plant compounds is one potential strategy to modify rumen fermentation and reduce emissions. Rumen protozoa play an important role in lowering nitrogen utilization efficiency (NUE) in cattle and the inhibition of rumen protozoa is a potential approach to increase nitrogen utilization in ruminants. This study aimed to evaluate red sorghum (*Sorghum bicolor* L. Moench; sweet sorghum) powder as a means to inhibit rumen protozoa and effect fermentation characteristics in vitro. Fresh rumen fluid was collected from 3 cannulated dairy cows and filtered to conduct the in vitro culture. The effect of red sorghum powder (5 doses: 0, 10.4, 20.8, 41.6, and 83.2 mg/g; $n = 6$) on rumen protozoal counts and fermentation characteristics was evaluated after 18 h of in vitro culturing. Rumen protozoal cells were morphologically identified and counted. Ammonia nitrogen (NH_3-N) and microbial protein (MCP) concentrations in the in vitro cultures were tested using colorimetric determination, and the concentrations of volatile fatty acids (VFA) were determined using gas chromatography. Methane production was calculated using a formula based on the VFA production profiles. Data were analyzed in a complete randomized design using R Studio (version 1.4.1717). The results revealed the red sorghum powder reduced *Entodinium* by 20.26% and 35.33% compared with the control at the doses of 41.6 and 83.3 mg/g ($P < 0.05$; $P < 0.01$, respectively), with no negative effect on the number of beneficial species of rumen protozoa, including *Diplodinium*, *Isotricha* and *Dasytricha*. Compared with control group, NH3-N was decreased by 32.15% at 83.2 mg/g inclusion and methane production was predicted to decrease by 14.99%, while total VFA production was unchanged. The dose of 83.2 mg/g increased the MCP concentration by 15% ($P < 0.01$). In conclusion, red sorghum may be a new feed additive to enhance NUE in ruminants without impairing fermentation characteristics, but this hypothesis will need to be tested in animal trials.

Key Words: rumen protozoa, red sorghum, dairy cows

Ruminant Nutrition: Protein and Amino Acids 1

1245M Stability of liquid 2-hydroxy-4-methylthiobutanoic isopropyl esters (HMBi) in compound feed and total mixed rations. S. Van De Craen[1], B. Vennekens[1], B. Janssens[1], E. Eren Gültepe[1], J. Salaklang[2], D. Martinez del Olmo[1], F. Nuyens[1], and F. Sun*[2], *[1]Kemin Europa N.V., Herentals, Belgium, [2]Kemin Industries Inc., Des Moines, IA.*

Isopropyl ester of hydroxy methionine analogs (HMBi) stand out as an alternative way to supply methionine in ruminant diets to encapsulated forms due to their stability against aggressive thermo-physical processing. But one of the big questions still unanswered is whether HMBi-based products are stable in total mixed rations (TMR) and in the presence of a high proportion of cereals, like grain mixes (GM). Two experiments were designed to evaluate the stability of HMBi in GM for up to 2 mo and in TMR up to 24 h. In Experiment 1, liquid HMBi (≥97% wt., KESSENT MF, Kemin Europa N.V., Belgium) was added at 3% to a GM consisting of wheat, barley, corn, and soybean (21% CP, 2.77 Mcal/kg ME, 42% starch). The HMBi concentrations were analyzed on d 1, 14, 21, 30, and 60 of storage. In Experiment 2, the HMBi was added at 1.5% to a corn silage-based-TMR (57.5% DM, 14.5% CP, 1.64 Mcal/kg ME, 16% starch). The HMBi concentrations were analyzed after 1 h, 4 h, 8 h, 12 h, and 24 h. The HMBi concentration was determined using the protocol based on RP-HPLC as described by EURL evaluation report No. 1831/2003, with minor modifications (n = 5). Samples were extracted using an (acidified) extractive solution and subjected for analysis. For methodology, coefficient of determination (R^2) ≥0.99 was satisfactory for both experiments. Quantitative results were expressed as HMBi recovery (%) and were evaluated using PROC MIXED of SAS. Relative HMBi concentrations (%, relative to time zero) of 99.3, 99.3, 100, and 100 were obtained at d 14, 21, 30, and 60 of storage in GM, and of 98.5 at 24 h, 100 at all other time points in TMR. The least squares means of HMBi recovery (%) showed no significant changes ($P > 0.05$) both in GM until d 60 (73.2, 72.7, 72.8, 72.2, 74.2 ± 1.2; at d 1, d 14, d 21, d 30, d 60, respectively) and in TMR compared with time zero (76.4, 78.9, 81.8, 80.4, 75.1 ± 1.4 after 1 h, 4 h, 8 h, 12 h, 24 h, respectively). Altogether, liquid HMBi from KESSENT MF remains stable in the grain mix and in TMR under real field conditions. This is the first stability report of HMBi in TMR to our knowledge.

Key Words: 2-hydroxy-4-methylthiobutanoic isopropyl esters (HMBi), feed stability, methionine

1246M Ability of three dairy feed programs to predict post-rumen outflows of essential amino acids (EAA) in dairy cows: A meta-analysis. R. Martineau[1], D. R. Ouellet[1], D. Pellerin[2], J. L. Firkins[3], M. D. Hanigan[4], R. R. White[4], P. A. LaPierre[5], M. E. Van Amburgh[5], and H. Lapierre*[1], *[1]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [2]Department of Animal Science, Laval University, Québec, QC, Canada, [3]The Ohio State University, Columbus, OH, [4]Department of Dairy Science, Virginia Tech, Blacksburg, VA, [5]Department of Animal Science, Cornell University, Ithaca, NY.*

Accurate prediction of EAA post-rumen outflows is crucial for balancing dairy rations for AA. Our objective was to compare reported flows (unadjusted for the effect of study) and predictions from 3 dairy feed programs [National Research Council (2001, NRC), Cornell Net Protein and Carbohydrate System (CNCPS, v6.5.5), and National Academies of Sciences, Engineering and Medicine (2021, NASEM)]. The data set included 359 treatment means (71 duodenal and 26 pre-duodenal sampling studies). Mean (as is) and slope (assessed through the difference detailed in Table 1) biases were deemed biologically relevant and discussed if statistically significant and representing ≥5% of the observed mean. Results are detailed for His, Lys and Met and discussed for the other EAA. Mean and slope biases were relevant for His with NRC and CNCPS, and for Lys and Met with CNCPS; mean biases were relevant for Met with NRC and for Lys with NASEM. The NRC had only mean bias for Arg, Phe, Thr, Ile, and Val, whereas CNCPS had mean and slope biases for Arg, slope bias for Ile and Val, and mean bias for Leu. The NASEM has the best performance for predicting EAA outflows; NRC mainly presents mean biases, whereas CNCPS has both mean and slope biases.

Key Words: histidine, lysine, methionine

Table 1 (Abstr. 1246M). Differences between observed and predicted EAA post-rumen outflows with 3 dairy feed programs[1]

	His			Lys			Met		
Variable	NRC	NASEM	CNCPS	NRC	NASEM	CNCPS	NRC	NASEM	CNCPS
Mean observed	56	56	56	156	156	156	49	49	49
Mean predicted	52	56	66	154	173	168	45	52	59
CCC, %	61.4	62.8	54.6	66.2	59.4	62.3	53.6	55.9	53.1
RPE, % obs.	27.5	26.2	33.8	21.9	24.7	24.4	28.1	27.1	33.7
ECT, % MSPE	5.7	0.3	27.8	0.2	20.4	9.5	8.8	3.1	37.6
ER, % MSPE	2.3	0.8	8.1	2.4	1.4	6.8	0.1	0.1	3.9
Mean bias	3.7**	−0.8	−9.9**	2.4	−17.4**	−11.7**	4.1**	−2.3**	−10.2**
Slope bias, mg/g	−155**	−92†	−306**	−143**	−128**	−248**	−46	−39	−231**
Difference	3.1	1.6	6.5	7.3	6.2	12.3	0.7	0.5	3.9

[1]In g/d unless otherwise stated. CCC = concordance correlation coefficient; RPE = relative prediction error (root mean squared prediction error, as % of mean observed); ECT and ER = error in central tendency and error due to the regression; MSPE = mean squared prediction error. Mean and slope biases were calculated according to St-Pierre (2003, JDS 86:344) The absolute difference was computed between the linear biases calculated at first (Q1) and third (Q3) quantile of predicted AA outflows. NRC = National Research Council; NASEM = National Academies of Sciences, Engineering and Medicine; CNCPS = Cornell Net Protein and Carbohydrate System.

**$P \leq 0.01$; † $P = 0.10$.

Table 1 (Abstr. 1247M). Duodenal digesta characteristics of cows fed canola meal (CM) or soybean meal (SBM)

Variable	SBM	CM	SEM
Phases,[1] % of DM			
Fluid	32.3	35.4	1.47
Small particles	22.5	26.7	1.78
Large particles	45.2	38.0	3.24
Bacterial source,[1] % of DM (flux, kg of DM/d)			
FAB[2]	19.9[b] (2.57)	24.7[a] (2.64)	0.52 (0.19)
PAB[3]	80.2[a] (10.58)	75.3[b] (8.18)	0.52 (0.62)
Apparent DM digestibility,[4] %	29.6	40.7	3.77

[a,b] Values within the same row with a different superscript differ ($P = 0.03$).

[1] Duodenal.

[2] FAB = fluid-associated bacteria.

[3] PAB = particle-associated bacteria.

[4] Pre-duodenal.

1247M Substitution of soybean meal by canola meal in dairy rations on fluid-associated and particle-associated bacteria at the duodenum.

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Canola meal (CM) has a positive effect on milk production even if NRC (2001) predicts a decrease in supply of metabolizable protein (MP) when CM replaces soybean meal (SBM) in dairy rations (Martineau, 2013, JDS 96:1701). The aim of this project was to estimate the effect of substitution of SBM by CM in dairy rations on duodenal proportion and flux of fluid-associated (FAB) and particle-associated bacteria (PAB). Four cows fitted with ruminal and duodenal cannulas were used in a crossover design (28 d/period). Cows were fed a total mixed ration (65.9% grass silage and 34.1% concentrate, DM basis). The SBM ration was formulated to supply 100% NE_L and MP requirements for a cow producing 25 kg/d of milk (NRC, 2001), contained 10.8% of SBM; substitution was made on a CP basis and the CM diet contained 14.7% of CM. After 14 d of adaptation, 18 duodenal samples were collected, covering 3 d. Duodenal digesta flows were determined using 3 markers. The FAB and PAB proportions were assessed by purine analysis and gravimetry (differential centrifugations). Statistical analyses were made using mixed model in SAS. Dry matter intake (18.6 ± 0.27 kg/d), milk production (15.9 ± 0.74 kg/d) and milk composition were not affected ($P > 0.33$). Results for digesta are detailed in Table 1. Proportion of FAB was greater for CM than SBM and the opposite was observed for PAB. Duodenal flux of FAB and PAB were similar. Pre-duodenal apparent DM digestibility was numerically greater for CM than for SBM. In conclusion, although proportion of FAB was greater for CM compared with SBM ration, their fluxes were similar, reinforcing the assumption that positive effect of CM could be attributed to the soluble fraction flowing at the duodenum.

Key Words: canola meal, fluid-associated bacteria, particle-associated bacteria

1248M Determining plasma lysine concentrations for lactating Holsteins supplemented with either infused lysine or two rumen-protected lysine prototypes using the in vivo plasma dose response method.

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Two novel rumen-protected lysine prototypes under development were tested using the in vivo plasma AA dose-response method. Our objective was to measure the relative Lys bioavailability of these 2 prototypes (Kemin Industries, Des Moines, IA). Eight multiparous Holstein cows (128 ± 38 DIM) fitted with ruminal cannulas were used in a replicated 4 × 4 Latin square with 7-d periods. Treatments were (g/d): 1) 0 (CON), 2) 60 g Lys abomasally infused (INF), 3) 60 g Lys from Prototype IL (IL), and 4) 60 g Lys from Prototype II (II). The Lysine prototypes were fed in 3 daily aliquots at 0500, 1300, and 2100 h with the infusion being continuously infused into the abomasum via the ruminal cannulas. Intake, milk yield and components were collected the last 3 d of each period. Blood was collected from the tail vein the last 3 d of each period at 2, 4, 6 and 8 h after the morning feeding (0500 h). Amino acids in plasma was determined using HPLC. Data were analyzed using PROC MIXED to generate means for Lys % of (total AA−Lys) which were then used in the REG procedures of SAS to generate the linear regression variables. Significance was declared at $P \leq 0.05$. Average milk yield was 45.3 kg/d and DMI was 28.0 kg/d. The relative bioavailability was 35.5% and 49.0% for prototypes II and IL respectively (Table 1). The bioavailability for prototype IL was significantly higher than prototype II.

Key Words: lysine, bioavailability, plasma

1249M Energy source and amino acids independently alter mammary extraction of nutrients.

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The objective of this study was to determine if metabolizable AA level (AAL) altered mammary extraction of nutrients based on energy source [ES, glucogenic (GE) vs ketogenic (KE)]. Twenty dairy cows (75 DIM) were enrolled in a replicated 4 × 4 Latin square with four 28-d periods and four treatments arranged as a 2 × 2 factorial. Factors were AAL: 10% metabolizable AA deficient (DAA) or sufficient, balanced for Met, Lys, and Leu (BAA); and ES: GE (29.5% starch, 3.5% fat) or KE (21% starch, 6% fat, soy hulls replacing corn). Blood samples were collected from a coccygeal vessel and the subcutaneous abdominal vein, 6 times across d 25–26 of each period, staggered to represent every 2-h sampling between a.m. and p.m. milkings. Plasma was analyzed for insulin, glucose, β-hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), triacylglycerol (TG), and AA. Data were analyzed with a mixed model containing ES, AAL, ES × AAL, period, and square as fixed effects and cow within square as random. Mammary plasma flow, based on the Fick principle, was not affected by ES ($P = 0.25$) but tended to decrease in response to BAA (−54 L/h, $P = 0.13$). Plasma insulin was higher for GE than KE (0.60, 0.54 μg/L $P = 0.02$) and increased more in response

Table 1 (Abstr. 1248M). Bioavailability calculation using changes in plasma Lys [% of (total AA−Lys)]

Item	Infusion	Prototype II	Prototype IL
Slope (SE)	0.02846 (0.0007)	0.01010 (0.0005)	0.01394 (0.0008)
Bioavailability of RP-Lys[1]	—	35.5 ± 1.7	49.0 ± 3.0

[1] Calculated as [(slope of RP-Lys/slope infusion) × 100]. RP = rumen-protected.

to BAA under GE than under KE (0.16, 0.06 µg/L $P = 0.03$). Plasma concentration and mammary extraction and uptake of glucose were not affected by ES or AAL ($P > 0.15$). Plasma concentration of BHB, NEFA, and TG increased by KE ($P < 0.01$) but were not affected by BAA ($P > 0.15$). Mammary extraction and uptake of TG were increased ($P < 0.001$) by KE, while BAA increased mammary extraction of BHB (+5%, $P < 0.001$) and uptake of NEFA (+9.2%, $P = 0.01$). Plasma concentration of essential AA (EAA), except Phe and Thr, increased for KE vs GE ($P \leq 0.01$), but mammary uptake on KE increased only for Arg ($P = 0.01$) and decreased for Leu ($P = 0.02$). Plasma concentration of His, Leu, Lys, Phe, and Val increased ($P < 0.001$) and Thr and Trp decreased ($P < 0.001$) for BAA. Mammary uptake of EAA increased ($P < 0.01$) for BAA, except for Arg ($P = 0.3$) and Trp that tended to increase ($P = 0.13$). In conclusion, there was no interaction between ES and AAL on mammary uptake of individual nutrients.

Key Words: mammary gland, energy, amino acids

1250M Effect of oat variety on total truly digestible protein supply and degraded protein balance in dairy cows in comparison with barley grain. M. R. Tosta[1], L. L. Prates[1], M. E. Rodríguez-Espinosa[1], J. He[2], and P. Yu*[1], [1]*Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada,* [2]*Inner Mongolia Academy of Agriculture and Husbandry Science, Hohhot, China.*

The objectives of this study were to determine the effect of oat variety on total truly digestible protein supply (DVE) and degraded protein balance (OEB) in dairy cows in comparison with common barley grain. Total DVE and OEB values were determined based on the DVE/OEB Dairy Nutrition System. Total truly digestible protein supply (DVE value) consisted of truly digestible rumen undegraded protein (ARUP), truly digestible rumen microbial protein (AMCP), and correction for endogenous nitrogen loss (ENDP). The OEB value was a balance between microbial protein synthesis from rumen degradable CP and that from the energy extracted during anaerobic fermentation in the rumen. Four Holstein cows fitted with rumen cannula were used in this study. Three oat varieties were used in comparison with common barley grain. The experimental design was a RCBD with grain varieties as a fixed effect and cows as a random block effect. The data were analyzed using the Mixed model procedure in SAS 9.4 with the analysis RCBD model. The results showed that there was no significant effect on AMCP, ARUP, and ENDP among oat varieties with average of 73, 74, and 33 g/kg DM, respectively. There was also no oat variety effect on total DVE and OEB values with average of 101 and −44 g/kg DM, respectively. However, when compared with common barley grain, barley grain was higher ($P < 0.05$) in total DVE value (127 vs 101 g/kg DM) and lower in OEB value (−94 vs −44 g/kg DM). In conclusion, there was no significant effect on total truly digestible protein supply to dairy cow among oat varieties. Barley provided higher true protein supply to dairy cows than oat varieties. Both oat and barley had negative degraded protein balance.

Key Words: true protein supply, oat and barley variety, degraded protein balance

1251M Effects of two commercial rumen protected methionine products on milk yield and composition on lactating cows. N. P. G. Viana[1], L. Obialeski[2], J. M. Ebeling[1], E. S. Vaz[1], J. C. Grossmann[1], M. Poczynek[1], J. T. R. Carvalho[1], L. S. Nogueira[1], F. H. Dalmass[3], L. B. Los[3], and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brazil,* [3]*Frísia Cooperativa Agroindustrial, Carambeí, PR, Brazil.*

The aim of this study was to evaluate the effects of 2 rumen protected methionine (RPM) products on milk yield and composition on lactating cows. A total of 278 multiparous Holsteins (185 ± 129 DIM) producing 42.0 ± 10.4 kg/d were used from a commercial herd at Paraná State, Southern Brazil. Cows were blocked by parity, milk yield, and DIM and randomly assigned to 1 of the 3 treatments, consisting of 120 cows supplemented with Smartamine M (SM; Adisseo Inc., France), 116 cows Mepron (MP; Evonik Nutrition and Care GmbH, Germany), and 42 control cows (CT). In a randomized block design, the treatment consisted on daily supplementation of 15 g of RPM (SM or MP) mixed with 85 g of corn meal provided top-dressed and divided on 2 daily meals. Control animals received an equivalent amount of corn meal. The total experimental period was 33 d, with the first 3 d used as a covariate and 30 d of supplementation. Milk samples were collected in the last 3 d of the experimental period. The data were analyzed through MIXED procedure of SAS containing the fixed effects of treatment, time and their interaction, the covariate, and the random effects of block and cow within treatment. There was no difference for milk yield (40.54 vs. 39.91 kg/d; $P = 0.62$) between treatments SM and MP, but both groups of RPM-treated cows showed higher milk yields (40.23 vs 37.75 kg/d; $P < 0.01$) than CT. Concerning milk composition between SM- vs. MP-treated cows, no differences ($P > 0.05$) were detected for all components. However, comparing SM and MP vs. CT, the RPM treatments increased ($P < 0.01$) fat yield by 0.193 kg/d, protein yield by 0.115 kg/d, casein yield by 0.089 kg/d, lactose yield by 0.133 kg/d, and milk total solids yield by 0.460 kg/d. Cows supplemented with RPM also showed higher milk protein content (3.56 vs 3.51%; $P < 0.01$), casein content (2.83 vs. 2.79%; $P < 0.01$) and total solids content (13.65 vs 13.51%; $P = 0.05$) than CT, with no differences for milk fat and lactose contents ($P > 0.05$). Our results suggest that there was no difference between RPM products on milk yield and composition, and both RPM-supplemented groups showed greater performance than controls.

Key Words: amino acid, milk solids

1252M Bioavailability assessment of a rumen-protected methionine considering performance, plasma methionine concentration, and total-tract digestibility. J. Guyader*[1], L. Zhou[2], Y. Zhang[3], C. Parys[1], and Y. Cao[2], [1]*Evonik Operations GmbH, Hanau, Germany,* [2]*Northwest A&F University, Yangling, Shaanxi, P.R. China,* [3]*Evonik (China) Co. Ltd., Beijing, P.R. China.*

Rumen-protected methionine (RPM) products should be compared on the basis of their metabolizable methionine (Met) content. In this work, we measured the total-tract digestibility of a commercial RPM (Mepron, 85% DL-Met, Evonik Operations GmbH) to further estimate its bioavailability. This study was completed at the Northwest A&F University, China, as a completely randomized design with 16 Holstein cows (48 ± 12 DIM and 39.3 ± 7.2 kg milk/d at start; mean ± SD). Cows were fed for 5 weeks a corn silage-based diet alone (15.7% CP, 28.1% NDF, 1.90% of Met in metabolizable protein; n = 8) or with 15 g/d of Mepron (2.22% of Met in metabolizable protein; n = 8). During the last week of the experiment, individual DM intake, milk yield and milk composition were measured. Total-tract digestibility of Met was calculated from analyzing Met content in feed and pooled fecal samples by HPLC from 3 d of total collection. Total-tract digestibility of Mepron was estimated from the difference in Met digestibility between diets. In parallel, plasma Met concentration was measured in blood samples taken the last experimental day at 0, 3 and 6 h after the morning meal. Data were analyzed in R with a mixed model using the fixed effects of treatment, time and their interaction (day for milk yield and hour for plasma Met concentration), and the random effect of cow. Initial milk yield was included as a covariate to test the impact of treatments on milk performance. The effect of time (alone or combined with treatment effect) was not significant. Fat- and protein-corrected milk was numerically higher for cows fed Mepron (+0.7 kg/d) even though no significant difference was observed between treatments for DM intake and milk yield. Daily fecal Met excretion was similar between diets while daily Met intake was logically higher for Mepron-supplemented cows ($P < 0.001$). Total-tract digestibility of Mepron was calculated at 94.5%. Assuming a rumen protection of 80%, intestinal digestibility of Mepron was estimated at 92.6%, leading to a final bioavailability of 74.1%. Interestingly, in this study, plasma Met concentration was similar between diets, demonstrating the absence of relationship between RPM bioavailability and Met concentration in blood.

Key Words: rumen-protected methionine, digestibility, bioavailability

1253M Effects of buffer composition on in vitro disappearance of rumen-protected amino acid products.

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The objective was to evaluate the effect of buffers on in vitro disappearance of N in encapsulated amino acids (AA). Four different buffers were used: clarified rumen fluid (CRF), Kansas State buffer (KS), McDougall's buffer (MD), and Van Soest buffer (VS). Nine AA products, comprised of true lipid encapsulation, lipid and polymer, lipid, and methylcellulose, and mostly polymer, were used. The CRF was considered the positive control as the encapsulation was developed to protect against rumen conditions. A 0.5-g sample of each product was weighed into 125 Erlenmeyer flasks in triplicate. In each flask, 40 mL of the respective buffer solution was added and then flushed with CO_2 and inoculated with 10 mL of filtered rumen fluid. Rumen fluid was obtained from 2 lactating dairy cows and blended. Flasks were kept in a 39°C water bath under continuous CO_2 until filtration at respective time points. Samples were fermented for 4, 8, 12, 16, and 24 h. Samples were filtered through a Whatman AH-934 filter paper, rinsed, and dried. The filter residue was analyzed for nitrogen (N) content by Kjeldahl using a Foss Kjeltec 8200. The rate of disappearance of N by product and time was analyzed in a mixed model (R Studio) using fixed effects of buffer and time. Samples were replicated on 2 different days, and the model included estimates of the residual variance and random effect of day. There were differences in disappearance by buffer and products ($P < 0.01$). There was no effect on disappearance of polymer coated products 2, 6, and 7 in any buffer. All buffers demonstrated increased N disappearance among lipid-based and combination encapsulated products 1, 3, 4, 5, and 8 ($P < 0.05$). In all buffers, product 3 showed increased disappearance compared with CRF ($P < 0.01$). For product 8, VS and MD showed increased disappearance ($P < 0.01$) compared with CRF and KS buffer. Product 9 showed increased disappearance only in KS ($P < 0.05$). The CRF had the least impact on N disappearance among products. In conclusion, the results indicate buffer composition of mono- and divalent ions independently increases N disappearance of encapsulated AA products.

Key Words: encapsulation, in vitro, disappearance

1254M Use of the Se-yeast method to estimate absolute bioavailability of rumen protected methionine.

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Bioavailability (BA) of rumen protected Met (RP-Met) can be estimated by the selenomethionine (Se-Met) method, but this method provides relative BA because the absorption of the tracer is not usually measured, which is needed to estimate digestible Met (dMet). We determined the absolute BA of RP-Met by estimating dMet either by calculation (NASEM, 2021; ESTIM) or by measuring the marker absorption by quantifying Se excretion (MEAS). Twenty-four multiparous cows were used in a randomized complete block design, consisting of 3 periods: 10-d basal period (basal diet without Se supplementation), 14-d backgrounding period (basal diet with Se-Met; 0.16% of DiaMune Se), and 10-d experimental period (basal diet with Se-Met), during which cows in each block were randomly assigned to commercially available RP-Met 1, 2, and 3. During the basal and experimental period, spot feces (6 samples within 24 h) were collected, and Se excretion was quantified. The NASEM (2021) model estimated the dMet flow from the backgrounding diet which was divided by specific activity (SA; milk Se:N) of the experimental period to obtain dMet during the experimental period, allowing to calculate absolute BA of each RP-Met (ESTIM). For the MEAS approach, dMet during the backgrounding and experimental period were obtained from Se absorption divided by corresponding SA. The MIXED procedure of SAS was used for data analysis. The ESTIM approach estimated BA of 51, 41, and 48% for RP-Met 1, 2, and 3 respectively ($P = 0.71$); whereas the MEAS approach estimated BA of 49, 45, and 39%, respectively, without a difference among products ($P = 0.70$). When the approaches were compared, dMet during the backgrounding (ESTIM vs. MEAS; 55 vs. 53 g/d of dMet; $P = 0.66$) and experimental period (68 vs. 66 g/d; $P = 0.70$) were not different. The BA of products were not different (47 vs. 44%) as well as between approaches. In conclusion, the Se-Met method with estimating dMet by NASEM (2021) can be used to estimate absolute BA of RP-Met. Although product BA were not significantly different, ESTIM and MEAS agreed that RP-Met 1 numerically ranked the highest in BA.

Key Words: NASEM, Se absorption, digestible methionine

1255M Effects of feeding controlled-energy and high-energy diets with rumen-protected lysine and methionine prepartum on performance and of Holstein cows.

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The aim of this study was to determine cows' performance when rumen-protected lysine (RPL) and methionine (RPM) were fed in the same amino acid to metabolizable energy (ME) ratio in prepartum diets (1.21 g of digestible methionine/Mcal of dietary ME and 3.21 g of lysine digestible/Mcal of dietary ME) with different net energy of lactation (NE_L) concentrations. Sixty-two multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, and body condition score (BCS) during the far-off dry period were assigned to 1 of 3 dietary treatments. Prepartum (−21 d to expected calving), animals were fed a controlled-energy diet (straw-based diet, 1.45 NE_L, Mcal/kg of DM) with RPL (Kemin Industries Inc.) and RPM (Kemin Industries Inc.) [CEAA; 0.15% RPL and 0.09% RPM of dietary dry matter intake (DMI)], controlled-energy diet without RPL and RPM (control; CENAA), or high-energy diet (corn silage based diet, 1.71 NE_L, Mcal/kg of DM) with RPL and RPM (HEAA; RPL 0.22% and RPM 0.12% of dietary DMI). Dry matter intake was recorded daily and body weight (BW) weekly. Prepartum energy balance (EBAL) was calculated weekly according to the NASEM (2021) equation. Statistical analyses were performed using the MIXED procedure of SAS. A contrast CONT1 (average of CEAA and HEAA vs. CENAA) along with the treatment effects were compared. Tukey's adjustment was used for comparison

of means. There was no treatment by week interaction for any variables (*P* > 0.19). There was no treatment effect (*P* > 0.37) for DMI (CEAA = 12.46 ± 0.3; CENAA = 12.63 ± 0.4; and HEAA = 13.04 ± 0.3 kg/d). Cows that received HEAA tended (*P* = 0.11) to have higher DMI as a percentage of BW than CEAA and CENAA (CEAA = 1.51 ± 0.1; CENAA = 1.44 ± 0.1; and HEAA = 1.70 ± 0.1%). There was a treatment effect (*P* = 0.01) for EBAL (CEAA = 1.82; CENAA = 0.6; and HEAA = 4.16 Mcal/d). Cows that received RPL and RPM had higher (CONT1; *P* = 0.02) EBAL and a tended to have higher DMI as a percentage of BW (CONT1; *P* = 0.13) than cows in CENAA. Cows in CEAA had lower (*P* = 0.05) EBAL than cows in HEAA. In conclusion, providing RPL and RPM in either prepartum dietary energy content improved EBAL and appeared to improve DMI as a percentage of BW of Holstein cows.

Key Words: methionine, lysine, transition

Animal Behavior and Well-Being 2

1300T Dairy cull cows: Assessing transport characteristics between farm and auction in Québec, Canada. G. Després*[1], M. Puerto-Parada[1], S. Buczinski[1], J. Dubuc[1], L. Blouin[2], and M. Villettaz-Robichaud[1], [1]*Université de Montréal, Saint-Hyacinthe, Québec, Canada,* [2]*Producteurs de Bovins du Québec, Longueuil, Québec, Canada.*

Transportation of culled dairy cows can greatly impact their welfare since it may cause considerable stress for the animals. Interestingly, the commercial transportation conditions of these cows have not been thoroughly studied in Canada. For this observational cross-sectional study, data on the transportation conditions of culled dairy cows were collected upon arrival at 3 commercial livestock auctions in Québec, Canada. Data collection was performed during a total of 12 one-day visits between July 2022 and January 2023. Data collected included the type of hauling trailer, the number and type of animals transported per truck load, the use of bedding and its type, the level of cleanliness (from 0 being clean and 2 being very dirty) and ventilation (good being > 50%), and the fact that animals were tied or not during transportation. A total of 896 transportations were observed, for a total of 3,889 culled dairy cows. Most cows were transported in commercial long (70%), multi-levels (14%), and short (12%) trailers. On average, 3.5 dairy cows were transported per load (SD: 3.7, range: 1 to 29) and most transportations (86%) also carried other animals such as calves (77%), dairy bulls (3%), beef bulls (8%), beef cows (12%), or other species (26%). Most trailers had bedding (95%); the most commonly used one was wood shavings (87%). Also, 53% of the transportations were considered adequately clean, while 29% were dirty and 18% very dirty. Among all the transportations, 83% were adequately ventilated. Overall, 22% of culled dairy cows were individually tied using a halter during transportation. The results of this study show the variation in transportation conditions that may experience culled dairy cows. The effect of these transportation conditions on the animals' welfare, health and selling price deserve to be further investigated.

Key Words: animal welfare, cull dairy cow, auction market

1301T Benefits of tactile stimulation and environmental enrichment for the welfare of Gyrolando heifers in the weaning. A. C. A. R. Paz*[1], C. O. Miranda[2], J. A. Negrão[3], F. F. Simili[4], M. S. V. Salles[4], A. E. Vercesi Filho[5], L. El Faro[2], and D. P. Munari[1], [1]*Pos-Graduate Program in Animal Science–FCAV/UNESP, Jaboticabal, SP, Brazil,* [2]*Advanced Beef Cattle Research and Development Center–Instituto de Zootecnia, Sertaozinho, SP, Brazil,* [3]*University of Sao Paulo–FZEA/USP, Pirassununga, SP, Brazil,* [4]*Regional Research Center of Ribeirão Preto–Instituto de Zootecnia, Ribeirao Preto, SP, Brazil,* [5]*Genetics and Biotechnology Research and Development Center–Instituto de Zootecnia, Nova Odessa, SP, Brazil.*

Weaning is known as a stressor that can trigger endocrine changes and can generate losses in the dairy production system. The objective of the study was to evaluate the weaning in Gyrolando heifers, managed with or without enriched environment (EE and WEE, respectively) and that received or not tactile stimulation (TS and NTS, respectively). The experiment was conducted at the Milk Quality Laboratory of the Dairy Cattle Research Center, Ribeirao Preto, SP. The 33 calves were housed with an average age of 30 d, 17 animals on EE (9 animals in TS) and 16 animals on WEE, (8 animals in TS). The animals were housed indoors, and the adaptation period was 7 d. Feed was the same for all animals, as well as water ad libitum. Weaning was initiated gradually from d 45 until d 60. The EE treatment consisted of providing objects such as ball, tires, chains, and hanging scratchers. The TS occurred until d 31 when the animals were brushed on the scapulae, loin, and hindquarters for 4 min. Stress was measured at pre-weaning (PW: d 55), at weaning (W: d 60) and post-weaning (PO: d 65) with the following variables: heart rate (HR), rectal temperature (RT), and physiological parameters (glucose, lactate, cortisol, and oxytocin). Variables were analyzed using linear mixed models as repeated measures by the MIXED procedure (SAS, SAS Institute Inc., Cary, NC). The animal was considered as random effect, and treatments (EE, WEE, TS, and STS) and days (PW, W, and PO) were fixed effects. The means were estimated by the least squares means and compared by Tukey's test. In the W the animals from WEE had higher HR than from EE ($P = 0.026$). The lactate in the W of the EE animals was lower compared with the WEE animals ($P = 0.014$). The cortisol of the animals in the EE had increased in the W when compared with WEE ($P = 0.045$). In the W the animals that received TS and were in EE had higher oxytocin levels ($P < 0.01$). The TS along with EE left the animals calmer and more relaxed, which was evidenced by the lower lactate levels, higher oxytocin levels, and lower HR in the W, which is a moment of stress for the animal.

Key Words: animal behaviour, zebu dairy cattle, weaning

1302T Effects of preweaning social housing on dairy heifer social networks and behavior on pasture. D. Clein*, K. Burke, and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Social housing for has implications for dairy calf social development, yet there are gaps in our knowledge of longer-term effects on social bonds. We examined the effects of pre-weaning social housing, with heifers reared individually or in pairs, on social networks following introduction to social groups on pasture. Heifers raised in individual pens (n = 17) or paired pens (n = 20; 10 pens of 2) were mingled between treatments and moved into groups (10 ± 2 heifers/group; total of 4 groups observed) on pasture following weaning (8.8 ± 0.4 weeks of age; mean ± SD). When heifers were 17.8 ± 1.0 (mean ± SD) weeks of age, we conducted live observation over a period of 5 d (6 h/observation day; morning: 0800 to 1100 h and afternoon: 1200 to 1500 h) for a total of 30 h observation/group. Using instantaneous scans at 10 min intervals, we recorded activity (feeding, lying, or standing) and social proximity (<3 body lengths of another heifer, with neighbor identity noted). Data were analyzed in a general linear mixed model with fixed effects of previous housing treatment, time of day (morning or afternoon), and random effects of heifer and group. Social network position was calculated from nearest neighbor data across the observational period using eigenvector centrality. Duration of standing was subject to an interaction between previous housing treatment and time of day, with heifers reared in pairs standing for longer in the morning (30% vs. 24% of scans; previously PH vs. IH; SE = 0.031; $P = 0.042$). Lying time (59% vs. 65% of scans; previously PH vs. IH, SE = 0.036, $P = 0.11$) and feeding time (9 vs. 10% of scans; previously PH vs. IH, SE 0.018; $P = 0.82$) did not differ between previous housing treatments. Eigenvector centrality while standing was great for previously PH heifers (0.89 vs. 0.84; previously PH vs. IH; SE = 0.0297; $P = 0.079$), with no differences in centrality while lying or feeding ($P > 0.82$). These results provide evidence of increased sociality in heifers reared in pairs, compared with individual pens, after 2 mo of group housing on pasture.

Key Words: social housing, social network, dairy heifer

1303T Feeding behavior of group-housed preweaned dairy calves can be used to predict disease using machine learning algorithms.

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Automated milk feeders (AMF) can provide individual monitoring of calves housed in groups while allowing them to express natural behaviors and partake in social interactions. Research has shown that feeding behaviors recorded by AMF can be a useful screening tool for detecting disease in dairy calves. These data from AMF could potentially be used to create a more robust and efficient model to predict disease before clinical symptoms of illness are apparent. Therefore, the objective of this observational study was to use AMF feeding behavior data and machine learning algorithms to predict disease in preweaned dairy calves. This study was conducted on a 2,500-cow dairy farm located in the Upper Midwest USA. The farm was visited on a weekly basis from May 2018 to May 2019 by study personnel in which AMF data and calves' treatment records were collected. In addition, researchers visually scored calves for health status. The final data set consisted of 741 (healthy = 560 and sick = 484) calves with 1,044 observations. Data used for the analyses included primarily daily calf treatment records in addition to weekly health scores. Calf visit-level feeding behaviors from AMF data including milk intake (mL/d), drinking speed (mL/min), visit duration (min), rewarded (with milk being offered) and unrewarded (without milk) visits (number per d), and interval between visits (min) were used to predict health status using 3 predictive approaches: generalized linear model (GLM), random forest (RF), and gradient boosting machine (GBM). A total of 16 models were built using different combinations of behavior parameters as predictor variables. Of all algorithms, RF and GBM had the best performance to predict health status of dairy calves (e.g., F1 scores of 0.775 and 0.784 for RF and GBM). This study suggests that machine learning was effective in determining specific visit-level feeding behaviors to predict disease in group-housed preweaned dairy calves.

Key Words: feeding behavior, preweaned dairy calf

1304T Effects of preweaning social housing on growth, estrus behavior, and age of onset to estrus in dairy heifers.

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While there is evidence of short-term performance and welfare benefits of social housing systems for dairy calves, there are gaps in our knowledge of long-term production consequences. The objective of this study was to assess the effects of dairy calf social housing systems during the pre-weaning period on heifer growth, expression of estrus behavior, and age at estrus. Heifers were housed either individually (IH; n = 30 heifers), in pairs (PH; n = 30; 1 focal heifer/pair) or in groups (n = 30; focal animals selected from 6 groups of 10 calves/group) from birth until 9 weeks of age. Beginning at 6 mo of age (185.7 ± 2.6 d of age, mean ± SD), body weight (BW), hip height, and anogenital distance (AGD) were recorded weekly for 24 weeks. Heifers were also placed with an Estrotect breeding indicator (Estrotect, Spring Valley, WI), which was examined once/week for evidence of mounting and standing estrus (score 1–4 based on color change indicating rubbing; 1 = no change, 4 = solid color change). Ultrasounds were performed weekly following a positive Estrotect reading (score 3 or 4) until confirmation of a corpus luteum (CL). Data were analyzed in general linear models to test effects of previous housing treatment while controlling for birth weight and season. Previous housing treatment did not affect BW (258.1 kg; SE = 5.5; $P = 0.93$) or AGD (12 cm; SE = 0.24; $P = 0.73$). However, hip height was subject to an interaction between previous housing treatments and week ($P < 0.001$), with treatment differences increasing over time, and previously PH calves having greater hip heights overall compared with the other treatments (124.8 vs. 123.3 vs. 123.2 cm; PH vs. IH vs. GH; SE = 0.81; $P = 0.04$). Age at indication of estrus behavior (positive Estrotect reading; 288.4 d; SE = 21.7; $P = 0.42$) and age at confirmed CL (324.9 d; SE = 14.1; $P = 0.22$) did not differ between housing treatments. These results suggest potential improved longer-term growth performance in heifers that were reared in pairs, but no differences in reproductive development associated with social housing.

Key Words: dairy heifer, social housing, estrus

1305T Associations between feeding behavior and social network centrality in group-housed dairy calves.

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With growing adoption of social housing for dairy calves, there is a need to understand how social behavior relates to calf performance. Using social network analysis, we examined the relationship between social behavior and milk feeding behavior. At 2 weeks of age, calves (n = 90; 62 heifers and 28 bulls) were housed in groups (9 groups; 10 calves/group) until after weaning at 8 weeks of age. Calves received 8 L/d of milk replacer via an automatic milk feeder and milk intake and frequency of rewarded and unrewarded visits were recorded. Health scores (Wisconsin Health Scoring Chart) were recorded twice weekly. Calf position within the pen was recorded continuously using an ultra-wideband positioning system generating pairwise proximity estimates. Social network position was measured using 2 centrality measures: strength, reflecting connectedness, and eigenvector, reflecting influence in the network. We tested whether milk intake and autofeeder visit frequency during the pre-weaning period (wk 2 to 6 of age) were associated with these centrality measures using general linear mixed models, controlling for fixed effects of season (warm or cool), birth weight, sex, and health status, and random effects of week and calf within group. Calves with greater strength centrality tended to consume more milk (estimate = 106.7, SE = 60.4; $P = 0.08$) and had more frequent unrewarded visits (estimate = 0.27, SE = 0.11; $P = 0.02$). There was no association between feeding behavior and eigenvector centrality. Milk intake ($P = 0.40$) and visit frequency ($P = 0.50$) were not influenced by health status during the preweaning period. These results suggest a relationship between connectedness in a proximity-based social network and feeding behavior in group-housed dairy calves.

Key Words: social behavior, milk intake, meal frequency

1306T Effect of preweaning social housing on development of cognition in weaned heifers.

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Given evidence of immediate effects of environmental complexity on cognition, our objective was to assess longer-term behavioral flexibility of dairy heifers reared in different housing systems. At birth, calves were assigned to either: 1) individual pens (IH; n = 22), 2) pair pens (n = 22; 1 focal heifer/pair), or 3) group pens (GH; n = 22; 5 ± 1 focal heifer/group of 10). After weaning, at 8 weeks of age, calves were mixed between treatments and moved to group pens. At 2 mo (10 ± 0 weeks; mean ± SD) and 5 mo (23 ± 2 weeks), calves were tested in a T-maze with a reward (ability to exit the maze) located in one arm to assess initial learning ability, with a subsequent switch in reward side to assess reversal learning ability, an indicator of cognitive flexibility. Calves received 5 sessions/day for 5 d or until the learning criterion (moving directly to correct side for 3 consecutive sessions) was met. Pass rate and number of sessions to reach criterion were analyzed in a general linear mixed model with fixed effects of previous housing treatment and initial reward side (balanced by treatments). At 2 mo of age, pass rate for initial (82 vs. 96 vs. 96%; IH vs. PH vs. GH; SE = 4; $P = 0.29$) and reversal learning (82 vs. 95 vs. 82%; IH vs. PH vs. GH; SE = 9; $P = 0.40$) did not differ significantly between treatments. Sessions to pass did not differ for initial learning (9.1 vs. 7.2 vs. 7.6; IH vs. PH vs. GH; SE = 1; $P = 0.35$) but tended to differ for reversal learning (7.5 vs. 7.0 vs. 9.8; IH vs. PH vs. GH; SE = 1; $P = 0.07$). At 5 mo of age, previous housing did not affect overall pass rate ($P = 0.47$) or sessions to pass initial learning ($P = 0.39$) but sessions to pass reversal learning differed (6.6 vs. 8.4 vs. 12.4; IH vs. PH vs. GH; SE = 1.9; $P = 0.03$). Sessions to pass were correlated between time points for initial learning ($P = 0.037$) and tended to be correlated for reversal learning ($P = 0.10$). These results suggest persistent effects of preweaning housing system, possibly reflecting differences in behavioral flexibility or response to the testing environment, and provide evidence of individual consistency in responses over time.

Key Words: social housing, behavioral flexibility, dairy cattle

1307T Effects of willow bark (*Salix*) on post-disbudding behaviors in dairy calves under organic management. M. Bacon*[1,2], B. Heins[2], and M. Endres[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*West Central Research and Outreach Center, University of Minnesota, Morris, MN.*

White Willow Bark (*Salix*) is commonly used to alleviate pain in disbudded calves under organic management, but there is no scientific evidence that willow bark (WB) has an analgesic effect. Previous studies have evaluated the effects of WB on the physiological stress responses of organic dairy calves, but behaviors have not yet been examined. The objective of this study was to evaluate the effect of an oral WB bolus on the behavior of 25 Holstein and crossbred heifer calves disbudded between 5 to 7 weeks of age. Calves were randomly assigned to one of 3 treatments: hot iron disbudding with lidocaine (5 mL per horn bud; n = 8), hot iron disbudding with oral WB (200 mg/kg; n = 10), or a “sham” disbudding (n = 7) in which an unheated iron is used to simulate the disbudding procedure as a control for handling stress. Tablets continuously recorded behaviors beginning 1 h before and ending 4 h after disbudding. Three observers, blinded to treatment, recorded the frequency of 7 behaviors (ear flicks, head rubs, head shakes, head scratches, tail flicks, transitions between lying and standing, and grooming) in 10-min intervals using BORIS event-logging software. Logistic regression was used for analysis with the fixed effects of disbudding treatment, hour of observations, and the interaction of treatment and hour, with calf included in all models as a random effect. The frequency of transitions was affected by treatment ($P < 0.05$); the adjusted log mean number of transitions was 0.72 higher in calves receiving WB compared with sham disbudding. Hour of observation had a main effect on head rubs ($P < 0.001$) and grooming ($P < 0.001$). Calves across treatments rubbed their heads more frequently in the first hour after disbudding than in hours 2 and 3 and groomed themselves more in the hour before disbudding than in hours 2 and 4 after. There was a main effect of the interaction of treatment and hour in head shakes ($P < 0.05$). No effect of treatment, hour, or their interaction was observed in ear flicks, head scratches, or tail flicks. These results indicate that WB alone does not provide sufficient relief from disbudding pain.

Key Words: calf welfare, disbudding, organic farming

1308T Effects of pain following disbudding on cognitive performance of dairy calves. S. Yoo*, M. A. G. von Keyserlingk, and D. M. Weary, *The University of British Columbia, Canada.*

Some studies reported lasting pain in the weeks after disbudding. Studies on humans and rats have demonstrated that lasting pain can inhibit formation and recall of memories. Our objective was to assess, using a modified hole-board apparatus, whether hot-iron disbudding has a long effect on learning and memory in calves. The apparatus was a square arena holding 15 milk bottle holders. At 14 d of age Holstein heifer calves (n = 30) were randomly assigned to 3 treatments: hot-iron disbudding, hot-iron disbudding with ongoing analgesic treatment, and sham disbudding. All calves received multimodal pain relief (sedation, cornual local block, and an NSAID). Starting on the day after disbudding, calves were brought into the modified hole-board apparatus once daily and allowed to find the 4 bottles containing milk (versus 11 other bottles that were empty). The location of milk-containing bottles did not change for 12 d, but on d 13 the locations of the 4 milk-containing bottles were changed and calves were able to learn the new locations over 6 d. We measured working memory (WM), general working memory (GWM), reference memory (RM), and the number of visits to the bottles (VISIT). Data were analyzed using linear mixed models (treatment, days after disbudding, and their interaction were included as fixed effects; calf was included as a random effect). For the 12 d after disbudding, WM, GWM, and RM increased ($P < 0.001$) while VISIT decreased ($P < 0.001$). When the locations were changed on d 13, GWM and RM scores dropped ($P < 0.001$) and VISIT increased ($P < 0.001$). For the next 6 d, WM, GWM, and RM again increased ($P = 0.046$, $P < 0.001$, and $P < 0.001$, respectively) and VISIT decreased ($P < 0.001$). We found no effect of treatment on any measure. These results indicate that any pain over this period was insufficient to impair these measures, or that the modified hole-board test was not sufficiently sensitive to detect these effects.

Key Words: hot-iron, memory, calf welfare

1309T Effect of group composition on agonistic interactions received by subordinate cows: A pilot study. J. Krahn*, B. Foris, K. Sheng, D. M. Weary, and M. A. G. von Keyserlingk, *University of British Columbia, Vancouver, British Columbia, Canada.*

Social dominance influences resource access in dairy cows, and subordinate cows often receive a greater number of agonistic interactions when competing for resources compared with more dominant cows. Little is known about how grouping practices influence the social experience of these subordinate individuals. We investigated the effect of group composition on agonistic interactions received by subordinate cows while at the feeder. In each of 4 replicates, we followed 50 cows through an initial grouping phase and 2 treatment phases, automatically recording agonistic interactions using electronic feed bins and a validated algorithm. These data were used to calculate Elo-ratings and then categorize cow dominance into quintiles. In the initial grouping phase, all 50 cows were housed as a single group for 7 d. In the first treatment phase, cows were housed in groups of 10 for 5 d; these groups were created by randomly distributing 2 cows from each quintile (as based upon the Elo-ratings from the initial grouping phase), ensuring variation in dominance scores. In the second treatment phase, the 10 most subordinate cows were housed together in a single group for 5 d. For all phases stocking density remained constant. We used the last 3 d in each phase for analysis, which was conducted in SAS. Subordinate cows, on average received 40.7 ± 5.9 (mean ± SE; 95% CI: 22.1–59.2; n = 4) agonistic interactions in the first treatment phase (i.e., heterogeneous group), and 23.6 ± 3.4 (mean ± SE; 95% CI: 12.7–34.5; n = 4) in the second treatment phase (i.e., subordinate-only group). We used a linear mixed model to compare the mean number of agonistic interactions received across treatment phases including replicate as a random effect. Despite our underpowered sample, we found that subordinate cows tended to receive fewer agonistic interactions when housed as a subordinate-only group ($F_{1,3} = 6.4$ and $P = 0.09$). These results suggest that there is merit in larger-scale investigations of whether housing subordinate cows in subordinate-only groups reduces the number of agonistic interactions that they receive.

Key Words: competition, behavior, management

1310T Activity behaviors and relative changes in activity patterns were associated with diarrhea status in individually housed calves.

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The objective of this case-control study was to quantify if there was an association of daily activity behaviors and relative changes in activity patterns (lying time, lying bouts, step count, activity index) with diarrhea status in preweaned dairy calves. Individually housed calves sourced from auction were health scored daily for signs of diarrhea (fecal consistency loose or watery for 2 consecutive d) for 28 d after arrival. Calves with diarrhea were pair matched to healthy controls (n = 13 matched by arrival date, arrival weight, and diagnosis d to diarrheic calves). Mixed linear regression models were used to evaluate the association of diarrhea status, and the diarrhea status by d interaction with activity behaviors (d −3 to d 4) and relative changes in activity patterns (d −3 to d 4) surrounding diagnosis of diarrhea status. The serum Brix % at arrival and daily THI from the calf barn were explored as quantitative covariates, with d as a repeated measure. The baseline for relative changes in activity patterns was set at 100% on d 0. Diarrheic calves averaged less steps (119.1 ± 18.8 steps/d) than healthy calves (227.4 ± 18.8 steps/d, LSM ± SEM). Diarrheic calves also averaged lower activity indices (827.34 ± 93.09 daily index) than healthy calves (1,396.32 ± 93.09 daily index). There was also a diarrhea status by d interaction for lying time on d −3, with sick calves spending more time lying (20.80 ± 0.30 h/d) than healthy calves (19.25 ± 0.30 h/d). For relative changes in activity patterns, there was a diarrhea status by d interaction on d −2, where diarrheic calves had greater relative changes in step counts (diarrhea 634.85 ± 87.58% vs. healthy 216.51 ± 87.58%) and activity index (diarrhea 316.83 ± 35.69% vs. healthy 150.68 ± 35.69%). Lying bouts were not associated with diarrhea status. These results show that diarrheic calves were more lethargic, and they had relative changes in activity patterns at −2 d. Future research should explore the potential of an activity alert to identify calves at risk for a diarrhea bout using relative changes in activity patterns.

Key Words: precision technology, disease

1311T Dairy cows' social rank influences their drinking behavior.

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Providing sufficient access to clean drinking water is essential for the productivity and welfare of dairy cows. However, dairy farms show high variability in how they provide water to cows. Insufficient water access could lead to competition for this resource and agonistic interactions between cows. Little is known about how the social rank of cows influences individual drinking behavior, particularly drinker choices and drinking times. The most subordinate cows could avoid high water competition times and places by developing adaptive strategies. We monitored drinking behavior and replacements (an actor cow butting or pushing a receiver cow, leading to the receiver being replaced by the actor) at the drinker in a group of 22 lactating Holstein dairy cows for 9 d by video recording. Cows had access to 6 electronic drinkers. We recorded 257 replacements during this period. Based on these replacements, we used the normalized David's score to calculate the dominance score of each cow. We classified cows into 4 dominance categories using dominance score quartiles. Comparing categories in terms of dynamic of drinking, we found that subordinate cows modified their drinking behavior by shifting their drinking time from 2 to 3 h after dominants drank. Results also show subordinates and dominants used different drinkers among the 6 available, with subordinates mostly using the furthest from the feeding area. Our results indicate that cows' social rank can determine specific drinking strategies to avoid greater water competition peaks within the group, even in experimental conditions with a high number of drinkers per cow.

Key Words: drinking behavior, social hierarchy, dairy cow

1312T Effect of lameness on location preference within a pen for lactating dairy cows.

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Lameness in dairy cows may affect locational preference within a pen, and indoor positioning systems (IPS) record the location of individual cows continuously. Our objective was to evaluate the effect of lameness status (LS) on location preferences within a free stall pen. Thirty-five mixed-parity (2.0 ± 0.9 lactations; 219 ± 45 DIM; 45.3 ± 5.7 kg milk/d) Holstein cows were housed in a head-to-head free stall pen, milked 3×/d (0515, 1315, and 2115 h) and fed 1×/d (0530 h). Lameness status (Lame, non-Lame) was assigned based on locomotion scores using a 1–5 scale (1: normal, 5: severely lame) before the start of the study. Final breakdown was 15 Lame cows (score of 3–4) and 20 non-Lame cows (score of 1–2). Location preference was determined by time spent in each location within the pen for the entire d and for each 8-h intermilking period (IMP). Areas of interest (AOI) were: feed bunk (FB; 10 bays with 4, 76-cm headlocks each), 4 quadrants of 10 stalls (SQ), and alley (Feed, Back, Cross). The 3 IMP were: IMP1, 0545–1315 h; IMP2, 1345–2115 h; and IMP3, 2145–0515 h. Location was recorded continuously for 3 d. Data were summarized by day and IMP and analyzed with GLIMMIX (SAS v 9.4). The model included fixed effects of LS, AOI, IMP, and random effect of cow. Time spent in AOI across whole day for all cows was: FB, 225 min/d; SQ, 896 min/d; alley, 132 min/d. There was a significant ($P = 0.02$) LS × SQ interaction where lame cows spent more time in the SQ nearest the pen exit. There was no interaction between LS × AOI × IMP ($P > 0.65$). We did observe a significant interaction between FB × IMP ($P < 0.001$) whereby cows spent more time in the FB sections closest to the exit gate during IMP1 and IMP2 compared with IMP3. Cows spent more time in both Feed and Back versus Cross alley across the whole day: 51 and 46 vs. 35

min, respectively, and within an IMP ($P < 0.001$). Lameness status had little effect on location preference within the pen. However, there were significant differences in location preferences for both SQ and FB closest to the pen exit that varied with time of day.

Key Words: lameness, location, preference

Animal Health 2

1313T Characterizing *Staphylococcus aureus* from mastitis cases on Maine dairy farms. E. Roadcap*[1], A. Lichtenwalner[2], B. Kennedy-Wade[2], G. Adjapong[2], A. Chakrawarti[1], F. Machado De Santanna[1], and J. Barlow[1], *[1]University of Vermont, Burlington, VT, [2]University of Maine, Orono, ME.*

Staphylococcus aureus (SA) is an important intramammary pathogen causing mastitis in dairy cattle, reducing milk yield, causing economic loss, and posing a risk to public and herd health. We aim to assess the prevalence of SA among samples submitted to the University of Maine Veterinary Diagnostic Laboratory (UMVDL) over a period of 5 years and to determine antimicrobial susceptibility with genetic confirmation for a subset of these samples. Bovine, ovine, and caprine milk samples submitted to the UMVDL between July 2017 and June 2022 were evaluated for patterns of SA mastitis. Of 6,118 mastitis milk samples analyzed at UMVDL, 467 (7.6%) were positive for SA based on routine morphological and biochemical testing. These isolates were collected from 433 animals on 79 farms in 15 counties. The proportion of monthly mastitis cases positive for SA increased from 6.8 ± 0.70% from July 2017 to December 2019 to 12.3 ± 1.8% from January 2020 to June 2022 ($P = 0.008$). Over the 5 years, 91 of the 467 SA isolates had been tested for antibiotic susceptibility per farmer request; 25 (27.5%) were resistant to oxacillin. We conducted disk diffusion assays on 29 isolates to assess sensitivity to 12 antibiotics. Genomic DNA of the 29 isolates was extracted and submitted for whole genome sequencing. A hybrid assembly method will be used to assemble the sequences. We aim to use these assembled genomes to identify AMR genes using the 'ABRicate' tool and to identify sequence types using the 'mlst' tool. All 29 SA isolates were phenotypically susceptible to all antibiotics tested. Identification of AMR genes from whole genome sequences of the 29 isolates will allow us to report the relationship between phenotypic antimicrobial susceptibility and identified AMR genes. We recognize the biased nature of our study which drew upon opportunistic, voluntary samples provided by clients of UMVDL in the course of regular dairy farm operations. However, analyzing these samples will provide a preliminary understanding of SA in Maine's dairy industry. Our analysis will better clarify the risk posed to public and herd health by SA from dairy sources in Maine.

Key Words: *Staphylococcus aureus*, antimicrobial susceptibility

1314T Staphylococcal enterotoxins play an important role in clinical and subclinical bovine mastitis. S. Dantas[1], L. Takume[1], B. Rossi[1], E. Bonsaglia[1], I. Castilho[1], J. Pantoja[1], A. Fernandes Junior[1], J. Gonçalves[3], M. Santos[2], and V. Rall*[1], *[1]Universidade Estadual Paulista, Botucatu, Sao Paulo, Brazil, [2]Universidade de São Paulo, Pirassununga, Sao Paulo, Brazil, [3]Michigan State University (MSU), East Lansing, MI.*

Staphylococcus aureus is one of the main pathogens causing contagious bovine mastitis, being more frequently isolated from subclinical form although the clinical form also occurs. *S. aureus* presents many important virulence factors for the establishment of its pathogenicity in animals, such as enterotoxins, which are also responsible for foodborne poisoning. Our objective was to perform a comparative analysis among 103 isolates of *S. aureus*, obtained from milk of cows with clinical mastitis and more 103, from subclinical type, in relation to the presence of these enterotoxins and verify if their presence plays an important role in the signs of illness. We investigated all enterotoxins described until now, such as *sea-see*, *seg-sez*, *sel26*, *sel 27*, *se01*, and *se02*. Considering the isolates obtained from clinical mastitis, we observed up to 14 enterotoxin genes and the most frequent were *selw* (99.03%); *selx* (78.64%); *seh* (50.48%); *seg* (16.5%); *selm* (15.53%); *selu1* (10.68%); and *sei* (9.71%). In subclinical mastitis, only 5 genes were found, *selw* (82.52%); *selm* (15.53%); *selx* (14.56%); *seh* (7.76%); and *selz* (3.88%). Using Fisher exact test or chi-squared test, *seg*, *seh*, *sei*, selo, *selu*, *selw*, and *selx* genes presented statistical difference ($P < 0.0001$). Other interesting results were the low number of genes in each isolate from subclinical mastitis, that was up to 3 genes and most of the isolates presented one gene (55/53.4%). On the other hand, among the clinical cases, we observed up to 9 genes in one isolate (obtained from a severe case) and most of them presented at least 3 genes (38/36.9%). Based on these results, we can conclude that enterotoxins indeed play an important role in clinical signs in cattle with mastitis.

Key Words: superantigen, enterotoxin

1315T Risk factors associated with clinical mastitis in certified organic dairy herds. P. Munoz Boettcher*[1], A. De Vries[2], E. Miller-Cushon[2], B. J. Heins[3], V. Cabrera[4], E. Silva[4], R. A. Lynch[5], G. M. Schuenemann[6], D. Manríquez[1,7], J. Velez[8], and P. Pinedo[1], *[1]Colorado State University, Fort Collins, CO, [2]University of Florida, Gainesville, FL, [3]University of Minnesota, St. Paul, MN, [4]University of Wisconsin–Madison, Madison WI, [5]Cornell University, Ithaca NY, [6]The Ohio State University, Columbus OH, [7]National School of Veterinary Medicine of Toulouse, Toulouse, France, [8]Aurora Organic Dairy, Platteville, CO.*

The objective was to investigate the association between events occurring during the previous lactation, the dry period, and the peripartum period and the incidence of clinical mastitis (CM) within 30 DIM in Holstein cows maintained in large organic dairies. This retrospective study included information from 8 organic certified herds in northern CO and west TX. Cow information consisted of completed lactations with calvings occurring from 2016 to 2018. Potential risk factors included parity category, milk yield in the previous lactation (PLMLK), season of dry-off (DS), access to grazing at dry-off (DG), dry period length (DPL), close-up period length (CUL), season of calving (SC), access to grazing at calving (CG) and other disease ≤ 30 DIM (DZ). Data were analyzed using multivariable logistic regression and the models included SC, parity, and farm as covariables. After edits, 64,666 lactation records were analyzed in 40,920 cows, with 1 (55.6%), 2 (31.2%), and 3 (13.2%) lactations available. Overall, 13.8% of the lactations (primiparous = 12.3%; multiparous = 14.6%) had at least one CM case within 30 DIM. Parity, PLMLK, DS, DG, DPL, CUL, SC, and DZ were significantly associated with CM (Table 1). The identified risk factors for CM provide opportunities for interventions, especially focused on dry-off management, estimation of due dates, and disease prevention.

Key Words: mastitis, organic, risk factor

1316T Post-milking application of a *Lacticaseibacillus paracasei* strain on the bovine teat skin: Impact at the microbial, immune, and physiological levels. C. Goetz[1], L. Rault[1], J. Cuffel[1], P. Poton[2], S. Philau[2], G. Boullet[2], A. Mottin[2], J. Orinel[2], M. Boutinaud[2], and S. Even*[1], *[1]INRAE, Institut Agro Rennes-Angers, UMR 1253 STLO, Rennes Cedex, France, [2]INRAE, Institut Agro Rennes-Angers, UMR 1348 PEGASE, Saint-Gilles, France.*

Table 1 (Abstr. 1315T). Adjusted odds ratios of CM within 30 DIM by variable of interest; interactions were not significant and were removed from the models

Variable	Category	OR	95% CI	*P*-value
Parity	Primiparous vs. multiparous	0.83	0.79–0.87	<0.0001
Milk yield 305ME	Low vs. high	1.35	1.19–1.52	<0.0001
	Intermediate vs. high	0.99	0.90–1.10	0.91
Season of dry-off	Spring vs. winter	0.89	0.81–0.98	0.01
	Summer vs. winter	0.86	0.78–0.96	0.0045
	Fall vs. winter	0.81	0.74–0.90	<0.0001
Grazing at dry-off	No vs. yes	1.13	1.05–1.22	0.002
Dry period length (d)	<30	1.03	0.89–1.19	0.72
	30-70	Ref.	—	—
	>70	1.39	1.30–1.47	<0.0001
Close-up length (d)	<7	1.21	1.10–1.32	<0.0001
	7-14	Ref.	—	—
	>14	1.14	1.06–1.23	0.0004
Calving season	SP vs. WI	1.03	0.97–1.11	0.33
	SU vs. WI	1.16	1.08–1.24	<0.0001
	FA vs. WI	1.13	1.06–1.21	0.0003
Disease ≤30 DIM	Yes vs. no	1.21	1.12–1.31	<0.0001

Bovine mastitis is a major disease in the dairy industry. The current approaches are not entirely effective and may contribute to the risk of dissemination of antimicrobial resistance, supporting the need for alternative treatments. Here, a microbial strategy aiming to shape the teat microbiota toward a protective one through the application of a lactic acid bacteria (LAB), was explored on 23 Holstein cows in mid-lactation. Treatment (*Lacticaseibacillus paracasei* CIRM BIA 1542 sprayed at $\sim 1.10^9$ cfu/mL [L; n = 8], iodine [I; n = 7], or no treatment [N; n = 8]) was applied post-milking twice a day on the 4 teats of healthy animals for 15 d. Blood and milk samples and teat skin swabs were collected at D1, D8, D15, and D26 before morning milking and at D15 before evening milking (D15e) to evaluate the LAB treatment impact at the microbial (presence of pathogens, microbiota analysis), immune (somatic cell count) and physiological levels (milk production, markers of mammary epithelium integrity). Data were analyzed using an ANOVA with day and treatment as fixed effects and cow as a random effect. *L. paracasei* was transiently present on teat skin and in foremilk during treatment, as illustrated by a LAB population in foremilk 1.5- and 29-fold higher in L than I at D15 and D15e, respectively ($P < 0.01$). The total microbial population in cisternal milk, foremilk and on teat skin was significantly higher during treatment ($P < 0.05$) in L (1.79–4.84 log [cfu/mL]) compared with I (1.01–3.95 log [cfu/mL]). However, no pathogen was found in cisternal milk. Besides, LAB treatment did not trigger any major inflammatory response in the mammary gland (no significant impact on milk somatic cell scores) and had no impact on the functionality and the epithelium integrity (no significant impact on milk yield and composition, mammary epithelial cell exfoliation rate into milk or Na$^+$:K+ ratio). Altogether, these results indicate that LAB treatment is safe with regard to mammary gland functionality and immune system while impacting its microbiota.

Key Words: bovine mastitis, lactic acid bacteria, preventive treatment

1317T Association between the temperature-humidity index and microorganisms isolated from milk of healthy cows and cows with mastitis in different regions of Brazil.

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This work aimed to evaluate the relationship between temperature-humidity index (THI) and microorganisms isolated from healthy cows and cows with mastitis in 5 different states of Brazil. A total of 1,468 milk samples were collected from healthy cows, and cows with subclinical and clinical mastitis during summer and winter in the States of São Paulo, Santa Catarina, Paraíba, Pará, and Goiás, totaling 17 farms. All samples were inoculated on blood agar, and then, up to 3 colonies characteristic were isolated. Biochemical tests were performed to identify *Staphylococcus* sp. and *Streptococcus* sp. Proteomic analyzes were performed using the MALDI-TOF (matrix associated laser desorption-ionization–time of flight) technique for species identification. The following formula was used to calculate the THI: $THI = 1.8 \times Ta - (1 - RH) \times (Ta - 14.3) + 32$, where Ta is the average ambient temperature in degrees Celsius and RH is the average relative humidity. Statistical analyzes were performed using the SAS Studio statistical software, a chi-squared test for independence was performed and odds ratio was performed to quantify the probability of occurrence in relation to the evaluated factors. Statistical significance was declared at $P < 0.05$. The results of the number of identified microorganisms and the mean THI of each microorganism can be seen in Table 1. Although there was a statistical difference between the THI and the probability of isolating coagulase-positive *Staphylococcus* (CPS), coagulase-negative *Staphylococcus* (CNS), and *Streptococcus* sp., we cannot conclude that the THI influenced the probability of isolation of the microorganisms found. This is due to the great variability of temperature, precipitation,

Table 1 (Abstr. 1317T). THI refers to the groups of microorganisms isolated from milk samples from healthy cows and cows with mastitis in two seasons of the year, summer and winter

Group of microorganisms	Number isolated	Label	Median
Coagulase-positive *Staphylococcus* (CPS)	346	Average temperature (°C)	22.4
		Precipitation (mm/month)	84.0
		THI	70.2 ($P = 0.0003$)
Coagulase-negative *Staphylococcus* (CNS)	109	Average temperature (°C)	19.6
		Precipitation (mm/month)	80.1
		THI	66.2 ($P < 0.0001$)
Streptococcus sp.	29	Average temperature (°C)	26.3
		Precipitation (mm/month)	80.0
		THI	73.2 ($P = 0.0420$)

and relative humidity in the states where the samples were collected, even in the same seasons of the year.

Key Words: temperature-humidity index, milk, mastitis, pathogen

1318T The effects of bimodal milk flow on mastitis infection in dairy cows.

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Several factors, including the animal, milking machine, and milking routine, can affect the shape of the curve that describes the flow of milk over time in dairy cows during milking. In particular, inadequate pre-stimulation can lead to a delay in the milk ejection reflex, resulting in a temporary reduction in milk flow and bimodal milk flow curves. This phenomenon not only affects milking efficiency by extending machine run time but can also negatively impact teat and udder health due to its similarity to overmilking. Previous research has also linked bimodality to increased milk somatic cell counts in Holstein cows. This study aimed to assess the effect of bimodal milking profiles on mastitis infections in dairy herds. We analyzed 11 mo of individual milking records from 2,630 cows in 12 commercial robotic dairy farms across Canada, USA, the Netherlands, and Germany (Feb. 2022–Dec. 2022). We created a classification model to identify bimodal profiles based on the first phase of the milk flow of each teat, and an automated model to detect mastitis in each teat at every milking. A contingency analysis to examine the relationship of bimodal milking and mastitis infections was assessed in R software. For all cow parities, results showed that bimodality affected the mastitis infections odd ratio (OR) (P-value <0.0001). Primiparous and multiparous cows with bimodality were 1.98 (OR, 95% CI: 1.86–2.11) and 1.16 (OR, 95% CI: 1.13–1.19) times more likely to have a mastitis infection than those with milking profiles without bimodality, respectively. The results from this study suggest that there is a relationship between the presence of bimodal milking profiles and the possibility of having a clinical case of mastitis for both primiparous and multiparous cows. Reducing bimodality could help avoid udder problems while improving milking efficiency.

Key Words: bimodality, udder health, automatic milking system

1319T Intramammary liposome-toll-like receptor agonist (LTC) dose titration: Effect on differential somatic cell count.

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The development of alternative treatments capable of upregulating the immune system can contribute to the control of mastitis and enhance antimicrobial stewardship on dairy farms. A liposome-toll-like receptor (TLR) agonist (LTC) immune stimulant generates synergistic immune activation through a combination of synthetic nucleic acids, such as CgG oligonucleotides (TLR9 agonist) and polyIC (TLR3 agonist), with charged liposomes and a mucosal adhesive agent. Our objective with this pilot project is to determine the lowest effective dose of LTC that induces a mammary gland immune response without inducing signs of clinical mastitis. Eight mid-lactation (90 ± 3 DIM) dairy cows, without clinical mastitis and a SCC <200,000 cells/mL, were assigned to two 4 × 4 Latin squares with experimental periods of 7 d separated by a 28-d interval. Intramammary administration of treatments were randomly assigned to cows and consisted of LTC (0.50 mL [HI], 0.25 mL [MED], 0.125 mL [LOW], and 0 mL [CON]) diluted to a 10-mL volume using LTC diluent (Tris buffered, 10% sucrose, pH 7.2). All treatments were administered to the right rear quarter. On the first day of each experiment period, before treatment infusion, a milk sample from each quarter was collected. Additional samples were collected every 12 h during the following 7 d. The effect of LTC on the total leukocyte count (TLC), neutrophils (NEU), lymphocytes (LYM), and macrophages (MAC) during the study period was determined using a linear mixed-effects model accounting for repeated measures. After 2 iterations of treatments, we observe evidence of an extended immune response (defined as increased number of immune cells after 12 h of infusion) only in the LOW group. The average TLC (753 vs. 51 ± 112 × 1,000 cells/mL; $P < 0.01$), NEU (532 vs. 30 ± 82 × 1,000 cells/mL; $P < 0.01$), LYM (136 vs. 15 ± 12 × 1,000 cells/mL; $P < 0.01$), and MAC (97 vs. 19 ± 18 × 1,000 cells/mL; $P < 0.01$) were different when comparing the LOW to the CON group. Our data indicate that the LOW dose used in this experiment is an appropriate initial dose for a dose titration experiment. Lower doses will be investigated during the next iterations of this experiment to determine the most appropriate dose treatment.

Key Words: immune stimulant, immune response, antimicrobial stewardship

1320T Evaluation of a phage cocktail for the treatment of *Staphylococcus aureus* bovine intramammary infections.

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Bovine intramammary infections (IMI) are a major concern for dairy producers and *Staphylococcus aureus* (SA) is one of the predominant pathogens inducing this disease. With the rise of antimicrobial resistance and the want to reduce antibiotic use, alternatives are needed. Phages are viruses that specifically target bacteria. A cocktail of phages targeting SA showed promising results when previously tested in a murine mastitis model. The aims of this study were to (1) assess safety of this phage cocktail following intramammary infusion, and (2) evaluate its efficacy for the treatment of SA IMI. For safety assessment, 3 quarters of 3 cows were infused with PBS (negative control, 10 mL) or 1 dose of phages (10 mL of either 10^{10} or 10^9 pfu/mL). Milk samples were collected over 24 h to measure indicators of inflammation: somatic cell counts (SCC) and milk haptoglobin. The higher dose of the phage cocktail induced an elevation of SCC 12 and 24 h after infusion when compared with PBS (at 12 h: 7.1 $\log_{10}$ vs. 5.3, $P < 0.01$; at 24 h: 7.1 vs. 5.4, $P < 0.001$). The same response pattern was observed for haptoglobin. The lower dose was thus selected to evaluate the phage cocktail for the treatment of SA IMI. Eleven cows were infected in 2 quarters with 54 cfu of SA. Treatment was initiated after 2 weeks of infection. Each cow received 10 mL of either PBS or phage cocktail (10^9 pfu/mL) in their infected quarters, twice daily for 7 d. During the treatment period, SCC increased in SA infected quarters treated with phages (6.55 $\log_{10}$ vs. 6.14, $P < 0.05$). Two weeks after the treatment period, SCC of phage-treated quarters were intermediate between the PBS-treated quarters (5.54 $\log_{10}$ vs. 5.95, $P = 0.21$) and the uninfected quarters (5.54 vs. 5.07, $P = 0.14$); and SCC of PBS-treated quarters remained significantly higher than the uninfected quarters (5.95 vs. 5.07, $P < 0.01$). This is the first time that this phage cocktail was used in a trial for treatment of experimental SA bovine IMI. The cocktail was safe when used for infusion in cows. Adjustments are needed to improve the efficacy of this treatment.

Key Words: mastitis, phage, safety

1321T Lipopeptides as a potential anti-virulence therapy to prevent bovine mastitis.

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Bovine mastitis is a common disease in dairy farms worldwide that severely affect milk production and quality. Among the etiological agents, *Staphylococcus aureus* is considered a major cause of clinical mastitis and long-lasting infections in dairy cattle. Traditional therapeutic approaches based on antibiotics can leave residues in milk and contribute to the selection of antibiotic-resistant strains. Here we aimed to identify novel compounds that could reduce hemolysin production by *S. aureus*, an important virulence factor associated with mastitis severity. Thirty-three cultures of *Bacillus* spp. isolated from soil, plants, and water in different regions of Brazil were screened for compounds that attenuate the hemolytic activity of *S. aureus*. The bioactive molecules were purified and identified, and differences in gene expression and hemolysin activity were evaluated using the Mann–Whitney Wilcoxon test. Crude lipopeptide extracts obtained from different strains of *Bacillus* spp. inhibited *S. aureus* hemolytic activity by more than 90% at concentrations varying from 31.25 μg/mL to 250 μg/mL. Among the lipopeptides produced by *Bacillus* spp., the iturins showed the highest (up to 76%) anti-hemolytic activity. Oligomerization assays and SDS-PAGE analysis indicated that the lipopeptides were affecting hemolysin activity, not production. Although iturins did not prevent hemolysin oligomerization in vitro, molecular docking analysis revealed that these compounds could form hydrogen bonds with Glu665, an amino acid residue known to be important for hemolysin binding to the ADAM-10 receptor. Furthermore, an iturin variant that binds inside the hemolysin pore was identified, suggesting that it might block hemolysin activity. Our findings reveal for the first time the anti-hemolytic activity of lipopeptides and provide insights into their mechanism of action. This study demonstrates the potential application of lipopeptides as an anti-virulence therapy to prevent bovine mastitis caused by *S. aureus*.

Key Words: *S. aureus*, hemolysin, iturin

1322T Association between clinical mastitis and body condition score pattern and pregnancy at first artificial insemination.

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The objective was to investigate the association between the occurrence of clinical mastitis (CM) within 30 DIM, 60 DIM, and 7 d before and after the first artificial insemination (AI1), considering changes in body condition score within 21 DIM (CHBCS21), and the probability of pregnancy at AI1 (PAI1). This retrospective study included information from 12,042 lactations in 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy in Windsor, CO, USA. Cows were submitted to AI1 at about 80 DIM (primiparous) and 60 DIM (multiparous), following a double OvSynch protocol. Daily BCS were generated by video cameras (DeLaval International AB, Tumba, Sweden) mounted on the sorting gate at each exit (n = 2) of the milking parlor. Potential predictors of PAI1 included CM within 30 and 60 DIM, CM pre-AI1, and CM post-AI1. Data were analyzed by multivariable logistic regression and the models included change in BCS category (loss; no loss), calving season, parity, and average milk production at

Table 1 (Abstr. 1322T). Adjusted odds ratios (OR) of PAI1 for cows with or without CM by period of time and category of CHBCS21

	Loss in BCS		Maintained or gained BCS	
Variable[1]	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Mastitis ≤30 DIM	0.76 (0.62–0.92)	0.004	0.82 (0.51–1.30)	0.39
Mastitis ≤60 DIM	0.77 (0.68–0.88)	0.0001	0.76 (0.55–1.04)	0.09
Mastitis pre-AI	0.48 (0.31–0.74)	0.0009	0.56 (0.17–1.79)	0.36
Mastitis post-AI	0.38 (0.25–0.58)	<0.0001	0.24 (0.07–0.88)	0.032

[1]Absence of CM was considered as the reference category.

30 DIM as covariables. Overall, 4.53% and 9.77% of the lactations had a CM case within 30 and 60 DIM, respectively. In addition, 0.89% and 1% of the lactations had a CM case within 7 d before and after AI, respectively. Furthermore, 14.1% of the lactations maintained or gained BCS during the first 21 DIM. Mastitis ≤ 30 DIM, mastitis ≤ 60 DIM, mastitis pre-AI1, and mastitis post-AI1 were significantly associated with PAI1 in cows that lost BCS within 21 DIM (Table 1). The association between CM and PAI1 was dependent on the category of CHBCS21. The results highlight the relevance of BCS change on the reproductive efficiency of cows affected by disease.

Key Words: BCS, mastitis

1323T The effect of selective dry cow therapies based on two different algorithms on udder health and lactation performance in herds not using internal teat sealant. D. Paiva*, P. R. Menta, L. Bielamowicz, and V. S. Machado, *Texas Tech University, Lubbock, TX.*

The objective of this study was to evaluate the effect of selective dry cow therapy (SDCT) strategies based on 2 different algorithms on udder health, milk yield, and culling in herds not using internal teat sealant, comparing with blanket dry-cow therapy. Cows from 2 commercial farms in west Texas were randomized into 3 different groups: Algorithm 1 (ALG1; n = 455) cows treated with an intramammary antibiotic infusion at dry-off if SCC >200,000 cell/mL at any test date or had 2 or more cases of clinical mastitis during the current lactation; Algorithm 2 (ALG2; n = 458) cows treated with intramammary antibiotic infusion at dry-off if SCC >200,000 cell/mL at last test date or at least one case of clinical mastitis during the current lactation; Control cows (CON = 447) received blanket dry cow therapy. Milk yield and SCC linear score (LSCC) during the first 6 mo of lactation was analyzed using repeated measures ANOVA models, while Cox's proportional hazards models were fitted to culling and clinical mastitis data. Farm was fitted as random effect in all models, and data was analyzed in SAS. The percentage of cows receiving dry cow therapy was 100, 51.3, 24.7% for CON, ALG1, and ALG2, respectively ($P < 0.01$). The interaction term parity × treatment was retained in the model evaluating the effect of treatment on LSCC ($P = 0.07$). Among primiparous, LSCC was lower for CON cows in comparison to ALG1 ($P = 0.01$) and ALG2 ($P < 0.01$) counterparts. Treatment did not affect LSCC among multiparous cows ($P > 0.88$). Milk production was not different between ALG1 and ALG2 cows ($P = 0.99$), and ALG1 and CON cows ($P = 0.11$), but it tended to be lower for ALG2 cows in comparison to CON counterparts ($P = 0.08$). Treatment did not affect clinical mastitis incidence ($P = 0.37$) and culling ($P = 0.39$). In conclusion, although teat sealant was not used in this study, SDCT strategies did not lead to an increased incidence of clinical mastitis and culling, they led to increased LSCC in primiparous cows. Also, numerical decreases in milk production were observed in cows receiving selective dry cow therapy.

Key Words: selective dry cow therapy, mastitis, dairy cow

1324T Effects of feeding *Saccharomyces cerevisiae* fermentation product on milk oxylipids prior to and during an intramammary *S. uberis* challenge. Q. K. Kolar*[1], K. C. Krogstad[2], V. Mavangira[1], T. H. Swartz[2], I. Yoon[3], B. J. Bradford[2], and P. L. Ruegg[1], [1]*Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI,* [2]*Department of Animal Science, Michigan State University, East Lansing, MI,* [3]*Diamond V, Cedar Rapids, IA.*

Oxylipids are inflammatory modulators produced through enzymatic or non-enzymatic oxidation of polyunsaturated fatty acids (PUFA). Our objective was to determine if *Saccharomyces cerevisiae* fermentation product (SCFP) altered the oxylipid profiles during mastitis to promote a more robust and properly resolved inflammatory response. Lactating cows (n = 37; 180 ± 21 DIM; 42 ± 8.1 kg/d of milk) with no clinical mastitis in the previous 60 d were enrolled in a randomized complete block experiment. Cows received 19 g/d of corn (CON) or 19 g/d of SCFP for 97 d which were top-dressed after feeding. Milk samples were collected before treatment administration, after 45 d of treatment administration but before the mastitis challenge with 2,000 cfu of *S. uberis* 0140J, and 3 and 5 d after mastitis challenge. Oxylipids and PUFA were analyzed by LC-MS. Pre-challenge oxylipids were analyzed with a linear mixed model which included effects of treatment, parity, bovine leukemia virus status, pre-treatment oxylipid concentration (covariate), and 2-way interactions with treatment. Oxylipids after challenge were analyzed with a repeated measures mixed model. Temperature-humidity index (THI; 68 ± 3.2) and its interaction with treatment during mastitis challenge were also included in the models for post-challenge milk oxylipids. Cow and cohort were random effects. The SCFP interacted with pretreatment PUFA; cows supplemented with SCFP tended to maintain PUFA concentrations compared with CON (interaction, $P = 0.08$). Mastitis challenge increased milk omega-3 concentrations ($P = 0.02$). After challenge, treatment interacted with THI such that cows receiving SCFP had increased 12,13-EpOME, 8, 9-DHET, and 9-oxoODE as THI increased ($P < 0.05$), and 17-HDoHE tended to be reduced in CON cows ($P < 0.10$) as THI increased. The SCFP tended to increase thromboxane 2 before challenge ($P = 0.07$), but not after challenge. These data suggest that SCFP altered milk PUFA and downstream oxylipid concentrations, indicating that SCFP may alter mammary PUFA metabolism and the local inflammatory response during mastitis.

Key Words: mastitis, inflammation, postbiotic

1325T Antimicrobial peptides as therapeutic alternatives against bovine mastitis pathogens. A. J. Moreira[1,2], A. Assumpcao[1], K. Camargo[1,3], K. Araujo[2], N. Aulik[1], and H. Mantovani*[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Universidade Federal de Vicosa, Vicosa, MG, Brazil,* [3]*Universidade Estadual Paulista, Jaboticabal, SP, Brazil.*

Bovine mastitis is the costliest disease in the dairy sector and represents the main cause of antibiotic use on dairy farms. This work aimed to evaluate the use of antimicrobial peptides (AMPs) derived from the rumen and human microbiomes as well as chemical adjuvants (CA) in antibiotic-free formulations to control mastitis pathogens. A panel of major mastitis pathogens (n = 35) was obtained from the Wisconsin Veterinary Diagnostic Laboratory (Madison, WI) and used as targets in inhibition assays. The etiological agents included bacteria associated with both clinical and environmental mastitis such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus chromogenes*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*. Minimum inhibitory concentrations (MIC) were determined using the microdilution method with cultures (5×10^5 cfu/mL) grown in cation-adjusted Mueller-Hinton broth. The highest concentration of AMPs was 128 µg/mL and less than 2.7 mg/mL for the CAs to avoid solubility and turbidity problems. Plates were incubated at 37°C for 24 h, and the optical density at 600 nm (OD_{600}) was monitored to determine the MIC_{90}. Statistical analyses were performed using R version 4.2.2. Except for PEP43, all treatments caused a significant decrease in final OD_{600} values at the highest concentration tested. PEP1 and CA1 were the 2 most effective treatments ($P < 0.05$) against the panel of bacterial strains tested in the current work. On average, PEP1 decreased the final OD_{600} of the bacterial cultures by 95%

and CA1 by 86.8%. PEP2 showed high efficacy against gram-negative bacteria and the average reduction in the final OD_{600} was 81%. PEP60 and CA2 decreased biomass formation by approximately 40%. These findings indicate that PEP1, PEP2, and CA1 are potential candidates for developing antibiotic-free formulations against mastitis pathogens. Further experiments will focus on evaluating the toxicity of the selected molecules and their combined use against mastitis pathogens.

Key Words: inhibition, antimicrobial peptide, adjuvant

1326T Effects of rumen-protected niacin on inflammatory response to repeated intramammary lipopolysaccharide challenges. K. C. Krogstad, J. Fehn*, and B. J. Bradford, *Department of Animal Science, Michigan State University, East Lansing, MI.*

Specific nutrients may improve an animal's resilience to health or environmental challenges. Niacin has reduced inflammation in some species, but this has not been investigated in dairy cattle. Twenty lactating cows (232 ± 65 DIM; 39 ± 5.8 kg/d of milk) with no clinical mastitis at the time were enrolled in a randomized complete block experiment which lasted 70 d. Cows received 26 g/d of rumen-protected niacin (RPN) or no additional top-dress (CON) for the first 42 d of the experiment. On d 28 and 56, cows were challenged in their rear-right quarter (RR) with 100 µg of lipopolysaccharide (LPS). Milk yield, milk conductivity, and feed intake were measured daily, and milk composition was measured on d 14, 23, 24, 30, 37, 45, and 52. Blood samples were collected 8, 12, 24, and 48 h after each LPS challenge. Quarter level (RR) milk samples were collected at 0, 8, 16, 24, 48, 72, 96, 120, 144, and 168 h after each LPS challenge. Body temperature was measured continuously for 24 h after challenge with vaginal thermometers. Data were analyzed with linear mixed repeated measure models which included fixed effects of treatment, day, and their interaction, as well as parity; cohort, block, and cow were random effects. Before LPS challenge, RPN did not affect feed intake or milk production, but it reduced SCS (1.24 ± 0.41 vs. 0.05 ± 0.45; $P < 0.01$). After each LPS challenge, RPN did not affect feed intake, milk production, milk composition, body temperature, or plasma glucose concentrations. Both LPS challenges resulted in similar body temperature 24-h area under the curve (528 ± 49 vs. 512 ± 56°C × min, $P = 0.81$) The 2nd LPS challenge induced a greater peak SCS response than the first LPS challenge ($P < 0.01$). During the 1st challenge, primiparous cows had reduced plasma glucose at 12 h post-challenge compared with multiparous cows ($P < 0.01$); after the 2nd challenge, parity did not affect blood glucose ($P > 0.78$). Repeated LPS challenges did not promote tolerance among measured outcomes in late-lactation dairy cows. The RPN reduced SCS before challenge but it did not affect the response to repeated intramammary LPS challenges.

Key Words: mastitis, inflammation

1327T Renal metabonomic changes of Saanen goats exposed to different doses of aflatoxin B_1. D. Su, J. Peng, X. Bai, X. Wang, and H. Shi*, *Southwest Minzu University, Chengdu, Sichuan, China.*

Aflatoxin B_1 (AFB_1) widely exists in numerous moldy and deteriorated feed and food crops and has been considered a potent hepatotoxin. In addition to the liver, the kidney is also a major target organ of AFB_1 and its nephrotoxicity in ruminants deserves further evaluation. This study evaluated the effects of AFB_1 on the renal metabolic changes of Saanen goats using untargeted metabolomics strategies. Eighteen Saanen goats were randomly divided into 1 of the 3 treatments: control group (C; basal diet), low-AFB_1 group (L; 50 µg/kg AFB_1), and high-AFB_1 group (H; 500 µg/kg AFB_1). After the 14-d challenge period, the goats were slaughtered and kidney samples were collected for LC-MS/MS metabonomic analysis. Multivariate statistical analyses were performed to visualize differences among groups. A total of 3,214 and 3,150 metabolites were obtained in positive and negative ion modes, respectively. The organ weight/body weight ratio of the kidney tended to increase ($P = 0.067$) with increasing AFB_1 levels. Based on the screening criteria, 776 differential metabolites were detected between C and L. Leukotriene D4 and anandamide were the principal metabolites affected and the asthma, retrograde endocannabinoid signaling, and central carbon metabolism in cancer were the most impacted pathways. There were 638 differed metabolites between C and H. The representative metabolites including leukotriene C4 and volicitin, which were enriched in asthma, choline metabolism in cancer and α-linolenic acid metabolism pathways. Moreover, 26 metabolites including L-glutamate, glycocholate, and L-serine, were differed among all groups. The major pathways enriched were 2-oxocarboxylic acid metabolism and monobactam biosynthesis. In conclusion, alterations in renal metabolome after AFB_1 exposure were successfully revealed by untargeted metabolomics and the underlying causes of the changes deserve further research.

Key Words: ruminant aflatoxicosis, renal metabolomics, metabolic pathway

1328T The cytokine profile following an endotoxin challenge in early versus mid-lactation dairy cows. J. Opgenorth*, E. J. Mayorga, M. A. Abeyta, B. M. Goetz, S. Rodriguez-Jimenez, A. D. Freestone, J. L. McGill, and L. H. Baumgard, *Iowa State University, Ames, IA.*

Our previous data indicated that early lactation cows have a more intense immune response (measured by rectal temperature, acute phase proteins, and neutrophilia) than cows in mid lactation. Thus, objectives were to evaluate the cytokine profile of multiparous cows in early (EL; 20 ± 2 DIM; n = 6) and mid (ML; 144 ± 22 DIM; n = 6) lactation following an endotoxin challenge. Cows were intravenously administered 0.09 µg lipopolysaccharide (LPS) from *Escherichia coli* O55:B5/kg body weight. Serum was harvested from blood collected at 0, 3, and 6 h relative to LPS infusion where the 0 h sample was utilized as a baseline measurement. Interferon (IFN)-γ, interleukin (IL)-6, IL-8, IL-10, IL-36, macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF)-α were analyzed utilizing the MILLIPLEX bovine cytokine/chemokine 15-plex kit (BCYT1–33K-PXBK15; EMD Millipore Corporation). Data were analyzed with PROC MIXED in SAS 9.4. Following LPS, concentrations of IFN-γ increased (10-fold) whereas IL-8 decreased (54%) similarly in all cows relative to baseline ($P < 0.01$). Additionally, IL-6, MIP-1α, MCP-1, and TNF-α concentrations increased following LPS and these were exacerbated in EL relative to ML (4.8-, 1.9-, 1.6-, and 6.3-fold, respectively; $P \leq 0.01$). Further, EL had increased IL-10 relative to ML at 6 h (2.3-fold; $P = 0.05$). Concentrations of MIP-1β tended to increase in EL relative to ML at 3 h (2.8-fold; $P = 0.07$), and IL-36 tended to decrease in EL relative to ML at 6 h (34%; $P = 0.06$). Circulating VEGF-α concentrations did not differ by lactation stage ($P > 0.35$) but did increase from 3 to 6 h after LPS administration ($P = 0.05$). In summary, cytokine concentrations were markedly altered by LPS administration in both EL and ML cows, but EL cows appear to have had a more robust inflammatory response. This data set agrees with our previous evidence that EL cows are more sensitive to intravenously administered LPS and suggests that EL cows do not have a suppressed—but rather exacerbated—antigen response relative to ML cows.

Key Words: inflammation, lactation stage

1329T Significantly lower milk production among intensively managed dairy cows with subclinical *Theileria orientalis* infection. H. Espiritu*[1], H. Lee[2], S. Jin[1], M. D. Aftabuzzaman[1], E. J. Valete[1], J. Pioquinto[1], Sangsuk Lee[1], and Y. Cho[1], [1]*Sunchon National University, Sunchon-si, Jeollanam-do, South Korea,* [2]*Mari Animal Medical Center, Yongin-si, Gyeonggi-do, South Korea.*

Theileria orientalis is the major tick-borne parasite affecting cattle in many regions of East and Southeast Asia, Asia-Pacific, and even the United States. In Korea, the vast majority of reports indicate that oriental theileriosis primarily results in asymptomatic to moderate clinical disease; however, there is limited data on productivity losses caused by subclinical *T. orientalis* infection. Therefore, this study assessed the impact of subclinical *T. orientalis* on the average daily milk production (ADMP) with regard to the lactation period and parity of intensively managed dairy cows in Korea. Blood samples were taken from 163 clinically healthy Holstein-Friesian cows from 19 non-grazing herds and milk data were provided by the Dairy Cattle Improvement Center (DCIC). Lactation stages were classified as early, mid, and late lactation. Parity was grouped as first to ≥fifth parity. Molecular detection by PCR was done for *Anaplasma* spp., *Babesia* spp., and *T. orientalis*. Red blood cell (RBC) was measured using an automated hematology analyzer. The statistical difference for these parameters between infected and noninfected was measured using *t*-test. *Theileria orientalis* affected 32.52% of the observed population, while *Anaplasma* and *Babesia* were not detected. The RBC of the majority of animals was within the normal range (infected: 95%; noninfected: 100%); however, statistical comparison of means of the RBC between groups showed the infected to be significantly lower. The ADMP of the infected group which measured 25.63 L/d was significantly lower than that of the noninfected group (34.53 L/d). Infected cows in early and mid lactation exhibited significantly lower ADMP than noninfected animals. As compared with noninfected cows, animals on the first to third parity also displayed significantly lower ADMP. This is the first study to show that subclinical *T. orientalis* infection in cows might cause decreased ADMP, which may be brought on by the interplay of the infection with physiological and environmental stresses. As it is uncommon to diagnose and cure asymptomatic cows, the economic impact of these losses could be significant for the dairy industry.

Key Words: dairy cattle, *Theileria orientalis*, milk production

1330T Effects of β-caryophyllene on bovine monocyte-derived macrophage proteome and phosphoproteome in response to lipopolysaccharide challenges *in vitro*. K. C. Krogstad[1], L. K. Mamedova*[1], G. Kra[2,3], Y. Levin[4], M. Zachut[2], and B. J. Bradford[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Department of Ruminant Science, Institute of Animal Sciences, Agriculture Research Organization Volcani Institute, Rishon LeZion, Israel,* [3]*Department of Animal Science, the Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot, Israel,* [4]*The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel.*

The endocannabinoid system regulates immune responses. The cannabinoid receptor 2 (CB2) is expressed in immune cells and has anti-inflammatory effects. We investigated the effects of a CB2 ligand, β-caryophyllene (BCP), on the response of bovine monocyte-derived macrophages to 2 lipopolysaccharide (LPS) challenges. Blood was collected from 5 lactating Holsteins (175 ± 37 DIM). Peripheral blood mononuclear cells (PBMC) were collected using the Ficoll-Paque centrifugation technique and seeded at 1×10^6 cells/mL. After 2 h to allow adherence of monocytes, remaining cells and supernatant were removed. Cells were incubated with or without 1 µ*M* BCP for 6 d. On d 6, cells were challenged with 0.1 µg/mL of LPS and 24 h later the cells were challenged again; 30 min after the 2nd LPS challenge, cells were collected and protein lysates were extracted for proteomic and phosphoproteomic analysis (LC-MS/MS and Fe immobilized metal ion affinity chromatography enrichment of phospho-peptides; Weizmann Institute, Israel). Ingenuity Pathway Analysis was conducted to determine enriched pathways affected by treatment. In total, 650 proteins and 1,406 phosphosites were identified; 10 proteins and 22 phosphopeptides were affected ($P \leq 0.05$ and fold change [FC] ± 1.5) or tended ($P < 0.1$) to be affected by treatment. Proteomics revealed that BCP reduced tyrosine phosphatase ($P = 0.01$; FC = −2.7 ± 1.2 [SED]), latexin ($P = 0.05$; FC = −3.9 ± 1.6), and secreted phosphoprotein 24 ($P = 0.10$; FC = −2.0 ± 1.4). Calpain-1 catalytic subunit (FC = 3.2 ± 1.4) and fatty acid binding protein (FC = 2.2 ± 1.4) tended to increase in BCP vs. control (CTL; $P < 0.07$). A tendency for lower phosphorylation of the phosphopeptides in secreted phosphoprotein 1 (FC = −6.3 ± 2.1) and Serine/Threonine Kinase 10 (FC = −1.8 ± 1.3) was observed in BCP-macrophages compared with CTL ($P < 0.07$). Top canonical pathways according to phosphoproteomics were apoptosis signaling and integrin signaling which were enriched in BCP vs. CTL. The BCP may reduce immune cell migration which may benefit dairy cows experiencing inflammation.

Key Words: proteomics, phosphoproteomics, cannabinoid

1331T A multivalent vaccine for mastitis and Johne's disease in dairy cattle. D. F. Díaz Herrera*[1], C. Ster[2], P. Lacasse[2], and F. Malouin[1], [1]*Universite de Sherbrooke, Biologie, Sciences., Sherbrooke, QC, Canada,* [2]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Staphylococcus aureus (SA), is one of the main causes of mastitis, and *Mycobacterium avium* ssp. *paratuberculosis* (MAP) causes Johne's disease. Both pathogens are challenging to control. In the past, we designed a SA vaccine that was evaluated with an experimental challenge in dairy cows (Ster, 2021). The aims of this study were to create a SA-MAP vaccine for mastitis and paratuberculosis and to design a vaccination schedule for optimum protection at the beginning of lactation. Our vaccine contained 3 SA proteins (SA1, SA2, SA3) and one MAP recombinant polypeptide. Ten Holstein cows (1.9 ± 1.0 lactations) received 2 vaccine injections, 10 weeks apart, with the first dose about 6 weeks before dry off and the second dose 4 weeks before predicted calving (W0). Pre-immune and immune sera, milk, and colostrum were collected, and total IgG, IgG_1, and IgG_2 titers against each antigen were measured by ELISA. Efficacy of vaccination was evaluated by comparing the pre-immune data to data following the second immunization (*t*-test or ANOVA, no control group). Serum total IgG responses against SA1–3 were higher at calving (W0) vs. pre-immune serum ($P < 0.0001$). SA1 had higher antibody titers ($P < 0.0001$) up to 8 weeks postpartum (W8). The antibody titers were also comparable to those previously obtained using a monovalent SA vaccine, which only contained the 3 SA proteins and that was administered following the same timeline but during lactation, denoting that the MAP protein and the vaccination schedule had no adverse effect on the cow's immune response to SA. For all antigens, a predominant Th1 response (IgG_2/IgG_1 ratio > 1) was observed in serum samples at calving. On the opposite, the response in colostrum displayed a Th2 pattern (IgG_2/IgG_1 ratio <1). Anti-MAP antibodies in colostrum reached a very high titer ($>6.2\ \log_{10}$) and antibody levels were significantly higher than those found in serum at calving ($P < 0.01$). The vaccine yielded a great immune response at the start of lactation. We can infer that in this period of vulnerability, the cows could be better protected against SA mastitis, and could provide passive immunization to calves against MAP.

Key Words: intramammary infection, mastitis, paratuberculosis

1332T Evaluating tart cherry pit extracts for antioxidant and anti-inflammatory function as a feed additive using RAW 264.7 cells. H. L. Reisinger*[1], L. K. Mamedova[1], K. C. Krogstad[1], E. C. Alocilja[2], B. Aliakbarian[3], and B. J. Bradford[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, [3]The Axia Institute, Michigan State University, East Lansing, MI.*

Tart cherry pits are a byproduct of cherry processing with abundant polyphenols that have antioxidant and anti-inflammatory properties. Incorporating pit extracts into animal feed may reduce food waste and support animal health. We aimed to evaluate the antioxidant and anti-inflammatory potential of extracts in an in vitro model. Two extracts were studied; one utilizing water (CPW) and the second utilizing 50:50 water:ethanol (CPE) as solvents. Chemical composition (GC/MS) and antioxidant function were assessed. Anti-inflammatory effects of CPW and CPE were assessed using RAW 264.7 murine macrophages transfected with a vector driving alkaline phosphatase (AP) expression on activation of the inflammatory transcription factor, nuclear factor κ-light-chain-enhancer of activated B cells. Cells were treated with extracts (0.061%, 0.125%, 0.25%, 0.5%, and 1% [wt/vol] concentrations in cell culture media) with or without lipopolysaccharide (LPS, 100 ng/mL) stimulation, and metabolic activity, measured by resazurin metabolism, and AP activity were measured. Data were analyzed by mixed models with treatment as a fixed effect and plate as a random effect. Total phenolic content and Trolox-equivalent antioxidant capacity of CPW and CPE samples were 7.20 ± 0.25 and 15.36 ± 1.88 mg gallic acid equivalents and 7.47 ± 0.47 and 17.93 ± 0.03 mg/g DM, respectively. Cells treated with 0.25%, 0.5%, and 1% CPE showed reduced metabolic activity compared with untreated cells, potentially indicating impaired cell viability. In contrast, cells treated with CPW at concentrations of 0.25%, 0.5%, and 1% had increased metabolic activity compared with controls ($P < 0.05$). Treatment with CPW reduced LPS-stimulated AP activity by 15–17 ± 3.5% at 0.25% and 0.5% concentrations, and those treated with CPE reduced LPS-stimulated AP activity by 18–22 ± 3.8% at all concentrations. For comparison, 1% red and white grape pomace reduced LPS-stimulated AP activity by 15 and 19 ± 5%, respectively ($P < 0.05$). The anti-inflammatory and antioxidant capacity of tart cherry pit extracts point to potential use as a feed additive to improve animal health.

Key Words: polyphenol, inflammation, macrophage

1333T Integrated metabolomics and lipidomics analysis reveals lipid metabolic disorder in NCM460 cells caused by aflatoxin B_1 and aflatoxin M_1 alone and in combination. X. Yang[1,2], Y. N. Gao[1,2], J. Q. Wang[1,2], and N. Zheng*[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China, [2]Key Laboratory of Quality and Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Aflatoxin B_1 (AFB1) and aflatoxin M_1 (AFM1) are universally found as environmental pollutants. AFB1 and AFM1 are group 1 human carcinogens; previous sufficient toxicological data show that they posed health risks in humans and animals. The intestine is vital for resistance to foreign pollutants. The enterotoxic mechanism of AFB1 and AFM1 have not been clarified at the metabolism levels. In the present study, the toxic effects of 2.5 μ*M* AFB1 and AFM1 were determined by comprehensive metabolomics and lipidomics analyses on NCM460 cell. Combination of AFB1 and AFM1 treatment induced more extensive metabolic disturbances on NCM460 cells than either aflatoxin alone, AFB1 exerted a greater effect in the combination group. Metabolomic pathway analysis showed that glycerophospholipid metabolism, fatty acid degradation, and propanoate metabolism were dominant pathways that were interfered by AFB1, AFM1, and AFB1+AFM1, respectively. It can infer that lipid metabolism played a critical role in AFB1/AFM1-induced enterotoxicity. Those results suggest that attention should be paid to lipid metabolism after AFB1 and AFM1 exposure. Further, the lipidomics was used to explore the fluctuation of AFB1 and AFM1 in lipid metabolism. The 34 specific lipids that were differentially induced by AFB1 mainly attributed to 14 species, in which cardiolipin (CL) and triacylglycerol (TAG) accounted for 41%. AFM1 mainly affected CL and phosphatidylglycerol, approximately 70% of 11 specific lipids, while 30 specific lipids were found in AFB1+AFM1, mainly reflected in TAG up to 77%. This research found for the first time that the lipid metabolism disorder caused by AFB1 and AFM1 was one of the main causes contributed to the enterotoxicity, which could provide new insights into the toxic mechanisms of AFB1 and AFM1 for animals and humans.

Key Words: aflatoxin B_1, aflatoxin M_1, enterotoxicity

1334T Peripheral blood mononuclear cell culture for Treg cell isolation in dairy cows. Q. Jiang* and J. J. Loor, *Department of Animal Sciences, University of Illinois, Urbana, IL.*

The isolation and culture of T regulatory (Treg) cells, producing the anti-inflammatory cytokine IL-10, can help us understand the inflammatory response around parturition in dairy cows. Before fluorescence-activated cell sorting (FACS) to isolate Treg, there is a need to optimize the marker selection via routine flow cytometry and protocols for peripheral blood mononuclear cell (PBMC) preservation and the proliferation of Treg cells. Thus, our objective was to increase the percentage of Treg cells in isolated PBMC from dairy cows through the stimulation in culture with CD3 and CD28 antibodies plus IL-2. Whole blood was collected in EDTA Vacutainers from 4 clinically healthy multiparous lactating cows (115.5 ± 8.5 d in milk). The PBMC were isolated by Histopaque and Red Blood Cells Lysis Buffer and stored in liquid nitrogen. Then, PBMC were thawed and separated into 3 groups. The first group (negative control, NC) was prepared for routine flow cytometry after thawing. The second and third groups of cells were cultured in different media for 24 h in 6-well plates: a positive control (PC) and a treatment group (TRT). The PBMC in PC were cultured in RPMI Medium (SLM-240, Sigma). The PBMC in TRT were cultured in the RPMI media with 2 μg/mL CD28 antibody, 2 μg/mL CD3 antibody, and 10 ng/mL IL-2. All PBMC were stained with CD4-FITC, CD25-Alexa Fluor 647, and Fixable Viability Dye and run on an Attune NxT flow cytometer (Thermo Fisher Scientific). Data were analyzed using FlowJo software (v10.7.2) and SAS (OnDemand for Academics). The model included the fixed effect of group and the random effect of cow. In addition, the preplanned CONTRAST *P*-values for the comparisons of NC vs. PC and PC vs. TRT were obtained. The percentage of live cells in NC was greater than PC and TRT ($P < 0.05$). The percentage of $CD4^+CD25^+$(Treg) cells in living PBMC cells in PC tended to be lower than NC ($P = 0.10$). However, this percentage tended to increase ($P = 0.13$) from 2.11%, 0.26%, 0.21%, and 0.32% in PC to 7.07%, 0.42%, 1.56%, and 1.11%

in TRT, respectively. Ongoing research is examining if longer culture times help boost Treg cell numbers in PBMC.

Key Words: flow cytometry, regulatory T cell, lactation

1335T Withdrawn.

1336T Antimicrobial activity of a bacterial secondary metabolite against pathogens impacting animal health. T. B. Gupta*, K. Subharat, S. K. Gupta, J. Singh, and G. Brightwell, *AgResearch Ltd., Palmerston North, New Zealand.*

There has been an increased demand for natural antimicrobial compounds that can be used in pharmaceuticals, for food preservation as well as enhancing animal health. Previously, we demonstrated that an anaerobic spore forming bacteria produced a secondary metabolite possessing antimicrobial properties against several spoilage and pathogenic bacteria. In this study, the efficacy of this metabolite was tested against *S.* Bovismorbificans and *S.* Give, which are zoonotic bacteria that cause salmonellosis in animals and can also be transferred to humans as well as against the fungus *Pithomyces chartarum,* which causes facial eczema in animals. We are unable to disclose the name of the metabolite due to IP and privacy reasons and hence is designated as HA1. HA1 was commercially procured and used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the selected bacteria and fungus using the microplate turbidimetric assay. A final bacterial inoculum of 10^5 cfu/mL and 200 fungal spores were used for the experiments. The microtiter plate containing pathogens and different concentrations of HA1 was incubated in a spectrophotometer at 35°C for bacteria, for 24 h or 25°C for fungus, for 5 d and growth was determined by measuring the optical density at 600 nm. MIC was the lowest concentration of HA1 at which there was no bacterial or fungal growth. The MBC was assessed for only bacteria, by removing the suspension from each well after 24 h assay and subculturing them on sheep blood agar (SBA) plates. The concentration at which no bacterial growth was obtained following incubation was recorded as the MBC. The results showed that HA1 was highly effective (ANOVA, P-value < 0.05) in inhibiting the growth *S.* Bovismorbificans and *S.* Give at the concentration ranging between 0.9 and 32 mg/mL and it also inhibited the germination of *P. chartarum* spores at the concentration ranging between 4.0 and 10 mg/mL. The findings from the study suggest that the secondary metabolite HA1 has the potential to be used as an antibacterial and antifungal agent in improving animal health and wellbeing.

Key Words: salmonellosis, facial eczema

1337T Effect of systemic ceftiofur on cows diagnosed with metritis and classified as high risk for spontaneous cure using a predictive model with farm variables on metritis cure, reproduction, culling, and milk yield. P. R. Menta*[1], E. B. Oliveira[3], J. G. Prim[2], K. N. Galvao[2,4], F. S. Lima[3], M. A. Ballou[1], N. R. Noyes[5], and V. S. Machado[1], *[1]Department of Veterinary Sciences, Texas Tech University, Lubbock, TX, [2]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, [3]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, [4]D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL, [5]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.*

Our objective was to compare the effect of ceftiofur treatment on metritic cows classified as high risk for spontaneous cure using an algorithm including farm variables on metritis cure, reproduction, culling and milk yield. A total of 412 cows diagnosed with metritis from 4 different farms located in TX, CA, and FL were enrolled in a randomized clinical trial. Cows were examined for metritis using the Metricheck device. Metritis was defined as the presence of red-brownish, watery, and fetid vaginal discharge, while cure was defined as the absence of metritis 14 d after initial diagnosis. Cows diagnosed with metritis were randomly allocated to receive ceftiofur (CEF) or to remain untreated (CON). An algorithm for spontaneous cure was created using data from CON cows (dystocia, retained placenta, twin, calf sex, stillbirth, DIM, and rectal temperature at metritis diagnosis), with sensitivity and specificity of 61.1 and 66.1, respectively. Only rectal temperature and days in milk at diagnosis were retained in the final algorithm. A total of 237 cows (CON = 127 and CEF = 110) were classified as high risk of spontaneous cure and included in the final analysis. Cure risk at 14 d after diagnosis, reproduction, culling, and milk yield were evaluated until 300 DIM. Data were analyzed in SAS using GLIMMIX, MIXED, and PRHEG procedures. Cure risk was 85% and 74% for CEF and CON, respectively. Cows assigned to CEF had 1.95 increased odds of cure (95% CI = 0.91–4.19; P = 0.08). No effect of CEF on milk yield up 300 DIM was observed (CEF = 39.7

kg/d; CON = 39.9 kg/d; $P = 0.79$). Ceftiofur therapy did not affect hazard of pregnancy ($P = 0.46$) and culling ($P = 0.86$). In conclusion, ceftiofur treated cows classified as high risk of spontaneous cure tended to have increased cure rate but overall benefits to productivity and longevity were not observed. This data suggests that this algorithm can be used to reduce the use of antibiotics for the treatment of metritis in dairy cows.

Key Words: predictive model, metritis, cure

1338T Integrating genotypic and phenotypic characterization with *in vivo* efficacy for probiotic therapy of endometritis in bovine.

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Introduction: Endometritis in bovines can result in reduced fertility, extended calving interval, decreased milk production, and early culling. The threat of AMR highlights the need for alternative treatments. Probiotics offer a potential solution by restoring normal microorganism balance, reducing inflammation, and promoting healing without the risk of AMR. The use of probiotics can improve the health and fertility of dairy cattle, reduce antibiotic resistance, and support the sustainability of the dairy industry. Objectives: To reduce the spread of antibiotic-resistant bacteria and support reproductive health of the bovine population. Method: The study involved a total of 34 healthy cows and 17 buffaloes. Healthy vaginal swabs (n = 50) were taken to analyze microbial diversity, and probiotic bacteria were screened and identified. Experiments were conducted to assess the phenotypic probiotic characteristics of the identified bacteria. Whole genome sequencing was performed on 2 probiotics, *L. plantarum* (LP) and *P. pentoseus* (PP). In a follow-up in vivo efficacy study, approximately 100 animals with clinical endometritis were selected and randomly allocated into 3 groups. Two groups, LP (n = 53) and PP (n = 45), received individual probiotics, while the control group (n = 63) received normal saline (NS). The response rate of estrus within 21 d was monitored and recorded. The discharge score on the day of estrus was recorded and AI/natural service was performed. The pregnancy diagnosis was performed 45 d after AI/natural service. Results: The study found that the identified probiotics possess the necessary probiotic characteristics phenotypically and genotypically too and are effective in fighting infections. Results showed a significant improvement in endometritis symptoms in buffalo when probiotics were administered (LP = 50% estrus induction; PP = 42% estrus induction) compared with control group. The in vivo study revealed that *L. plantarum* produced a stronger response compared with *P. pentoseus*. Conclusion: This finding indicates that these probiotics can effectively enhance the fertility and milk output of dairy buffalo. Further investigation is necessary to establish the optimal dosage and combination of probiotics that can effectively improve the reproductive health of bovines.

Key Words: endometritis, probiotics, AMR

1339T Withdrawn.

1340T Effects of heat stress and dietary organic acid and pure botanical on hepatic transcriptome.

V. Sáinz de la Maza-Escolà*[1,2], G. Li[1], F. Ghiseli[2], X. V. Yu[1], A. B. P. Fontoura[1], A. Javaid[1], N. S. Salandy[1], S. L. Fubini[1], J. E. Duan[1], E. Grilli[2,4], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy,* [3]*Tuskegee University, Tuskegee, AL,* [4]*Vetagro S.p.A, Reggio Emilia, Italy.*

Our objective was to evaluate the effects of heat stress (HS) and dietary organic acid and pure botanical (OA/PB) supplementation on measures of hepatic inflammation and oxidative stress. Following a 7 d acclimation in thermoneutrality (temperature-humidity index [THI] 68), 46 Holstein cows were randomly assigned to 1 of 4 groups: thermoneutral conditions (TN-Con, n = 12), HS conditions (HS-Con, n = 12; diurnal THI 74 to 82), TN conditions pair-fed to match HS-Con (TN-PF, n = 12), or HS fed OA/PB (HS-OAPB, n = 10; 75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlus R; Vetagro S.p.A) for 14 d. Plasma was collected on d −1, 3, 7, and 14 and analyzed for serum-amyloid A (SAA) and reduced glutathione (GSH) concentrations. Liver was biopsied on d 12 and analyzed by transcriptomics (RNA-Seq). Data were analyzed using a mixed model including random effect of cow, and the fixed effects of treatment, time, and their interaction. Planned contrasts included HS-Con vs. TN-Con, HS-Con vs. TN-PF, and HS-Con vs. HS-OAPB. HS-Con had greater concentrations in plasma SAA ($P < 0.01$) and GSH ($P < 0.08$) concentrations relative to TN-Con, TN-PF, or HS-OAPB. Hepatic expression of genes related to inflammatory signaling (MYD88, SAA3, and NFKb1), were increased in HS-Con relative to TN-Con and TN-PF ($P < 0.01$) and HS-OAPB ($P < 0.12$). HS-OAPB tended to downregulate the initial enzyme in the transulfuration pathway (CBS; $P = 0.06$), while upregulated glutathione peroxidase 1 (GPX1; $P = 0.01$), relative to HS-Con. HS-Con overexpressed the taurine pathway (CDO1), relative to all treatments ($P < 0.05$). Regarding transmethylation pathway genes; AHCY and BHMT were upregulated in HS-Con compared with TN-Con and TN-PF ($P < 0.01$) and HS-OAPB ($P < 0.07$), while CHDH and MAT1A were downregulated in TN-PF compared with HS-Con ($P < 0.01$). Phosphatydilcholine degradation through GDPD5 was downregulated in HS-Con compared with all treatments ($P < 0.04$). Observed alterations at gene level may explain HS detrimental effects and suggest OA/PB supplementation as a means to improve hepatic health and metabolism.

Key Words: heat stress, liver, transcriptome

1341T Barn air particles as a pro-inflammatory predisposing factor for bovine respiratory disease.

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Poor air quality increases the occurrence of respiratory disease in dairy and veal calves. It was previously considered that ammonia interferes with innate immunity in the respiratory tract, but newer evidence suggests proinflammatory effects of dust particulates may play a role in BRD. The objectives were to describe changes in air quality parameters over time in a veal barn, and to determine effects of barn air particulates on in vitro pro-inflammatory responses of bovine monocyte-derived macrophages. The concentrations of PM 5, 2.5, 1, and 0.3 particles (measured with a particle counter), ammonia, CO_2, and relative humidity were measured weekly in a veal barn for 5 mo. Changes in these parameters were described over time. Particulate matter was sampled from barn air and used in vitro to stimulate interleukin-1β production (measured by ELISA) by bovine monocyte-derived macrophages, determining effects for different particle sizes ($PM_{0.5–1}$ and $PM_{1–3}$) and different times of exposure. $PM_{1–3}$ and $PM_{0.5–1}$ particles induced monocyte-derived macrophages to secrete IL-1β after 6, 12, or 24 h of exposure. Dose-dependent stimulation of IL-1β secretion were detected for both particle sizes, which were significant for $PM_{0.5–1}$ particles ($P = 0.046$) but not for $PM_{1–3}$ ($P = 0.864$) (1-way ANOVA, Prism). The data showed that fine particulates in barn air stimulate inflammatory responses in bovine macrophages, which might affect how these cells react to subsequent bacterial infection. These findings may be relevant to how barn air predisposes to bacterial pneumonia and may suggest improved methods to control respiratory disease in calf barns.

Key Words: air particle, respiratory disease, bovine

1342T Withdrawn.

Breeding and Genetics 2: Genetics of Health

1343T A comprehensive characterization of longevity and culling reasons in Holstein cattle. T. Souza*[1], L. F. Pinto[1], V. Cruz[1], H. Oliveira[2,3], V. Pedrosa[3,4], G. Oliveira Junior[2], F. Miglior[2,5], F. Schenkel[2], and L. F. Brito[2,3], *[1]Federal University of Bahia, Salvador, Bahia, Brazil, [2]University of Guelph, Guelph, Ontario, Canada, [3]Purdue University, West Lafayette, IN, [4]State University of Ponta Grossa, Ponta Grossa, Parana, Brazil, [5]Lactanet Canada, Guelph, Ontario, Canada.*

Culling directly impacts the profitability of dairy farms. Understanding the primary causes of culling of cows will improve management and selection goals in dairy cattle breeding programs to avoid early culling. This study aimed to analyze the temporal frequencies of 34 culling reasons in Canadian Holstein cows. After data editing and quality control, records from 3,096,872 cows culled from 9,683 herds across Canada were used in the analyses. Reproductive issues were the main culling reason accounting for 23.0%, followed by milk production (20.8%), health (20.4%), conformation problems (13.7%), economic factors (13.1%), accidents (5.7%), age-related issues (1.7%), and workability (1.6%). Reproduction issues were the highest reason for culling in fall (24.5%), winter (24.0%), and spring (22.5%), while health issues were the most frequent (22.5%) culling reason in the summer period. Health issues (25.5%) and milk production (27.7%) were the most frequent culling reasons in Quebec and Ontario, respectively. Reproductive issues showed the highest frequency across climates, except for warm-summer Mediterranean climate and tropical and subtropical steppe climate, where production issues showed the highest culling rate (29.4% and 21.6%, respectively). Reproductive and production performance issues were the main culling reasons in most ecozones, except in Boreal Shield and Atlantic Marine, where health issues had the highest frequencies (25.1 and 23.7%, respectively). These results can contribute to improving management practices and selection decisions to reduce early culling of Holstein cows in Canada.

Key Words: culling reasons, dairy cattle, longevity

1344T Genetic and genomic evaluation for lameness in US Holstein cattle. A. Sewalem*, S. Arens, L.-Y. Chang, B. Shonka-Martin, J. Nani, M. McClure, and K. Olson, *ABS Global, Deforest, WI.*

Lameness is one of the costliest diseases ($150–300 per incident) in the dairy industry. The aims of this study were A) to assess the incidence of lameness and evaluate its risk factors; B) examine this trait's association with other traits of economic importance; C) to develop genetic and genomic evaluations for lameness. The data set from 946 herds in ABS's Real World Data (RWD) consisted of 9.6M cows from 25,682 AI sires with lameness incidence data from 2000 to 2020. Incidence of lameness were recorded whenever a cow was examined for lameness or received preventive foot-trimming. Cows were assigned a value of either 0 as healthy or 1 as lame per lactation. The overall lameness incidence rate was 11.6%. Incidence rate increased with parity with rates of 10.7%, 13.8%, 16.9%, 19.7%, and 21.6% for parities 1, 2, 3, 4, and 5+, respectively. (Co)variances were estimated using single trait restricted maximum likelihood (REML) with a sire linear model. The model included the fixed effects of parity by stage of lactation and herd-year-season with random effects of sire and the residual. The estimated heritability value was 16%. The sire evaluation results were expressed as an estimated transmitting ability (ETA) representing the expected lameness rate differences among daughters with the average ETA set to 100 and std of 5. The ETAs had a normal distribution ranging from 84 to 124. Traditional evaluation results for lameness were de-regressed and used as pseudo-phenotypes to predict the direct genomic values for animals with 42K SNP genotypes. No markers with large effects were observed. A sire's lameness ETA had low to moderate correlations (4–45%) with CDCB type, health, and production trait breeding values. In conclusion, this study showed large variation in the predicted lameness breeding values indicating that the implementation of genetic and genomic evaluation for lameness will help producers make breeding decisions to reduce their herd's economic risk from lameness.

Key Words: lameness, Holstein, genetic and genomic evaluations

1345T Genome-wise association study and functional genomic analyses for hoof lesion traits in Holstein cattle. L. P. B. Sousa Junior[1], L. F. B. Pinto[1], V. A. R. Cruz[1], H. R. Oliveira[2,3], V. B. Pedrosa[3,4], G. A. Oliveira Junior[2], F. Miglior[2,5], F. S. Schenkel[2], and L. F. Brito*[2,3], *[1]Federal University of Bahia, Salvador, BA, Brazil, [2]University of Guelph, Guelph, ON, Canada, [3]Purdue University, West Lafayette, IN, [4]State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [5]Lactanet, Guelph, ON, Canada.*

Hoof lesions are a serious problem for the dairy industry due to their negative impact on milk production and on fertility, welfare, and longevity. Therefore, we performed a GWAS for 8 hoof lesions and for the overall hoof health index used in Canada. Pedigree, phenotypic, and genomic information were provided by Lactanet. From 10,361 to 16,389 animals with deregressed estimated breeding values for digital dermatitis (DD), heel horn erosion (HHE), interdigital dermatitis (ID), interdigital hyperplasia (IH), sole hemorrhage (SH), sole ulcer (SU), toe ulcer (TU), white line disease (WL), and hoof health (HH) index were used. Genotype imputation of 25,580 animals to high-density SNP panel (HD, 311,725 SNPs) was performed from a medium-density SNP panel (44,315 SNPs) using the Minimac3 software. A mixed linear model was used to estimate the SNP effects in the GCTA software. Bonferroni correction based on the expected number of independent chromosome segments was applied. The GALLO R package was used to detect genes located within ±100 Kb of the significant SNPs and QTL regions previously reported in the Animal QTLdb. Functional enrichment analyses of the identified candidate genes were performed using the DAVID platform. A total of 70 genes were found to be associated with the analyzed traits. The most promising ones for each trait were *NELFE* (DD), *CSMD3* (HHE), *NFYC* (ID), *TROAP* (IH), *TMEM130* (SH), *XKR7* (SU), *MYT1L* (TU), *CLEC14A* (WL), and *WWP1* (HH). These genes are associated with bone, metabolic, infectious, and immunological diseases. In summary, several regions of the genome were found harboring compelling candidate genes for hoof lesion traits and may contribute to the selection of animals with less predisposition to these diseases.

Key Words: digital dermatitis, dairy cattle, GWAS

1346T Assessing genetic variants as indicators of susceptibility to digital dermatitis infection in lactating Holstein cattle. Z. Macon*[1], J. Waddell[1], B. Jones[1,2], K. Wellman[1], and C. Runyan[1], *[1]Tarleton State University, Stephenville, TX, [2]Texas A&M Agrilife, Stephenville, TX.*

Lameness caused by digital dermatitis (DD) leads to major loss in the dairy industry every year and is difficult to combat once a herd is infected. The objective of this study was to determine if identified single nucleotide polymorphisms (SNPs) in the *Bos taurus* genome influence individual susceptibility for digital dermatitis (DD) infection. The sample population consisted of 45 lactating Holstein cows from one dairy farm in central Texas. Cattle were scored on a M0 (no lesions present) to M4 (chronic lesions) scale and were further grouped as having a lesion (n = 35) and not having a lesion (n = 10). Blood samples were taken from cattle (n = 45) from the coccygeal vein to extract and purify DNA. Two previously reported SNPs were evaluated to determine relation to DD susceptibility by qPCR melt curve analysis. SNPs of interest included rs208894039, located downstream from cytidine/uridine monophosphate kinase *2* (*CMPK2*), and rs109521151 located within ankyrin repeat and SOCS box containing 16 (*ASB16*). Data from qPCR melt curves were analyzed using chi-square analysis in SAS (Version 9.4 SAS Institute Inc., Cary, NC) to determine the relationship of amplicon melting temperature to DD infection status. The *CMPK2* associated SNP was found to be significantly related to infection ($P = 0.0025$), as well as ASB16 SNP ($P = 0.022$). These significant relationships between both SNPs and DD infection in the sample population suggest a PCR-based test would prove useful in selecting for cows resistant to digital dermatitis in dairy herds.

Key Words: digital dermatitis, genetic, susceptibility

1347T Analysis of neuromuscular disorders prevalence in Holstein dairy herds in Canada: A preliminary study. G. Condello*[1], C. M. Rochus[1], F. S. Schenkel[1], B. J. Van Doormaal[2], F. Miglior[1,2], and C. F. Baes[1,3], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Lactanet, Guelph, ON, Canada,* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Two neuromuscular disorders, bovine spastic syndrome (Crampy) and bovine spastic paresis (Paresis) are raising concern in the Canadian dairy industry due to the detrimental impact they have on cattle health and welfare. Crampy and Paresis are similar disorders, as they are both progressive, affect the hind limbs of cattle and are characterized by involuntary and uncontrollable muscle cramping when animals are attempting movement. Paresis is usually seen in animals up to 2 years of age, while Crampy generally has a later onset of signs, seen in cattle 2 years or older. The objective of this study was to estimate the population and within herd prevalence of Crampy cases in cattle 2 years of age and older, as well as neuromuscular disorders (Crampy or Paresis) in cattle one year and older in the Holstein population to provide a better understanding of the impact these disorders have on Canadian dairy farms. Lactanet Canada (Guelph, ON, Canada) collected and provided data from 759 dairy herds reporting at least one case of Crampy or Paresis in their herds. To estimate within herd prevalence, only animals meeting the age restrictions from herds with greater than 30 registered Holstein cattle were included. Animals reported to have signs of a neuromuscular disease that were 2 years or older on September 1, 2021, were assumed as Crampy cases. Animals reported to have signs of a neuromuscular disease that were one year or older on September 1, 2021, were assumed Crampy or Paresis cases. Our overall data set of cattle 2 or more years of age contained 90,185 animals, with 2,731 of those with signs of Crampy resulting in an estimated population prevalence of 3.03%. Our data set of cattle one or more years of age contained 119,026 animals, with 2,797 of those affected by a neuromuscular disorder, resulting in an estimated population prevalence of 2.35%. To allow us to better understand the genetic nature of these neuromuscular disorders, our next steps will include estimating genetic parameters using this data set.

Key Words: crampy, paresis, neuromuscular

1348T Haplotype inheritance and livability of recumbent Holstein calves. A. Al-Khudhair*[1], P. M. VanRaden[1], D. J. Null[1], M. Neupane[1], and C. D. Dechow[2], [1]*USDA, Beltsville, MD,* [2]*The Pennsylvania State University, University Park, PA.*

A recessive condition affecting calves' ability to stand, termed recumbency, was reported by Dechow in 2022. The haplotype identified on chromosome 16 traced back 13 generations via several very popular sires back to 1952. For 70 years, it has been one of the most common haplotypes in the Holstein population, but the observed number of homozygotes was only slightly less than expected in national data. A potential explanation was that a new mutation had occurred within that popular haplotype. Computer code to sort out the mutated from the original version of the cholesterol deficiency haplotype was also applied to recumbency by requiring pedigree connections to a recent common ancestor of recumbent calves. For 5.1 million genotyped Holsteins, haplotype codes included 0.01% homozygotes tracing only to that ancestor on both sides (code 2), 0.44% other homozygotes (code 4) tracing to that ancestor on both sides but also to other sires having the original version, 12.4% not sure carriers (code 3) where the normal or mutated status of the haplotype could not be determined, 2.3% sure carriers (code 1), and 85% noncarriers (code 0). Haplotype status for 558K calves matched to heifer livability records; those were analyzed without adjusting for birth year or herd incidence. Only 46 code 2 homozygotes had records and 52% of those died before 18 mo at an average age of 1.7 ± 1.6 mo. That death rate may be underestimated if only healthier calves were genotyped. The death rate was only 4.2% for the 2,211 code 4 homozygotes and 2.4% for the code 0 noncarriers. Of the 5 oldest code 2 heifers that lived beyond 18 mo, most had 3 or 4 lactations with good production, but one died at 2 years of age. Thus, calves that recover from recumbency can have normal, productive lives. Instead of identifying known or suspected homozygotes from genotypes and then checking phenotypes, a more proactive strategy would be to identify carrier's mating and carefully monitor the resulting calves, but that would require more effort. Different reporting methods or dominance effects may be needed to include recumbency and other partially lethal effects in selection and mating.

Key Words: calf, recessives, recumbency

1349T Network of dysregulated miRNA-mRNA reveals candidate regulatory miRNAs in bovine mastitis caused by *Staphylococcus chromogenes*. F. A. Omonijo*[1,2], M. Wang[1], D. Gagné[3], M. Laterrière[3], Z. Xin[2], and E. M. Ibeagha-Awemu[1], [1]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada,* [2]*Department of Animal Science, McGill University, Ste-Anne-De-Bellevue, Quebec, Canada,* [3]*Quebec Research and Development Centre, Agriculture and Agri-Food Canada, Quebec, Quebec, Canada.*

Mastitis, an inflammation of the mammary gland, is caused by varied pathogens including *Staphylococcus chromogenes*. Despite numerous studies on the role of non-coding RNAs in diseases including mastitis, no study has investigated the involvement of miRNA in *S. chromogenes* mastitis. This study aimed to uncover the regulatory roles of miRNAs in cow's response to subclinical mastitis due to *S. chromogenes*. The miRNA and mRNA transcriptomes of milk somatic cells from *S. chromogenes* infected cows (n = 3) and healthy cows (n = 4) were sequenced and analyzed with bioinformatics tools. A total of 51 miRNAs (26 up- and 25 downregulated) and 7,744 genes (3,597 up- and 4,147 downregulated) were differentially expressed (DE) (FDR < 0.05). Networks of DE miRNA and DE mRNA (miRNA-mRNA) were constructed and 26 networks involving one miRNA and ≥2 mRNAs were further studied.

Functional analysis of network mRNAs revealed their enrichment in several disease and immune pathways, such as bacteria invasion of epithelial cell, TNF signaling pathway, NF-kB signaling pathway etc. suggesting the involvement of network miRNAs (candidate regulatory miRNAs) in immune modulation during *S. chromogenes* mastitis. Upregulated candidate miRNAs like bta-miR-454 and bta-miR-339b have been associated with bovine paratuberculosis and human diseases (cancer, leukemia). Also, downregulated candidate miRNAs like bta-miR-455 and bta-miR-23b-5p have been reported as modulators of immunity, inflammation and apoptosis in livestock diseases (e.g., foot and mouth disease, mastitis). Our data suggest that the candidate regulatory miRNAs potentially regulated the host response to mastitis caused by *S. chromogenes*.

Key Words: bovine mammary gland, *Staphylococcus chromogenes*, miRNA-mRNA network

1350T Identification of genetic variants affecting postpartum hypocalcemia in Holstein cows. L. C. Novo*[1], F. M. Rezende[2], J. E. P. Santos[2], C. D. Nelson[2], L. Hernandez[1], B. Kirkpatrick[1], and F. Peñagaricano[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

Periparturient hypocalcemia is a complex metabolic disorder that occurs at the onset of lactation because of a sudden irreversible loss of Ca incorporated into colostrum and milk. Some cows are unable to quickly adapt to this demand and succumb to clinical hypocalcemia, commonly known as milk fever, while a larger proportion of cows develop subclinical hypocalcemia. The main goal of this study was to identify causative mutations affecting postpartum blood calcium concentration in Holstein cows. Data consisted of 2,935 records of blood calcium measured in 2,935 Holstein cows on the first day after parturition. All cows had genotypic information for 79k SNP markers. Two consecutive rounds of imputation were performed: first, the 2,935 Holstein cows were imputed from 79k to 300k SNP markers. This imputation was performed using a reference set of 17,131 proven Holstein bulls with 300k SNP markers. Then, the 2,935 Holstein cows were imputed from 300k markers to whole-genome sequence data. This second round of imputation used 720 Holstein animals from the 1000 Bulls Genome Project as reference set. The identification of genetic variants associated with postpartum hypocalcemia was performed using a 2-step mixed model-based approach implemented in the R package MixABEL. Three genomic regions located on BTA3 (77.94 Mb, P-value = 2.6×10^{-07}), BTA6 (87.03 Mb, P-value = 2.0×10^{-06}) and BTA12 (58.99 Mb, P-value = 2.0×10^{-07}) showed the most significant associations with postpartum blood calcium concentration. Remarkably, these genomic regions harbor genes, such as *SREBP1*, *GC*, and *SPRY2*, that are closely related to calcium homeostasis, including calcium imbalance, vitamin D transport, and calcium signaling. This work is the foundation for the development of novel genomic tools for reducing the incidence of periparturient hypocalcemia in dairy cattle.

Key Words: calcium homeostasis, causal mutation, genomic scan

1351T Genetic evaluation of colostrum production in a commercial Jersey dairy herd. M. M. Schutz*[1], B. J. Heins[2], N. Lopez-Villobos[3], and D. L. Carr[4], [1]*University of Minnesota, St. Paul, MN,* [2]*University of Minnesota WCROC, Morris, MN,* [3]*Massey University, Palmerston North, New Zealand,* [4]*Wilbur-Ellis Nutrition, Kennewick, WA.*

Recently, Jersey cows have been noted to produce less, little, or no colostrum at calving in some herds, especially during months of short daylight adjacent to the winter solstice. Previous studies have implicated feed protein content, niacin, delayed release, and genetics as factors in reduced colostrum production by Jerseys in winter. The objective of this study was to determine levels of Jersey colostrum yield during spring and summer long-day light and additive genetic effects in a herd of Jerseys as a benchmark for later comparison during short-day light. Records of Jersey colostrum yields in the first milking following calving were from a 6,000-cow herd in Washington state from April to August 2022. For necessary convenience, calving barn managers measured and recorded colostrum produced by cows as number of 3L bottles filled, ranging from 0 to 8. Calving date and lactation number were also obtained along with sire and maternal grandsire identification. The 1,577 records of daughters from sires with 10 or more daughters were included, with mean colostrum yield of 2.6 (SD = 1.34) bottles. Data were analyzed using an animal model with fixed effects of calving week (1–17) and parity (1 to 5) and random animal and residual effects using ASREML. Animal effects included 1,577 cows with records, 38 sires, and 2,386 related ancestors. Range of weekly estimates ($P < 0.001$) was 1.17 bottles, with highest estimates during June. Parity ($P < 0.001$) estimates were 2.5 bottles for parity 1, increasing to 2.8 for parity 3 and 4. The estimate of heritability was 0.14 (SE = 0.06). Breeding values for bulls with progeny were from −0.77 to 0.66 bottles, a range of 1.43 bottles. Colostrum production is under moderate genetic control and affected by time of year. Future work must consider genetic and management factors impacting colostrum production across seasons for Jerseys.

Key Words: colostrum, genetic evaluation, Jersey

1352T Determination of genome regions and metabolic processes associated with tick resistance in Charolais and Limousin cattle from French New Caledonia. P. Martin*[1], T. Hüe[2], J. Mante[3], A. Lescane[4], D. Boichard[1], and M. Naves[5], [1]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [2]*Institut Agronomique néo-Calédonien (IAC), équipe ARBOREAL, Païta, New Caledonia, France,* [3]*France Limousin Sélection, Boisseuil, France,* [4]*Unité Néo-Calédonienne de sélection et de promotion des races bovines (UPRA Bovine), Païta, New Caledonia, France,* [5]*INRAE, ASSET, Petit-Bourg, French West Indies, France.*

Ticks cause significant production losses in cattle and consequences of infestation can even cause animal death. This parasite is a severe issue in tropical and sub-tropical areas and new regions will become at risk in the future due to climate change. For these reasons, a better understanding of the genetic control and the metabolic processes involved in host resistance appears of primary importance. To address this question, regular phenotyping have been implemented in 11 commercial farms of French New Caledonia from 2014 to 2021. Six different traits were considered: score of adult female ticks, score of juvenile ticks, total score, and the logarithm of the 3 previous traits. A total of 556 Limousin animals and 302 Charolais animals were phenotyped, with 1 to 8 visits per animal for the Limousin breed (mean = 2.3, s.d. = 1.6, total = 1,273), and 1 to 12 visits for the Charolais breed (mean = 4.2, s.d. = 2.8, total = 1,281). All the animals were genotyped with the 50K EuroGMD SNPchip. First, a generalized linear model was used to correct performances by the effects of sex, age, herd, technician, and period. Then, genome-wide association studies were performed on each breed and each trait using an average corrected performance per animal with the GCTA software. A total of 5 genomic regions in Charolais and 13 regions in Limousin were found to be associated with at least one trait (none shared between breeds), with 2 regions in Limousin being highly significant ($P < 10^{-11}$) and shared between traits, on chromosomes 11 and 13, respectively. Finally, all the significant regions have been included in a Gene Ontology analysis, using ClueGO and Cluepedia packages from the Cytoscape software. Results shows that 37 terms from 9 functional groups are overrepresented, with the 3 most overrepresented group being the linoleic acid metabolic process, the negative regulation of insulin secretion and the excitatory extracellular ligand-gated ion channel activity. The linoleic acid being implicated into the skin firmness, this process appears as a promising candidate that requires deeper investigation.

Key Words: GWAS, tick resistance, Gene Ontology

1353T Copy number variation accounts for complementary additive genetic variance of health traits in Holstein cattle. G. C. Ladeira*[1], P. J. Pinedo[2], J. E. P. Santos[1], W. W. Thatcher[1], and F. R. Rezende[1], [1]*University of Florida, Gainesville, FL,* [2]*Colorado State University, Fort Collins, CO.*

Copy number variations (CNVs) are structural genomic variants that can provide valuable insights into the genetic architecture of complex traits. Here, CNV information was included in a SNP-based threshold model to assess its contribution in estimating genetic parameters for mastitis and metritis in 2,900 Holstein cows. Both traits were analyzed as binary variables (1 = disease, 0 = no disease). A total of 3,952 CNVs derived from 546,802 autosomal SNP genotypes were used to build the CNV-derived genomic relationship matrix (CNV_GRM). CNV loci showing double deletions, single deletions, and normal state in the population were coded as 0, 1, and 2, respectively, whereas CNV loci presenting normal state, single duplications, and double duplications were coded as 0, 1, and 2, respectively. Given 21 CNVs presented both deletions and duplications at a same position in the population, these mixed-CNVs were treated as 2 distinct CNVs loci, one to represent deletions and other to represent duplications. The variance components were estimated by fitting either only a SNP-derived GRM (SNP_GRM) or jointly SNP_GRM and CNV_GRM in models that included female category and farm-year-season as fixed effects and were implemented in a Bayesian framework using THRGIBBS1F90. The additive genetic variances captured by the SNP_GRM were 0.18 ± 0.09 (mastitis) and 0.16 ± 0.06 (metritis). When SNP_GRM and CNV_GRM were considered jointly, the genetic variances accounted for by CNV_GRM were 0.02 ± 0.02 (mastitis) and 0.03 ± 0.02 (metritis), with no change in variance estimates associated with SNP_GRM. Heritability estimates considering SNP- and CNV-derived GRMs in the same model were 0.17 ± 0.09 for mastitis and 0.16 ± 0.06 for metritis, slightly greater than those obtained by the pure SNP_GRM model (0.14 ± 0.06 for mastitis and 0.13 ± 0.04 for metritis). In conclusion, CNVs account for some of additive genetic variance of complex traits which cannot be accounted for only by SNPs as genetic markers.

Key Words: CNV, dairy cattle, variance component

Dairy Foods 2: Production, Products, and Chemistry

1354T Physicochemical properties, antioxidant capacity and bioavailability of whey protein encapsulated coenzyme Q_{10} nanoparticles. Y. Sun*[1], J. Liu[1], X. Pi[1], S. Zhang[1], A. Kemp[2], and M. Guo[2], *[1]College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, China, [2]Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.*

Coenzyme Q_{10} (CoQ_{10}) is an important fat-soluble antioxidant in biological processes. Its preparations are widely used as a nutritional supplement. However, the bioavailability of CoQ_{10} is very limited due to its hydrophobic nature. To improve the bioavailability, CoQ_{10} was encapsulated in 10% (m/v) whey protein concentrate (WPC) or polymerized whey protein concentrate (PWPC) to prepare CoQ_{10} loaded nanoparticles with mass ratios of protein to CoQ_{10} from 100:1 to 20:1 through high pressure homogenization method, and the physicochemical properties, antioxidant capacity and bioavailability of the nanoparticles were characterized. The diameters (D_{43}) of WPC-CoQ_{10} and PWPC-CoQ_{10} particles ranged from 241 to 331 nm and zeta potential from 20.8 to −26.6 mV at the mass ratios of protein to CoQ_{10} from 100:1 to 20:1. The encapsulation efficiency of CoQ_{10} in all groups was above 90.7%. Differential scanning calorimetry analysis revealed that CoQ_{10} was successfully encapsulated in both WPC- and PWPC-based particles. Fourier transform infrared spectra indicated that CoQ_{10} and protein molecules interacted mainly through hydrogen bonding and hydrophobic interactions. In vitro antioxidant capacity and bioavailability evaluations for WPC-CoQ_{10} and PWPC-CoQ_{10} were further conducted under the optimal mass ratio of 20:1. Compared with free CoQ_{10}, significantly increased antioxidant capacity was observed in nanoparticles of both WPC-CoQ_{10} and PWPC-CoQ_{10} ($P < 0.05$). A gastrointestinal digestion in vitro study showed that WPC and PWPC encapsulated CoQ_{10} had 7.6- and 7.5-fold increase in bioavailability as compared with free CoQ_{10}, respectively. The results indicated that both WPC and PWPC matrix could be used as an effective delivery carrier to improve the antioxidant capacity and bioavailability of CoQ_{10}.

Key Words: coenzyme CoQ_{10}, whey protein concentrate, bioavailability

1355T Occurrence of fraud, alteration and adulteration in commercial milk in the Brazilian legal Amazon region. J. Ribeiro Júnior*[1,2], B. Dias[1], C. Nascimento[1], L. Lino[1], Y. Rodrigues[1], L. Silva[3], C. Lobo[1,2], R. Tamanini[2], and A. Alfieri[2], *[1]Federal University of North Tocantins, Araguaína, Tocantins, Brazil, [2]National Institute of Science and Technology for the Milk Production Chain, Londrina, Paraná, Brazil, [3]Tocantins Agricultural Defense Agency, Araguaína, Tocantins, Brazil.*

Part of Brazilian refrigerated raw milk has low microbiological quality, which, when combined with small-scale production, reduces its technological potential. In Brazil, few dairy producers are compensated for producing high-quality milk but instead are paid based on the volume of milk delivered. To mask the addition of water and to increase product shelf life, producers and dairy industries often add substances to reconstitute density, to neutralize acidity and to preserve the milk. This study investigated raw, pasteurized, and ultra-high temperature (UHT) commercialized fluid milk in Tocantins State, Brazil (located in the Brazilian legal Amazon region) for the presence of ethanol, chlorides, sucrose, starch, chlorine/hypochlorite, formaldehyde, hydrogen peroxide, and alkaline substances. The study was conducted between 2019 and 2021, and 39 milk samples were evaluated; 18 were raw, 15 were UHT, and 6 were pasteurized. The samples were collected from different producers/brands. Each substance was evaluated using standard qualitative methods established by the Brazilian Ministry of Agriculture, Livestock, and Supply. Every positive result was validated through confirmatory testing of additional samples from the same batch. Only ethanol was detected by colorimetric method by passage of steam in sulfochromic solution, which was positive in 23% (9/39) of the samples, with 3 samples from each type of fluid milk. Due to its low freezing point and ability to increase suspended particles in an emulsion, ethanol at low levels of inclusion can restore the cryoscopic index and density of milk after adding water. The positive samples were further evaluated using cryoscopy to detect water, and the results showed up to 26%, despite the presence of ethanol. These results indicate that commercialized milk sold in the Brazilian legal Amazon region may be subject to fraud and adulteration through the addition of water and substances that artificially improve milk's parameters. Therefore, it is imperative that agencies increase their monitoring and enforcement efforts to ensure the safety and quality of milk for consumers, particularly regarding dilution and the presence of potentially hazardous chemicals.

Key Words: cryoscopic index, ethanol, UHT milk

1356T Camel whey protein with enhanced antioxidative and antimicrobial properties upon simulated gastro-intestinal digestion. H. Kamal*[1,2], *[1]University of Nottingham, Loughborough, United Kingdom, [2]United Arab Emirates University, Al Ain, UAE.*

Whey proteins and their peptide derivatives have attracted a great attention in the pharmaceutical and nutritional fields, due to their numerous bio-functionalities. In the present research study, enzymatic camel whey protein hydrolysates (CWPHs) from camel whey proteins (CWPs) were produced and investigated for their antioxidant and antimicrobial potentials. For the production of CWPHs, 3.0% (wt/vol) CWPs were digested using pepsin (gastric), and trypsin and chymotrypsin (pancreatic) enzymes at an enzyme/substrate (E/S) ratio of 1/100 (wt/wt) under constant agitation of 90 rpm. Aliquots were afterward withdrawn at 3 h and 6 h and treated at 100°C for 10 min to inactivation. CWPHs were analyzed in triplicate for protein degradation, antimicrobial and antioxidant potential. Among all CWPHs, a complete degradation of all different protein bands was perceived with chymotrypsin-derived CWPHs, whereas, light bands of serum albumin and α-lactalbumin were observed with trypsin and pepsin-derived CWPHs as evident from degree of hydrolysis (DH), ranging from control (8.5%) and chymotrypsin 6 h (47.5%). Current results reveal that for unhydrolyzed CWP, an intact peak of α-lactalbumin was detected. However, after hydrolysis with pepsin for 3 and 6 h, α-lactalbumin underwent complete degradation. After enzymatic degradation, both CWPHs antioxidant and antimicrobial activities were improved. Chymotrypsin-derived CWPHs showed the highest free radical scavenging activities followed by trypsin and pepsin generated CWPHs ($P < 0.05$). Enzymatic hydrolysis of CWP for 6 h with trypsin was noticed to promote a 4-fold enhancement in the metal chelating activity, compared with control. No significant increase ($P < 0.05$) in the ferric reducing antioxidant power (FRAP) activities was noticed after CWPs hydrolysis using trypsin and chymotrypsin, while pepsin-derived CWPHs showed higher reducing power. In terms of antimicrobial activity, significantly higher bacterial growth inhibition rates were exhibited by CWPHs compared with control.

Key Words: simulated gastrointestinal digestion, camel milk, protein and bioactive peptide

1357T A sedimentation test for determination of heat-induced aggregation in milk protein beverages. C. M. Smits*[1], J. A. Hargrove[1], M. A. Drake[1], and D. M. Barbano[2], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Milk protein beverages have increased in popularity but heat induced protein aggregation and settling is a defect. Our objective was to develop a bench top method to determine differences in heat induced aggregation among milk protein beverages. Liquid milk protein concentrate (MPC) at 7.5% protein, with and without 0.15% (wt/vol) dipotassium phosphate (DKP), was given oil bath heat treatments to simulate direct steam injection (DSI) ultra-pasteurization (UP) (135 to 155°C for 30 s). The same MPC beverages were also processed by DSI UP at 142°C for 3 s. The entire experiment was replicated twice. The oil bath system consisted of capped stainless steel tubes (18 cm × 0.96 cm, filled with 6.6 g liquid), a mineral oil bath, and an ice bath. One tube had a modified end cap for a thermocouple to record temperature. At the end of the holding time, the rack of tubes was lifted from the oil bath and transferred directly into an ice bath. The cooled product was inspected and if not coagulated, was emptied into a container for further testing. Cooled liquid (ca. 1.5 g) was added to a microfuge tube, weighed, and centrifuged at 21°C for 5 min at 21,100 × *g*. The supernatant was decanted and the weight of sedimented protein determined. Results were expressed as the pellet weight as a percent of the sample weight, and data were evaluated by ANOVA with means separation. Sedimentation values for protein beverages without heat treatment were 2 to 3% both with and without added DKP ($P > 0.05$), as expected. For DSI UP, the MPC beverage with added DKP had a sedimentation value of 3%, consistent ($P > 0.05$) with oil bath sedimentation values at 135 to 155°C. Without added DKP, the 7.5% MPC beverage gelled by DSI UP processing. The same beverage was liquid after oil bath treatments at 135 and 140°C while at >145°C in the oil bath, the beverage gelled, similar to DSI UP. The combination of a small-scale benchtop oil bath thermal treatment of beverages followed by a quantitative centrifugal sedimentation test can be used to screen combinations of milk proteins, other ingredients, and flavors to determine heat stability before pilot plant or production scale batches.

Key Words: heat stability, beverage

1358T Seasonal influence on buffalo milk composition. C. F. Viana[1], I. L. S. Gomes[1], E. H. P. Andrade[1,2], M. R. Souza[1], C. F. A. M. Penna[1], B. M. S. Souza[1], A. C. C. Lopes[1], R. S. Conrrado[1,2], T. O. Santos[1], M. O. Leite[1,2], and L. M. Fonseca*[1,2], [1]*School of Veterinary Medicine/Federal University of Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*Laboratory of Milk Quality Analysis/School of Veterinary Medicine/Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.*

Buffalo milk represents an important share in the milk production in several countries, however not much is known about how its milk quality is affected by environmental factors in Brazil. The objective of this work was to investigate buffalo milk composition in Minas Gerais State, Brazil. Raw milk was collected from refrigerated tanks with a total of 837 samples from 34 farms (each farm with about 40 lactating cows on average), during 24 mo (July, 2020 to June, 2022). Compositional analyzes by Fourier transform infrared (FTIR) included fat, protein, lactose, total solids, and solids nonfat (Lactoscope; Delta Instruments). Results were analyzed using descriptive statistics and Tukey test for multiple comparison of data. Average minimum and maximum temperatures in this region were about 12°C and 24°C, respectively, in the winter, and 19°C and 29°C in the summer. Average relative humidity was about 73% and 84%, and average monthly accumulated precipitation was 20 mm and 240 mm in the winter and summer, respectively. Feeding is mainly pasture based during summer, while during winter, supplementation is necessary. Average results for fat were significantly higher during the spring (6.19 g/100 g ± 0.70 g/100 g) when compared with winter and fall (5.57 g/100 g ± 0.70 g/100 g for both seasons) ($P < 0.05$). Protein, and solids nonfat averages were higher during the summer (4.24 g/100 g ± 0.31 g/100 g and 10.03 ± 0.32 g/100 g, respectively). However, solids nonfat content average result during summer was similar to the average found on spring (9.96 g/100 g ± 0.28 g/100 g) ($P > 0.05$). Lactose content, on the other hand was higher in the winter season (4.94 g/100 g ± 0.18 g/100 g) with the lowest concentrations during the spring and summer (4.80 g/100 g ± 0.17 g/100 g, and 4.78 g/100 g ± 0.20 g/100 g, respectively). We conclude that seasonal conditions related to temperature, humidity, feed type, and availability, among others, are important factors for the characterization of buffalo milk composition. Moreover, buffalo species has peculiar reproductive patterns, with reproductive seasonality, which may affect milk composition unlike cows. Hence, more studies are necessary to establish milk quality parameters for this species. Acknowledgments: FAPEMIG APQ-02740–17; FEPE proj.882.

Key Words: buffalo milk, composition, milk quality

1359T Milk casein concentrate and serum proteins concentrate as the sources of bioactive peptides. M. Darewicz*, J. Borawska-Dziadkiewicz, A. Iwaniak, P. Minkiewicz, D. Mogut, M. Baranowska, K. Przybylowicz, and J. Zulewska, *University of Warmia and Mazury, Olsztyn, Poland.*

The aim of this research was to evaluate the angiotensin I-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) inhibitory as well as antioxidant activities of bovine micellar casein concentrate (MCC) and serum proteins concentrate (SPC) after digestion. In silico part of the study was carried out using computation tools: UniProt (http://www.uniprot.org/) and BIOPEP-UWM (http://www.uwm.edu.pl/biochemia/). The MCC and SPC were prepared by membrane filtration and digested using INFOGEST protocol. The degree of hydrolysis (DH) was determined according to the o-phthaldialdehyde method. The digests were analyzed for their enzyme inhibitory and antioxidant activities. The digests were used in a screening for bioactive peptides by reversed-phase high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS) method. All experiments were repeated at least 3 times. A high degree of defragmentation of milk protein concentrates was observed after the duodenal phase (from 63.96 ± 0.69% to 77.83 ± 0.97%). Digests of concentrates showed ACE and DPP-IV inhibitory as well as antioxidant activities. The highest ACE inhibitory activity was determined for digested MCC (IC_{50} = 1.856 ± 0.08 mg/mL) whereas also digested SPC revealed the highest DPP-IV inhibitory activity (IC_{50} = 0.512 ± 0.04 mg/mL). The latter was characterized with the highest antioxidant activity (IC_{50} = 4,616 ± 0.18 mg/mL). It was possible to find 35 biopeptides in MCC and SPC digests. The ACE inhibitory (e.g., Ile-Pro-Ala, Ile-Arg), DPP-IV inhibitory (e.g., Ile-Pro-Ala, Ile-Arg, Pro-Trp), and antioxidant fragments (e.g., Ile-Ala, Pro-Trp) were identified. It can be concluded that, bovine milk protein concentrates can be considered as an interesting source of peptides with biological activity, including ACE and DPP-IV inhibitors, as well as antioxidant peptides released after digestion.

Key Words: bioactive peptide, digestion, milk concentrate

1360T Developing a hybrid method for manufacturing whey protein nano-/micro-gel particles derived from a dispersion containing fibril/ribbon-shaped assemblies.

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The present study aims to produce colloid particles from an engineered parental protein dispersion system containing fibrillar/ribbon-shaped assemblies derived from whey protein isolate (WPI) via antisolvent precipitation followed by heat treatment. Briefly, hybrid assemblies were prepared in 3 steps. 1) Parental protein dispersion containing long fibers was prepared by injecting 20% (wt/vol) WPI protein solution (pH 7.0) at a rate of 0.05 mL/min into an antisolvent blend [ethanol: glycerol = 75:25 (vol/vol)] under shear (100 rpm). 2) The protein-antisolvent mixture was then subjected to a heat treatment at 80°C/20 min. Antisolvent was then removed through successive centrifugation and washing steps. 3) Obtained pellet was a mixture of fibril-like and other complex morphologies, which was re-dispersed in water and microfluidized under different pressures. All samples were prepared in no less than duplicates and each measurement was performed in no less than 2 times for each mentioned replicate. Results showed that the D[4,3] was 7.80, 5.83, and 3.28 μm for particles processed at 50, 100, and 150 MPa, respectively ($P > 0.05$). However, the D90 value decreased from 24.08 to 3.38 μm with increasing microfluidization pressure ($P < 0.05$). Mass weighted PSD profile derived from dynamic light scattering measurements showed a clear separation between particle dispersion and WPI solution, suggesting an effective aggregation induced by the hybrid process. Optical microscopic images revealed that the protein particles with diverse structures including fibril/ribbon and other irregular shapes were formed during the antisolvent-driven aggregation step, and anisotropic morphologies were found after the centrifugation steps. In conclusion, a method was developed that is able to generate hybrid whey protein assemblies with heterogeneous structures that might possess uniquely improved functionalities compared with the native WPI. Their potential for food applications may be investigated in the future.

Key Words: whey protein isolate, antisolvent precipitation, microfluidization

1361T The investigation of a rapid method for indicating powder properties based on the correlation analysis of interfacial parameters.

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Dairy powders are widely used as ingredients in diverse food products, meanwhile, characterization of powder properties (e.g., wettability and flowability) are of technical challenges to the manufacturers of ingredients and consumer foods. Rapid evaluation methods are needed to fill the mentioned technological gap. This study aimed to investigate correlations between a series of conventional powder properties and powder surface properties, which may be characterized using a relative rapid method. The samples were: milk protein concentrate 80 (MPC80), milk protein isolate 90 (MPI90), micelle casein concentrate (MCC), nonfat dry milk (NFDM), whole milk powder (WMP), and sodium caseinate (NaCN) with 2 different lots for each sample. Triplicated measurements were performed. The wettability of powder samples was determined by measuring wetting time based on a modified International Dairy Federation Standard (IDF) method. Flowability was determined using the Warren Spring (WS) cohesion measured by powder-rheometer. Regarding surface characterizations, the contact angle of powder samples was determined by sessile drop method using both water and diiodomethane. Subsequently, the surface free energy was computed through the Owens, Wendt, Rabel, and Kaelble (OWRK) model. Statistical analyses including one-way ANOVA and Pearson correlation were performed to reveal statistical significance. Among 6 powers, NaCN showed the longest wetting time and the lowest flowability (WS cohesion) at 1,993.50 ± 924.62 s and 1.33 ± 0.06 kPa, respectively ($P < 0.05$). The poor wettability of NaCN was also demonstrated by a relatively larger contact angle at 122.04 ± 2.66° as compared with MCC, MPC80, NFDM, WMP ($P < 0.05$). The overall surface free energy was significantly correlated with powder behaviors ($P < 0.05$) including WS cohesion, contact angle, and wetting time. In conclusion, this study demonstrated a rapid method using a series of surface characteristics of powder to indicate powder quality properties. The obtained findings have important implications for the dairy food industry.

Key Words: powder property, statistical correlation, interfacial study

1362T Lysozyme activity and total antioxidant capacity in raw and pasteurized donkey milk of the Sicilian *Ragusana* breed.

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Donkey milk can be considered a functional food, rich in lysozyme and antioxidants. This study aimed to determine the threshold conditions of different temperature-time combinations for milk pasteurization to preserve lysozyme activity (LA) and total antioxidant capacity (TAC). Individual samples of 6 *Ragusana* breed donkeys were collected 4 times in 2021, on March 18, April 27, August 25, and October 4. Especially during summer, the donkey farms in Sicily tend to use higher temperatures and longer times than those usually suggested for pasteurization, to guarantee milk safety. Thermal treatments included 63°C × 30 min, and both 72°C and 76°C × 15 s, × 2 min, × 10 min, and × 30 min, respectively, using a batch system. Raw and heat-treated samples were analyzed as follows: *Micrococcus lysodeikticus* cells were used as the substrate for

Table 1 (Abstr. 1362T). Results of study

Treatment		TAC[1] (e-BQC/mL)	LA[1] (U/mL)
Raw milk		568^{a}	8,263^{abc}
Temperature	Time		
63°C	30 min	457^{b}	9,784^{a}
72°C	15 s	478^{b}	9,089^{ab}
	2 min	486^{ab}	8,980^{ab}
	10 min	463^{b}	6,545^{c}
	30 min	435^{b}	3,633^{ef}
76°C	15 s	466^{b}	7,435^{bc}
	2 min	466^{b}	6,279^{cd}
	10 min	448^{b}	4,303^{de}
	30 min	435^{b}	2,128^{f}

$^{a–f}$Values in the same column with different superscripts differ by $P < 0.05$.

[1] TAC = total antioxidant capacity; LA = lysozyme activity.

the LA assay and TAC was determined with an electrochemical method using the e-BQC NI device (Bioquochem). Heat treatment was the fixed effect in the statistical model with a significance level set at 0.05. Heat treatment affected both LA and TAC (Table 1). Raw donkey milk had a TAC of 568 e-BQC value/mL that decreased by 14–23%, independently of the heat treatment. As for LA, raw donkey milk had an average activity of 8,263 U/mL. Compared with raw milk, LA was thermoresistant with standard treatments (63°C × 30 min and 72°C × 15 s). Only the treatments 72°C × 10 min, 76°C × 10 min, and 76°C × 30 min had lower LA. When only the heat-treated samples are considered though, milk pasteurized at 63°C × 30 min had similar LA compared with 72°C × 15 s and 72°C × 2 min but was higher compared with all other treatments. The treatments 76°C × 15 s and 72°C × 10 min reduced LA by 24 and 33%, respectively, compared with 63°C × 30 min. In that sense, higher temperatures than 72°C, even at very short times, may reduce LA, and should not be used. This applies also to times longer than 2 min when milk is pasteurized at 72°C.

Key Words: donkey milk, total capacity antioxidant, lysozyme activity

1363T Developing scalable enzymatic manufacturing process of lactobionic acid from permeate of milk protein concentrate.

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Lactobionic acid (LBA) as an oxidation product of lactose has received great interest regarding its food and pharmaceutical applications such as flavor enhancement and delivery of nutrients/drug molecules. Enzymatic synthesis of LBA has been investigated previously due to the advantages of cost efficiency and relatively less environmental impact. However, practical knowledge on enzymatic LBA production using dairy permeates as substrates is scarce in the literature. In this study, reconstituted milk protein concentrate permeates with 2 lactose levels (20% and 30%, wt/vol) were used as substrates, and LBA (Na-salt form) was produced using commercial cellobiose oxidase (LOXU/g) at 38°C. The objectives were to investigate the effects of substrate lactose level and reaction pH (6.4 and 6.8) on the LBA yield and enzymatic conversion kinetics. The production process was conducted in an automated (lab-scale) bioreactor, in which the processing pressure was monitored and temperature, pH, O_2 level were controlled. The LBA production rate was monitored in situ by the pH-Stat method for no less than 20 h per batch. Our results showed that regardless of pH, the total lactose content had a significant impact on LBA productivity (mmol/h). Samples “30%-6.8” and “30%-6.4” (lactose level-pH) had similar real-time productivities at ~68 and ~71 mmol/h, which were higher than the samples with 20% lactose (~48 mmol/h) after 7 h of reaction ($P < 0.05$). Although the productivity of samples under all conditions dropped significantly after 20 h of the bio-conversion process, the lactose conversion percentage reached to ~73% after 20 h reaction time. Interestingly, the normalized productivities based on the lactose amount (mmol/g/h) were similar among all tested samples at 20 h of reaction time (~0.06 mmol/g/h, $P > 0.05$) suggesting a relatively durable enzymatic activity. In conclusion, this study provides practical information regarding enzymatic production of LBA using dairy permeate streams as substrates. The processing control parameters generated from the lab-scale reactor should be scalable to industrial scale.

Key Words: lactobionic acid, Bioreactor, enzymology

1364T Modification of butterfat to alter its crystallization pattern.

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Butterfat has a unique crystallization pattern that dictates its functionality such as hardness and meltability. Changing the chemistry and structure of the fat molecules in butterfat can change its crystallization pattern. Previously, enzymatic transesterification has been used to alter the chemistry of butterfat. However, chemical transesterification can be achieved with mild conditions. Furthermore, non-dairy fat can be added to modify the fat crystal network as well. Therefore, the objective of this research is to perform a transesterification of butterfat, and to add corn oil to butterfat and monitor how these modifications change the crystallization patterns. For the transesterification, butterfat is mixed with glycerol and 3 different catalysts, potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$), and calcium carbonate ($CaCO_3$), and heated for 4 h at 210°C. For the fat mixture, melted butterfat is mixed with corn oil in various concentrations, and allowed to crystalize at 25°C. Polarized light microscopy (PLM), differential scanning calorimetry (DSC), and wide-angle x-ray diffraction (XRD) are used to measure the crystal morphology, thermal properties, and polymorphism, respectively. The shape and polymorphism of butterfat crystals, and transesterified butterfat crystals using KOH as a catalyst are similar, with spherulite, β’ crystals; however, the melting temperature of the transesterified crystals is higher, 49°C compared with 47.5°C. Furthermore, using $Ca(OH)_2$ as the catalyst, the reaction produces denser, more formed crystals, with a different β’ polymorph, d spacings of 4.5, 4.2, 4.1, and 3.8 Å compared with 4.2, 4.0, and 3.8 Å, and a lower melting point, 44°C. Therefore, changes are occurring in the butterfat from the transesterification reaction, and the type of catalyst used for the reaction impacts the changes in crystal development and formation.

Key Words: butterfat, crystallization, transesterification

1365T Physical chemical and microbiological quality control of raw milk.

W. E. Oliveira, M. Ramatiz Lima dos Santos*, and M. Dias França, *IF Goiano Ceres, Ceres, Goiás State, Brazil.*

The objective of this work was to evaluate the physicochemical and microbiological quality of in natura milk in the municipality of Uruana-GO. Three milk producers were selected and the physicochemical (fat, protein, lactose, ESD, and EST) and microbiological (SCC and TBC) properties of in natura milk were evaluated from 2019 to 2021. Data from the physical- Chemical and microbiological data were obtained from the reports of El Shaday EIRELI Dairy and were tabulated in an Excel spreadsheet and statistical analysis was performed and the results were evaluated by ANOVA and Tukey test at 5% of significance to verify the interaction between the averages. The results of the physical-chemical analysis indicated that, in the 3 years evaluated in the study, the variations in fat, protein, lactose, EST, and ESD were minimal and were within the values recommended by Brazilian legislation. For microbiological analysis, all milk samples did not meet the levels of SCC and TBC established by Brazilian legislation, being above 300 thousand cells/mL and 300 thousand cfu/mL, respectively. Overall, there was a trend in the progressive decrease and emphasis on P3 products with a reduction in CBT of almost 80% throughout the study. The monitoring and technical assistance of milk producers, especially those with lower technological level, must be a constant practice to achieve a better quality product that meets Brazilian legislation.

Key Words: quality control, milk production, SSC

1366T Preliminary studies of the development of a milk protein concentrate containing pre-aggregated whey proteins. A. Schnurr* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Milk protein concentrate (MPC) is one of the preferred ingredients in formulating high-protein products. Increasing the protein content can lead to undesirable functional and sensory attributes due to increased protein-protein interactions. Whey proteins and their interactions with caseins in MPC during heat treatment play a major role. Limiting the casein-whey protein interactions in MPC can result in a novel functionality. This study aims to understand the functionality of a model MPC containing pre-aggregated whey proteins. Two lots of milk whey protein isolate (mWPI) and micellar casein concentrate (MCC) were collected from a commercial manufacturer. The mWPI was hydrated to 8% (wt/wt) protein and stored overnight to ensure complete hydration. The next day, the pH of mWPI solution was adjusted to pH 3 or 7 as per the experimental design. The pH adjusted mWPI solutions were heated to 85°C for 10 min under constant stirring, cooled to 30°C, and viscosity and particle size were measured. No significant ($P < 0.05$) differences were found between the viscosity of mWPI solutions heated at pH 3 or 7. However, mWPI solution heated at pH 7 resulted in a significantly ($P < 0.05$) higher particle size (87.48 ± 3.22 nm) than mWPI heated at pH 3 (74.36 ± 1.33 nm). The pH of heated mWPI solutions were readjusted to 6.8 and viscosity and particle size were measured. Samples heated at pH 3 had a viscosity of 13.28 ± 0.98 mPas and particle diameter of 1,580.34 ± 25.30 nm, which were significantly ($P < 0.05$) higher than the samples heated at pH 7, which had a viscosity of 7.52 ± 0.53 mPas and a particle diameter 99.4 ± 9.86 nm. In the next phase, MCC was rehydrated to 14% (wt/wt) protein and mixed with pH adjusted mWPI heated at pH 3 and 7 to prepare a model MPC. Model MPC prepared with mWPI heated at pH 3 had a significantly ($P < 0.05$) lower viscosity and a significantly ($P < 0.05$) larger particle size than model MPC prepared with mWPI heated at pH 7. Overall, modified MPC containing pre-aggregated whey proteins showed promising differences and potential to use as an ingredient in tailoring the functionality of MPC.

Key Words: whey protein aggregation, functional modification, viscosity

1367T The physiochemical changes during storage of retort-sterilized dairy-based high-protein beverages. B. Zaitoun* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Milk protein concentrates (MPC) are the preferred ingredients in formulating low-acid high-protein beverages (HPB). This study aims to understand the physiochemical changes of low-acid HPB during storage. Two lots of commercial MPC85 were used to prepare beverages containing 8% protein (wt/wt). The formulation was filled in glass bottles, retort sterilized (121°C/15 min), and stored at room temperature. The HPB were analyzed on 0, 7, 14, 28, 42, 61, 88, 109, 140, 161, and 225 d of storage with no shaking applied. On each experimental day, the beverage in each bottle was carefully separated into 4 equal layers from top to bottom and labeled as L1 to L4. Selected physical and chemical analyses were performed on the separated layers, such as total protein (TP), soluble proteins, viscosity, particle size, and zeta potential. Data was analyzed as repeated measures design. The TP of the beverage for all layers at d 0 was 7.86 ± 0.09; as expected, there were not significantly different ($P > 0.05$). During storage, it was observed that the TP content of L1 and L2 significantly decreased to 6.03 ± 0.18 and 7.03 ± 0.58%, respectively. On the other hand, the TP content of L3 and L4 significantly increased to 8.70 ± 0.36 and 9.82 ± 0.69%, respectively. The increase in TP in L3 and L4 suggested the sedimentation of proteins during storage. On d 0, the viscosity of at a shear rate 100 s^{-1} (74.36 ± 3.19 mPa·s), particle size (187.77 ± 1.45 nm), and zeta potential (−34.17 ± 0.99) were not significantly different between the layers. Over storage, the viscosity of L1 significantly increased ($P < 0.05$) up to 140.23 ± 20.79 mPa·s. At the same time, no change was observed in the viscosity of L2. However, a different trend was observed in L3 and L4 as the viscosity in both layers significantly decreased to 47.52 ± 3.19 and 31.34 ± 12.98 mPa·s, respectively. The results conclude that there were compositional differences, especially in the TP content, due to gravity separation between the top and bottom layers during storage and consequently leading to significant changes in the viscosity and other physicochemical properties.

Key Words: sedimentation, beverage, protein

1368T Immunoglobulins concentration and major solids content of bovine colostrum can be accurately determined through mid-infrared spectroscopy. A. Goi[1], M. De Marchi[1], G. Visentin[2], C. L. Manuelian*[3], and A. Costa[2], [1]*Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Legnaro (PD), Italy,* [2]*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (BO), Italy,* [3]*Group of Ruminant Research (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain.*

Colostrum has to be administered to calves as soon as possible after birth to permit the passive transfer of the immunity and avoid negative effects on survival and performance. Moreover, bovine colostrum is an emerging ingredient used by functional food manufacturers and pharmaceutical industry. The narrow-sense quality of colostrum relies on the concentration of immunoglobulins G (IgG), whose determination via gold standard is expensive and time consuming, making the analysis difficult to implement on a large-scale and/or in routine. In the present study we evaluated the predicting ability of mid-infrared spectroscopy (MIRS) as an indirect method for the assessment of IgG and gross composition traits in Holstein cows colostrum (n = 714) collected within 6 h from calving in 9 commercial farms located in Northern Italy. Reference values of IgG concentration and fat, protein, and lactose content were determined through radial immunodiffusion, Verbands Deutscher Landwirdschaftlicher Untersuchungs und Forschungsanstalten VI C15.2.1 method, Kjeldahl, and high-performance liquid chromatograph, respectively. Spectral data, collected using a benchtop instrument (Milkoscan 7 RM, FOSS Electric A/S, Hillerød, Denmark), were used as predictor variables in the partial least square regression analyses. Both cross- and external validation were performed for each trait. Colostrum IgG, fat, protein, and lactose averaged 93.54 g/L, 14.71%, 4.61%, and 2.36 mg/100 mg with a coefficient of variation of 36.21, 23.86, 65.94, and 21.61%, respectively. Overall, the predictive ability of MIRS resulted promising. The coefficient of determination in external validation, in fact, ranged from 0.74 (fat) to 0.89 (protein) and was outstanding (0.84) for IgG. Root mean square errors were 13.39 (IgG), 1.16 (protein), 1.57 (fat), and 0.19 (lactose). Our findings represent a validation of the MIRS technology for a rapid and low-cost colostrum quality assessment and open the debate on the practicability of MIRS models implementation for acquisition of phenotypes of interest.

Key Words: immunity, novel phenotype, MIR

1369T Effect of slicing on the total coliform, *Escherichia coli* and toxigenic *Staphylococcus aureus* counts in mozzarella cheese produced in Tocantins, Brazil. J. Ribeiro Júnior*[1,2], D. Santos[1], Y. Rodrigues[1], B. Dias[1], E. da Silva[1], F. Nunes[1], and A. Alfieri[2], [1]*Federal University of North Tocantins, Araguaína, Tocantins, Brazil,* [2]*National*

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Mozzarella cheese is one of the most widely consumed dairy products both globally and in Brazil. Due to its organoleptic characteristics and for convenience, it is often sliced and packaged for consumption. However, slicing may increase the risk of microbiological contamination, decreasing its shelf-life and posing a potential health risk. This study evaluated the impact of slicing on the microbial quality of mozzarella produced at a dairy facility in Tocantins, Brazil, in October 2022. Ten samples of cheese were evaluated for microbial quality both before and after automated slicing. Aliquots were taken from each piece and homogenized in buffered peptone water. Decimal and serial dilutions were prepared for enumeration of total coliforms, *Escherichia coli*, and *Staphylococcus aureus* using the Compact Dry EC and X-SA (Nissui Pharmaceutical Co.), respectively. *S. aureus* isolates were recovered for the detection of staphylococcal toxins (A–E), toxic shock syndrome toxin, and enterotoxins (A and B) using a previously validated molecular approach. Counts were analyzed using the chi-squared test (Pearson). The mean total coliform count significantly increased from 73 to 109 cfu/g ($P > 0.05$). However, *E. coli* was not detected in either the whole or sliced sample (<10 cfu/g). *S. aureus* counts in whole and sliced cheese were not significant different ($P < 0.05$) at 108 and 127 cfu/g, respectively. A total of 48 and 46 *S. aureus* isolates were obtained from whole and sliced cheese, respectively; one isolate from a sliced sample was positive for staphylococcal toxin C (*seC*), and another isolate from a different sliced sample was positive for toxin D (*seD*). The study demonstrated the significant impact of automated cheese slicing on contamination and the presence of toxigenic *S. aureus* in mozzarella produced in northern Tocantins. Therefore, it is crucial to improve control programs, specifically the hygiene of the slicers and handlers, to reduce the risk of contamination with toxin-producing microbes that threaten consumer safety.

Key Words: environmental contamination, operational self-control, staphylococcal toxin

1370T Development of a novel probiotic dairy product with the mixture of soy and cow's milk.

S. Hekmat* and S. M. Fatima, *Brescia University College, Canada.*

Plant-based milk alternatives represent a growing sector of the functional food industry. Soy milk is a good source of proteins, unsaturated fatty acids, lecithins, minerals, and isoflavones and can potentially increase the health and nutritive values of cow's milk. To investigate the probiotic potential and sensory appeal of the mixture of soy and cow's milk, 4 mixtures were prepared by incorporating 0% (T1), 25% (T2), 50% (T3), or 75% (T4) soymilk in cow milk. The viability of *Lactobacillus rhamnosus* GR-1, and pH were evaluated during fermentation and 30 d of refrigerated storage. Additionally, consumer acceptability was determined through a sensory evaluation. *L. rhamnosus* GR-1 reached viable counts of 10^8 cfu/mL in all samples. Sensory panelists provided higher hedonic scores to T1 for appearance and texture compared with T2–T4, but flavor and overall acceptability ratings among T1–T4 were comparable. These results serve as an indication for the successful fortification of cow and soy milk mixtures with *L. rhamnosus* GR-1.

Key Words: functional food, probiotic

1371T Microstructure and physicochemical properties of goat milk yogurt with fucoxanthin.

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The food industry has increasingly added nutrients and other ingredients to products to enhance their health benefits. Fucoxanthin, a marine carotenoid, is recognized for its benefits in mitigating obesity, diabetes, hypertension, and inflammation. The aim of this study was to analyze the microstructure, particle sizes, chemical composition, water holding capacity (WHC), and firmness of goat milk yogurt with fucoxanthin during storage. Yogurts with and without fucoxanthin were manufactured from goat whole milk (82.85%, wt/wt), powdered goat milk (10.68%, wt/wt), and sugar (6.47%, wt/wt). Fucoxanthin at a concentration of 0.052 mg/g of mix was added to the treatment. The mix was heated at 80°C for 30 min, cooled, inoculated with a culture, and incubated at 43°C for 5 h. The microstructure images, particle sizes, WHC, and firmness were evaluated in the control yogurt (CY) and fucoxanthin supplemented yogurt (FXY) during 4 weeks of storage. Also, the chemical components of yogurts were determined at 0 week according to the AOAC procedures. Confocal laser scanning microscopy and a Multisizer 4E Particle analyzer were used to evaluate the microstructure and particle sizes of yogurts. The experimental data from the physicochemical analyses were analyzed by a 2-factorial design within split plot design. We observed that the mean particle size diameter of FXY was significantly larger ($P < 0.05$) than the CY. Moreover, the FXY had significantly lower WHC and was less firm ($P < 0.05$) than the CY. The microstructure of FXY gel exhibited a heterogeneous protein network with smaller clumps and more frequent empty spaces at both 0 and 4 weeks of storage compared with the control. Thus, this type of microstructure produces a porous protein network that could favor syneresis or a decrease in the WHC and firmness of the treated yogurt. It appears that the carotenoid molecules intervene with the formation of the protein network during processing. However, more investigations are needed to elucidate the details of the protein network formation of fucoxanthin supplemented yogurt and its structure during processing.

Key Words: goat milk yogurt, fucoxanthin, microstructure

1372T Effect of adding xanthan gum on low-fat probiotic yogurt functionality and microbiological quality.

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A wide range of novel dairy products relies, to a large extent, on the rheological functions of stabilizers. Xanthan gum (XG) is a soluble fiber and is used in dairy and food products as a stabilizer and thickener. Low-fat yogurt has a weak body, poor texture, and increased syneresis and these undesirable characteristics are not liked by consumers. The objective of this study was to see the effect of XG addition on low-fat probiotic yogurt (LFPY) properties. The pasteurized Skimmed buffalo milk was heated to 95 ± 2°C/16 s, cooled to 40 ± 1°C, and, then divided into 6 treatment lots. The treatments included: T1 (Control), T2 (0.2% XG), T3 (0.4% XG), T4 (0.6% XG), T5 (0.8% XG), and T6 (1% XG). The mixed starter culture (2%) from *Streptococcus thermophiles* (ST), *Lactobacillus bulgaricus* (LB), and *Bifidobacterium bifidum* (BB) in the ratio1:1:1 were added. Yogurt was manufactured following the standard manufacturing protocol. The experiment was replicated thrice. The samples were microbiologically analyzed for total bacterial count (TBC) on agar- agar, and count of ST, LB, and BB on MRS agar, M17 agar, and MRS-modified, respectively, at 0, 7, 14, and 21 d of storage. Chemical composition, texture, viscosity, and water-holding capacity (WHC) were measured at zero days whereas only viscosity and WHC were measured during the storage. The results obtained showed that the addition of XG in LFYP decreases the pH. There was an increase in the total protein and ash content. As the level of XG increased the viscosity, WHC, and firmness of LFPY increased. The addition of higher amounts of XG led to a significant ($P < 0.05$) decrease in the total bacterial count and led to a significant ($P < 0.05$) increase in counts of ST, LB, and BB during the first 2 weeks of the storage period. The results showed that XG can be used as a stabilizer in the manufacturing of LFPY as well as a prebiotic for starter culture and improve the quality of LFPY.

Key Words: yogurt, low-fat, probiotic

1373T Functional characterization of whey protein concentrates from cow and goat milk cheese whey. A. Syamala, M. Akter, and P. Salunke*, *Department of Dairy and Food Science, Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.*

The goat cheese market is expected to grow at a rate of 3.6% in the next 5 years, though its by-product utilization is moving at a slow pace due to the high acidity of whey. But legislative restrictions on whey disposal encourage the cheese industries to explore it back into the food system as whey protein concentrate (WPC). In addition, the global whey protein concentrate market is projected a rapid growth due to the increasing health consciousness of consumers. The literature on goat cheese whey protein's functional properties is limited. So, this study was designed to evaluate the functional characteristics of commercial goat cheese WPC (GWPC) and compare it with cow cheese WPC (CWPC) powders. Four commercial samples of both GWPC and CWPC were evaluated for their functional properties at 15% protein solution. The functional properties studied included solubility, complex viscosity, gelling ability, and foaming properties. Statistical analysis was performed using GraphPad Prism 5 software. The solubility of the CWPC (94–99%) powders was significantly ($P < 0.05$) higher than that of the GWPC (37–64%) at 5% protein solution. No significant ($P < 0.05$) difference was observed in the overrun and foam stability of GWPC and CWPC. The gel prepared from a 15% protein solution of CWPC (153–760 g force) showed significantly ($P < 0.05$) higher firmness as compared with GWPC (13–87 g force). In the shear strain sweep test (1–1,000 s^{-1}), the complex viscosity of the GWPC powders was significantly higher than that of CWPC at 15% protein concentration. Many of the functional properties were affected by the initial low pH of the GWPC. In conclusion, the GPWC had lower solubility and gel strength but higher complex viscosity and no difference in foaming characteristics compared with CWPC at similar protein concentration. However, some of these properties will open new opportunities for GWPC utilization in high-protein food products as a functional ingredient.

Key Words: goat milk cheese, whey protein concentrate, viscosity

1374T Development of a chocolate milk sensory ballot for use in the dairy industry with consumers in mind. A. Stelick*, R. Dando, M. Wiedmann, and N. Martin, *Department of Food Science, Cornell University, Ithaca, NY.*

Chocolate milk (CM) is an important dairy beverage with broad consumer appeal. Chocolate milk sensory profiles vary widely by formulation and processor, ultimately impacting consumer acceptance. The objective of this research was to improve on a historic expert sensory assessment (ESA) CM ballot that was focused on defect identification per ADSA guidelines for white milk and make it relevant to CM consumers. For this, 18 CM samples were evaluated instrumentally for color parameters and with a trained and a consumer panel (on 6 of 18 CM samples) for sensorial properties after storage for 5 d at 3°C (control [C]) and 8°C (accelerated shelf-life [ASL]). Analysis of variance followed by Tukey's HSD tests at 95% level of confidence and principal component analysis were performed. Significant differences (at 95% LOC) in L*, a*, b* color parameters were found between the samples. The trained quality panel assessed the samples and found they varied widely in color, chocolate character and other sensory attributes. Panelists noted the increase in defect detection and decrease in sweetness and chocolate character, attributes not included in the historic ballot, for ASL samples compared with C samples. Overall liking and quality scores were highly correlated and decreased significantly (at 95% LOC) between the C and ASL samples, when CM samples were evaluated by the consumer panel. While no significant differences in color were identified by consumers, chocolate aroma and flavor significantly weakened, and presence of atypical aromas and flavors significantly increased in the ASL samples. Based on these outcomes the historic ESA ballot was updated to monitor consumer-relevant product attributes across CM shelf-life in addition to defect monitoring in routinely tested chocolate milk samples for 2 years. Using the updated ballot, ESA data from 2 years before and 2 years after the ballot change was compared. While no significant differences (at 95% LOC) in assigned overall quality mean scores were noted between the 2 ballots, the updated ballot allowed for improved tracking of chocolate character changes over shelf-life, which were important to consumer CM overall liking and quality perceptions, and, therefore, provides important feedback to improve CM quality with consumer preferences in mind.

Key Words: chocolate milk, sensory quality, consumer research

1375T Effect of *Lactobacillus plantarum* TD109 on the quality and cholesterol lowering properties of yogurt. M. Zheng, Q. Zhao, C. Man, and Y. Jiang*, *Northeast Agricultural University, Harbin, Heilongjiang, China.*

Yogurt has become one of the popular choices and is considered to be a healthy food because it is an excellent source of nutrients. The increasing popularity of yogurt has led researchers to continuously investigate value-added ingredients such as probiotics, prebiotics and different kinds of bioactive substances to produce functional yogurt that is more beneficial than traditional yogurt. *Lactobacillus plantarum* TD109isolated from traditional Tibetan dairy products has good in vitro cholesterol lowering properties. In this study, *L. plantarum* TD109 was used as the fermenting strain and fermented with commercial starter (*Lactobacillus bulgaricus: Streptococcus thermophilus* = 1:1) to prepare functional yogurt. The effect of *L. plantarum* TD109 on the quality of yogurt and its cholesterol lowering properties was investigated. The results showed that the compound fermentation group (LY group) had stronger fermentation acid production ability, which could improve the curdling speed of yogurt and shorten the fermentation time from 8 h to 6 h. The water holding capacity of LY group was 78.23% and that of control group (CY group) was 72.75%, the water holding capacity of yogurt in the LY group was significantly higher ($P < 0.05$) and the stability of the system was better. The addition of *L. plantarum* TD109 could improve the cohesiveness (from 0.37 to 0.40) and springiness (from 0.89 to 0.96) of yogurt and improve the taste of yogurt ($P < 0.05$). The sensory evaluation showed that the overall score of LY group was higher than that of CY group, which was mainly reflected in the more uniform tissue state and more delicate taste. The addition of *L. plantarum* TD109 increased the live bacteria count of yogurt from 8.14 lg cfu/mL to 9.06 lg cfu/mL ($P < 0.05$), which was 8.32 times higher. The in vitro cholesterol lowering properties of fermented yogurt in the LY group was significantly higher than that in the CY group ($P < 0.05$). Therefore, the addition of *L. plantarum* TD109 is beneficial to improve the quality and cholesterol lowering properties of yogurt, and can provide a theoretical basis for the development of functional yogurt.

Key Words: *Lactobacillus plantarum* TD109, functional yogurt, cholesterol lowering

1376T The protective effects of iron free lactoferrin on lipopolysaccharide-induced intestinal inflammatory injury via modulating the NF-κB/PPAR signaling pathway.

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Research evidence shows that effective nutritional intervention could prevent or reduce intestinal inflammatory injury in newborn infants. Iron free lactoferrin (apo-LF), one of the main types of lactoferrin (LF), is a bioactive protein in milk that plays a vital role in maintaining intestinal health. The potential mechanism by which apo-LF modulates intestinal inflammation is, however, still unclear. In this study, we first explored key genes and pathways in vitro by transcriptome data analysis, and then validated them in vivo to reveal underlying molecular mechanisms. The specific preparation method of apo-LF used in this study is as described in the previous patent "A method for preparing lactoferrin with required iron saturation." Data analysis was performed using GraphPad Prism 8.0. Differences were statistically examined by one-way ANOVA tests followed by Tukey's multiple comparison test. $P < 0.05$ was considered as statistically significant. In addition, each experiment was independently repeated at least 3 times. The results showed that apo-LF pretreatment effectively inhibited lipopolysaccharide (LPS)-induced primary intestinal epithelial cells (IECs) inflammation in the coculture system (primary IECs and immune cells), which was specifically manifested as the reduction of the concentration of TNF-α, IL-6, and IL-1β and increased the concentration of IFN-γ. In addition, transcriptome data analysis revealed that the key pathway for apo-LF to exert anti-inflammatory effects was the NF-κB/PPAR signaling pathway. Further validation was performed using Western blotting in colonic tissues of 32 healthy young male mice and it was found that the major proteins of NF-κB signaling pathway (NF-κB, TNF-α, and IL-1β) were inhibited by apo-LF and the target proteins of PPAR signaling pathway (PPAR-γ and PFKFB3) were activated by apo-LF. Taken together, this suggests that apo-LF has a protective effect against LPS-induced intestinal inflammatory injury via modulating the NF-κB/PPAR signaling pathway, which provides new insights for further anti-inflammatory study of apo-LF.

Key Words: iron free lactoferrin, protective effect, intestinal inflammatory injury

1377T HS-GC-IMS, HS-SPME-GC-MS combined with electronic nose and electronic tongue to analyze the flavor of raw milk in different regions of China.

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Milk as one of the best food resources, played an important part of the human diet. Flavor was the main indicator contributing to sensory attributes as well as consumer acceptance, so aroma and taste were the important influential factors for dairy products quality assessment. Discrimination of origin and its complemented aroma features is important for milk authentication. A total of 24 raw cow milk samples from healthy Holsteins were collected from 24 large and medium-sized dairy farms in 17 Chinese provinces in March, and April 2022 (Herd sizes range from 300 to 30,000). In this study, volatile components and taste characters of samples were analyzed using HS-GC-IMS, HS-SPME-GC-MS, and intelligence sensory technology method (electronic nose and electronic tongue). The HS-GC-IMS results revealed that a total of 29 compounds had been detected. The discrimination effect of electronic nose on samples was consistent with Volatile organic compounds, and W6S (sensitivity to hydrogen) and W3S (sensitivity to long-chain alkanes) sensors played a major role. Electronic tongue results showed that the taste of saltness and richness in the northern sample was more prominent ($P < 0.05$), while the sensory attribute score of umami and bitterness in the southern sample was higher ($P < 0.01$). Partial least squares-discriminant analysis (PLS-DA) based on GC-MS data was revealed a good classifying tool for raw milk from different original habitats. The variable importance in projection (VIP) discussed together with the results of the correlation analysis. Chemometrics analysis showed that significant correlations between milk origin and volatiles compounds including pyridine, nonanal, dodecane, furfural, 1-decyne, octanoic acid, and 1,3,5,7-cyclooctetraene (VIP > 1). The results provided a deeper understanding of volatiles variation of milk in China from different geographic origins and further investigation to explore the mechanism of variation in aroma volatiles accumulation under different geographical origins.

Key Words: volatile organic compound, flavor, raw milk

1378T E-nose, E-tongue combined with GC-IMS to analyze the influence of key additives during processing on the flavor of infant formula.

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The taste development in early infants was closely related to the food composition during pregnancy and lactation, which also affects the food exposure degree and the ability of accepting food during childhood. In this study, we obtained key added ingredients in the production of stage 1 infant formula include raw milk, pasteurized milk, demineralized whey, demineralized whey powder, hydrolyzed whey protein powder, whey protein powder supplemented with α lactalbumin and infant formula and discussed the sensory quality differences through HS-GC-IMS combined with intelligent sensory technology. In total, 41 volatile substances were identified by HS-GC-IMS include 12 aldehydes, 2 alcohols, 11 ketones, 9 esters, 4 olefins, 1 acid, and 2 furans. The W5S (sensitivity to nitrogen oxides), W1S (sensitivity to methyl), and W2S (sensitivity to alcohols, ketones, and aldehydes) sensors of electronic nose had intense responses to the odors, and the differences in flavor were mainly distinguished by them. Partial least square regression analysis was used to simplify the data structure and further explained the potential relationship between the sensors and volatiles identified by HS-GC-IMS. Electronic tongue result shows that raw milk and pasteurized milk had lower response to astringency aftertaste. In terms of bitterness attributes, all samples had higher response compared with reference solution, and demineralized whey powder samples had the most prominent response. The response of

the samples to the sourness were all below the tasteless point, which was not within the perception range. The amount of characteristic volatile compounds in raw milk were much higher than others. Raw milk is an essential control point in the flavor formation process of stage 1 infant formula milk powder and determines the sensory quality. Demineralized whey powder is the primary source of potential off-flavor components in hydrolyzed milk protein infant formula. Through the rational application of HS-GC-IMS, combined with intelligent sensory technology, the in-depth understanding of the flavor formation of infant formula in the production line can provide potential ideas for improving processing methods, quality control, and optionally added excipients.

Key Words: infant formula, headspace-gas chromatography-ion mobility spectrometry, volatile organic compound

1379T The production of an infant formula with a minimally processed route impacts its nutritional, physiological and sensorial qualities. A. Deglaire[1], N. Leconte[1], A. Blais[2], J. Calvez[2], C. Delteil[2], F. Blachier[2], G. Lucchi[3], K. Gourrat[3], E. Szleper[3], V. Feyen[3], G. Gesan-Guiziou[1], R. Jeantet[1], D. Dupont*[1], S. Nicklaus[3], A.-M. Davila[2], *[1]1STLO, INRAE, Institut Agro, Rennes, France, [2]PNCA, INRAE, AgroParisTech, Université Paris-Saclay, Paris, France, [3]3CSGA, AgroSup Dijon, CNRS, INRAE, Université Bourgogne Franche-Comté, Dijon, France.*

Many infants receive infant formulas (IFs), the most nutritionally adequate substitute to human milk. However, health effects still differ between these 2 foods. IF production requires multiple unit operations, particularly heat treatments leading to protein denaturation/aggregation and Maillard reaction. Recently, a minimal processing route allowed the semi-industrial production, in duplicate, of a bacteriologically safe IF, with a high content of native proteins and stable over time. The objective of the present study was to evaluate its organoleptic, nutritional and physiological properties as compared with a high heat load IF. Two complete powdered IFs were produced from microfiltered bovine milk and whey protein concentrate (protein wt:wt ratio of 50:50), with no heat treatment (T−, i.e., minimally processed) or successive heat treatment (T+++; 72°C/30 s, 90°C/2 s, 85°C/2 min), resulting in a protein denaturation extent of 4.4 ± 0.4% and 60.0 ± 0.1%, respectively, based on soluble N % determined by the Kjeldahl method. Their organoleptic properties were determined by aroma analysis (gas chromatography/mass spectrometry) and sensory evaluation in human adults (n = 50). The digestive deconstruction of IFs was followed through in vitro dynamic digestion. The true protein digestibility and the impact on intestinal homeostasis and microbiota were determined in young rats (n = 6–8). The organoleptic quality of the IFs significantly differed (triangular test, $P < 0.0001$), with a higher aroma compound concentration in T− than in T+++. The IFs presented a different microstructure, leading to larger aggregates for T− during its in vitro gastric digestion. T− tended to have a higher in vivo true protein digestibility than T+++ (96.6 ± 5.4% vs. 91.9 ± 5.4%, P-value = 0.09) such as for plasma amino acid concentration 1-h postprandially (P-value < 0.001). T− increased dietary intake and weight gain, decreased the cecal ammonium content, and modified the cecal microbiota species in rats. This study demonstrated that the minimally processed IF (T−) had different organoleptic, nutritional and physiological properties, including an enhanced protein bioavailability. Such innovative IF may be a further step toward human milk biomimetics.

Key Words: infant formula, nutrition, digestion

1380T *In vitro* infant digestion model leads to similar conclusion as *in vivo* study: Focus on human milk and infant formula protein digestion. E. Charton[1,2], O. Menard[1], M.-F. Cochet[1], Y. Le Gouar[1], J. Jardin[1], G. Henry[1], J. Ossemond[1], P. Moughan[3], C. Montoya[3,4], A. Bellanger[5,6], D. Dupont*[1], I. Le Huërou-Luron[2], and A. Deglaire[1], *[1]UMR STLO, Institut Agro, INRAE, Rennes, France, [2]Institut NuMeCan, INRAE, INSERM, Univ Rennes, Saint Gilles, France, [3]Riddet Institute, Massey University, Palmerston North, New Zealand, [4]Smart Foods Innovation and Bioproducts Innovation Centre of Excellence, AgResearch Limited, Palmerston North, New Zealand, [5]CHU Rennes, Pediatrics Department, Rennes, France, [6]University of Rennes 1, Faculty of Medicine, Rennes, France.*

Human milk (HM) and infant formula (IF), differing in fine composition and structure, are assumed to present different digestion kinetics, while rarely directly compared. Studies on HM or IF digestion have been mainly conducted using in vitro digestion models in separate studies; however, only a limited number of studies have directly compared these 2 types of infant diet digestion and the 2 types of digestion models (in vitro vs. in vivo). Therefore, the present study aimed to compare food deconstruction and protein digestion of HM and IF in the mini-piglet or in an in vitro dynamic gastrointestinal system (DIDGI), both simulating human term infant digestion. Eighteen mini-piglets, randomly allocated into 2 groups, were fed a mature HM (pool 15–20 volunteer mothers) or a standard IF during 6 d. After being fed 6-times hourly, piglets were euthanized 30 min after their last meal to collect digesta along the digestive tract to study in vivo digestion. In parallel, the same meals were digested in triplicate using an in vitro dynamic model parametered to a term infant. Digesta were sampled regularly during the gastric and intestinal phases from 0 to 180 min. Digesta collected either in vivo or in vitro were analyzed in terms of structure, proteolysis degree, and peptide release. Differences between groups were assessed using a 2-way ANOVA for repeated measures for the in vitro data and a one-way ANOVA for the in vivo data. The microstructure of the digesta differed between HM and IF in a similar manner in vitro and in vivo along digestion. Proteolysis of the common HM and IF proteins, caseins and α-lactalbumin, was significantly slower for HM than for IF in both models. The intestinal degree of proteolysis was slower for HM than IF during the first 2 h in the in vitro model. In overall, the in vitro dynamic gastrointestinal digestion model and the in vivo digestion of HM and IF were in good agreement. Further research is required to better evaluate the in vitro protein digestibility.

Key Words: human milk, infant formula, digestion kinetics

1381T Preparation of synbiotic milk powder and its improvement on calcium absorption and bone microstructure in calcium deficient mice. M. Jia*[1], J. Luo[1], B. Gao[1], Y. Huangfu[1], Y. Bao[1], D. Li[1], and S. Jiang[2], *[1]Northeast Forestry University, Harbin, Heilongjiang Province, China, [2]Heilongjiang Feihe Dairy Co., Ltd., Beijing, China.*

With the aging population, osteoporosis has become one of the most common chronic diseases in the world. Calcium deficiency is the main cause of osteoporosis. Adequate calcium intake can improve calcium deficiency and prevent osteoporosis. This study selected 4 prebiotics of *Lactobacillus plantarum* JJBYG12 and inulin (INU), xylo-oligosaccharide (XOS), fructo-oligosaccharide (FOS), and isomalto-oligosaccharide (IMO). The results showed that the addition of 1.2% IMO had the best effect on the screening of prebiotics in vitro. The number of viable bacteria and calcium enrichment of *L. plantarum* JJBYG12 were 7.43 ± 0.036 cfu/mL and 60.79 ± 0.69 mg/g. Compared with the blank control group, it was significantly improved ($P < 0.05$).

Synbiotic milk powder (SMP) was prepared by combining *L. plantarum* JJBYG12, 1.2% IMO and milk powder. One hundred twenty (60 males and 60 females) 4-week-old Kunming mice were fed a low-calcium diet for 4 weeks to establish a calcium deficient mouse model. SMP was continuously administered for 4 weeks, the normal control group was fed a normal calcium diet and gavaged with deionized water. The remaining groups were fed low-calcium diets and gavaged with deionized water. The positive control group used $CaCO_3$, and the calcium content was the same as that of the SMP medium dose group. After 8 weeks, compared with the low calcium control group, the apparent calcium absorption and calcium retention rate were 58.5 ± 0.41% and 62.45 ± 0.43% ($P < 0.05$), the serum calcium and phosphorus levels of mice in the high-dose SMP group were 3.23 ± 0.11 mmol/L and 2.54 ± 0.19 mmol/L ($P < 0.05$). ALP activity and CTX-1 level were 9.85 ± 1.71 King unit/100 mL and 2.56 ± 0.16 ng/mL ($P < 0.05$), respectively. After dissection of mice, the length and diameter of femur were found to be 16.02 ± 0.66 mm and 1.66 ± 0.04 mm ($P < 0.05$), respectively. The femur index and bone calcium content were 0.62 ± 0.04% and 273.61 ± 1.87 mg/g ($P < 0.05$), respectively. The micro-CT results showed that the number of trabeculae in mice increased, the connection was tight, and the reticular structure was clear and dense. H&E staining showed that the number of osteoblasts in the femur increased and the bone trabeculae in the shaft were closely arranged. The above results show that SMP has good dietary calcium supplement ability, which can improve bone density, bone metabolism, and bone remodeling in long-term calcium deficient mice.

Key Words: milk powder, synbiotics, BMD

1382T Frequency of milk beta-casein gene in Holstein cows in Korea and its relationship with energy balance during early lactation period. E. Jeon[1], S. Cho[2], J.-K. Son[1], S. Kim[1], B. Chae[2], I. Cheon[2], H.-R. Park[3], and N.-J. Choi*[2], [1]*Dairy Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan, Korea,* [2]*Department of Animal Science, Jeonbuk National University, Jeonju, Korea,* [3]*Korea Agriculture Technology Promotion Agency, Iksan, Korea.*

The most common types of β-casein in Holstein cow's milk are A1 and A2, which differ in one amino acid (A1; histidine, A2; proline) at position 67 of the chain. The present study investigated whether cows producing milk with different types of β-casein genes would have different energy balance during early lactation periods. Data were collected from 307 Holstein cows on a dairy farm located in Cheonan, Korea. All cows were housed in free stall facilities and were milked by an automatic milking system (Lely, Astronaut) providing daily body weight and milk composition. For energy balance (EB), daily body weight changes (dBW, gain vs. loss), net energy lactation in milk (NEl, increase vs. decrease), and phase of days in milking (0 ~50 d, 51 ~100 d, 101 ~150 d) were investigated and a total of 12 variables (2´2´3) were generated. The frequency of dBW and NEl in milk for each phase of days in milking was calculated. The effect of β-casein gene types on EB was analyzed by permutational multivariate ANOVA using the distance matrix method. Out of 307 animals, 34 (11.07%) were of A1A1 genotype, 157 (51.14%) were of A1A2 genotype, and 116 (37.79%) were of A2A2 genotype. As a result, there was no significant effect of genotypes on EB variables in Holstein cows. This study was supported by a grant from the "Cooperative Research Program for Agriculture Science and Technology Development (project No. PJ01620002)" and 2023 the RDA Fellowship Program of National Institute of Animal Science, Rural Development Administration, Republic of Korea. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2022R1I1A3068293).

Key Words: beta-casein, dairy cow, energy balance

1383T A novel biorelevant in vitro dynamic digestion simulator reproducing the biomechanics of the gastrointestinal tract. I. Greco, O. Menard, J. Feng, J. Lee, S. Le Feunteun, R. Jeantet, and D. Dupont*, *INRAE, Institut Agro, STLO, Rennes, France.*

The biomechanics of the gastrointestinal tract influence food disintegration and digestion in vivo. Most currently available in vitro dynamic digestion models lack, however, the ability of mimicking the oro-gastro-intestinal morphology and contractions, possibly overlooking the effect of mechanical forces in the digestive process. Recently the near real digestive tract (NERDT), a biomechanically-relevant digestion simulator equipped with a silicon real-size model of the human stomach has been developed. The system mimics the oro-gastrointestinal morphology and anatomical structures, biochemical environments, peristaltic contractions and dynamic aspects present in vivo in adult humans through a set of rollers that can be controlled precisely. The objective of the present work was to investigate the in vitro gastric digestion of milk (200 mL + 50 mL simulated salivary fluid, SSF), yogurt (200 g + 50 mL SSF) and cheese (100 g + 100 g water + 50 mL SSF) using the NERDT system and compare the gastric emptying, the proteolysis and pH evolution in the stomach obtained with the simulator with data collected on humans available in the literature. Gastric emptying was assessed by quantifying the dry matter released from the stomach whereas pH was monitored in real-time thanks to a pH catheter. Overall, the kinetics of gastric emptying and pH evolution in the stomach obtained with the simulator were in good agreement with those observed in vivo on human volunteers in the literature. The initial pH values for each matrix were 6.6 ± 0.7 for milk, 4.3 ± 0.3 for yogurt and 4.7 ± 0.5 for cheddar cheese and pH 2 was reached at 27, 40 and 140 min for milk, yogurt and cheese, respectively. These differences were due to differences in gastric emptying rate, milk being emptied faster than yogurt itself emptied faster than cheese. Proteolysis was qualitatively investigated by SDS-PAGE. Quantification of released free amine groups gave values of 2.8 ± 0.2 mg for milk, 19.5 ± 2.8 mg for yogurt and 353.5 ± 4.5 mg for cheese. In the future, this novel biorelevant in vitro dynamic digestion simulator will allow to better understand the relative importance of biomechanical contractions compared with enzymatic and physicochemical reactions in the digestive process and how these mechanical forces change the structure of the food bolus over time.

Key Words: digestion, simulator, biomechanics

1384T Milk's fatty acid profile due to the inclusion of pasture in the diet of dairy cows fed with a total mixed ration. G. Casarotto*[1], P. Véliz[1], A. López[1], C. Bonfiglio[1], I. Vieitez[2], A. Britos[1], J. L. Repetto[1], S. Carro[1], and C. Cajarville[1], [1]*Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay,* [2]*Facultad de Química, Universidad de la República, Montevideo, Uruguay.*

Some studies showed that combined systems which partially replace a total mixed ration (TMR) with fresh grasses, improve milk composition, but there are still few studies with grazing alfalfa. The aim was to evaluate the impact of partially replacing a TMR with fresh alfalfa in lactating cows on the milk's fatty acid profile (FA). Twelve multiparous cows were used in a change-over design (2 treatments and 2 consecutive periods), and randomly assigned to one of 2 feeding strategies: T1) 100% TMR; T2) 60% TMR + fresh alfalfa grazed without limit of quantity. Each experimental period lasted 26 d (21 d adaptation, 5 d sampling). Fat was extracted from individual milk samples, and FA profile was determined by gas chromatography. Data were analyzed by ProcMixed and means separated by LSMeans (SAS). The model included the effect of the period (P1 and P2) and the sequence of treatment application. Differences between periods were observed for some FA, which could be due to some changes in the diet composition during the study, a common problem in this kind of design. On the other hand, no differences were found in the sequence of treatment. The UFA:SFA ratio did not increase, but important changes in the milk FA profile were observed. The levels of α-linolenic (18:3 *c*9, *c*12, *c*15), rumenic (CLA, 18:2 *c*9, *t*11), and vaccenic acid (TVA, 18:1 *t*11) were significantly higher on T2 ($P < 0.05$), consistent with the use of pasture (Table 1). The α-linolenic in humans reduces the risk of chronic health issues, and rumenic acid has been shown to bring strong physiological functions, especially anticarcinogenic effects.

Key Words: CLA, milk fat composition, feeding strategy

1385T Characteristic chromatographic fingerprint study of isotopes, mineral elements and flavor in milk in Ningxia province of China. H. Guo, D. Bu, and Lu Ma*, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Milk produced in Ningxia province of China has shown better quality and sensory attributes than the average level of China, which is mainly attributed to the particular location and environment. In this study, milk, soil, and water samples were collected from 29 farms in 5 regions (Shi Zuishan, Yin Chuan, Qing Tongxia, Wu Zhong, Zhong Wei) of Ningxia province and 3 farms in Beijing Changping suburb to investigate their regional differences in isotopes (δ^{13}C, δ^{15}N, δ^{2}H, δ^{18}O) mineral elements, and milk flavor. One-way ANOVA test, correlation analysis, principal component analysis (PCA) and stepwise canonical discriminant analysis (CDA) were administered by R studio 4.2.1. The results showed that most mineral element concentrations in milk were significantly regionally different except for the contents of B, Mo, Cr, and As. Milk δ^{2}H and δ^{18}O contents in milk were positively correlated with the contents those in water ($R^2 = 0.51$ and 0.65, respectively), and the δ^{15}N in milk was positively correlated with that in soil ($R^2 = 0.51$). Significant positive correlations among milk, soil, and water were obtained through typical correlation analysis based on element analysis ($R^2 = 0.99$, 0.99, and 0.76, respectively). Finally, 4 key variables (K, Na, Ca/Sr, δ^{2}H milk water) selected by CDA with 84.4% of classification rate were enrolled to distinguish the regional sources of milk. Raw milk flavor analysis which was conducted by SPME-GC-MS showed that the contents of aldehydes, acids, alkanes, and aromatic heterocyclic compounds in Ningxia province were higher than those in Beijing, while the number of volatile flavor compounds in Ningxia milk was also higher than those in Beijing milk (116 vs. 97). 2-nonanone, methyl 4-sunflowerate, propionamide, and ethyl acetate made the most contribution to the difference with higher F values (F = 6,279.83, F = 1,132.62, F = 6,279.83, F = 937.37, F = 153.56). Overall, the milk quality in Ningxia province is related to local soil and water features. K, Na, Ca/Sr, and δ^{2}H in milk and water, and 2-nonanone, methyl 4-sunflowerate, propionamide, and ethyl acetate were proper indicators to identify the geographical sources of Ningxia milk.

Key Words: isotope, mineral element, milk flavor

1386T Cheese aroma perception: The link between cognitive and autonomic nervous system responses. J. Snow*, D. Olmos, M. Pedroza, and C. Licon, *Fresno State University, Fresno, CA.*

Cheese is a product that can develop a wide range of texture, flavors, and aromas. It is well known for eliciting strong positive and negative reactions in consumers. Consumers find the taste or aromas to be pleasant, while others find them overwhelming in aged cheeses. This response to the aromas is not entirely understood, it may be influenced by intensity or the valence of the aroma compounds. In light of this, the objective of this study is to evaluate the relationship between behavioral responses and physiological responses to cheese aromas to gain a better understanding of consumers' acceptance. For this purpose, 20 panelists were recruited to smell 7 aroma compounds that are commonly found in cheese. The concentration of the aromas was calculated to be equal in intensity. The participants were asked to rate the intensity (scale 1 to 9) and pleasantness (scale −4 to 4). E-PRIME was used to time the presentation of the aromas. While the panelists were self-reporting their ratings, they were connected to a wireless BIOPAC System to simultaneously monitor the physiological reactions to the aromas. The 4 parameters of interest were electrodermal activity (EDA), electrocardiography (ECG), facial electromyography (EMG), and respiration rate (R). The results from the self-reported ratings showed that isovaleric acid and hexanoic acid had the highest intensity ratings and lowest pleasantness ratings. While ethyl

Table 1 (Abstr. 1384T). Milk fatty acid profile from individual cows (g/100 g of total FA)

						P-value	
Item	T1	T2	P1	P2	SEM	T	P
14:0	12.26	11.88	12.38^{a}	11.77^{b}	0.282	0.113	0.019
16:0	34.81	35.73	34.92	35.62	0.919	0.051	0.122
18:0	8.59^{a}	8.06^{b}	8.67^{a}	7.98^{b}	0.312	0.008	0.002
18:1 *c*9	16.26	16.27	16.09	16.45	0.477	0.979	0.359
18:1 t11 (TVA)	1.59^{b}	1.91^{a}	1.67	1.83	0.063	0.004	0.106
18:2 *c*9, *c*12	1.78	1.72	1.76	1.75	0.072	0.210	0.737
18:2 *c*9, *t*11 (CLA)	0.62^{b}	0.79^{a}	0.66^{b}	0.75^{a}	0.027	0.001	0.028
18:2 *t*9, *t*12	0.88^{b}	0.94^{a}	0.87^{b}	0.94^{a}	0.031	0.031	0.010
18:3 *c*9, *c*12, *c*15 (α-linolenic)	0.41^{b}	0.55^{a}	0.50	0.46	0.029	0.004	0.205
∑ 18:2 *trans*	2.46^{b}	2.85^{a}	2.54^{b}	2.77^{a}	0.082	0.003	0.041
SFA	71.57	70.90	71.62	70.85	0.618	0.194	0.142
MUFA	19.01	19.06	18.82	19.26	0.472	0.905	0.300
PUFA	2.20	2.27	2.26	2.20	0.091	0.214	0.278

^{a,b}Statistical differences ($P \leq 0.05$).

butyrate and 2,3 butanedione were rated the highest in pleasantness. These results are in line with results from a previous study. A Spearman's correlation showed no significant correlation between the intensity of the aroma and the rating of pleasantness (r = −0.4285714). A principal component analysis (PCA) was conducted on the self-reported ratings and processed physiological data. The correlations between variables and factors indicated positive correlations between pleasantness (0.612), ECG (0.619), and EMG (0.680 and 0.749) for component 1, whereas intensity was negatively correlated (−0.853). The results indicate that there is a strong influence of pleasantness in consumer determinations of preference and acceptance.

Key Words: cheese, aroma, sensory evaluation

1387T Texture survey of commercial butters and its correlation to their chemical composition.

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Butter is a dairy product which is eaten as a spread or used as a cooking ingredient. Spreadability is a characteristic desired by consumers. The objective of this study is to compare the texture and fat composition of commercial butters to explore potential principles that explain its rheological properties. A total of 23 samples of butter (12 salted, 11 unsalted) from 12 brands (5 organic, 7 non-organic) were purchased in Columbus, OH. The firmness and work of shear were analyzed with a TA.XT.plusC Texture Analyzer at 10°C. Fat content was determined volumetrically by centrifuging butteroil, and a gas chromatographer equipped with a flame ionization detector (FID) was used to analyze the fatty acids profile of the butters. The results were analyzed with an ANOVA and Fisher's LSD as post hoc test. There were not overall significant differences (P-value >0.05) in firmness, work of shear, total fat, or saturated fat between organic or non-organic butters, nor between salted or unsalted butters. However, there were significant differences (P-value <0.05) between individual samples. In the samples, firmness (in N) ranged from 106.00 ± 3.17 to 243.10 ± 8.02, work of shear (in N·s) ranged from 237.97 ± 12.45 to 565.70 ± 29.49, fat content (%) ranged from 81.15 ± 1.26 to 88.50 ± 0.69, the unsaturated fat content (%) ranged from 26.70 ± 0.46 to 30.78 ± 0.24. Surprisingly, there were non-organic butters that had a lower shear force than some organic butters despite having a similar unsaturated fat content. Also, it's interesting to note that when comparing the salted to unsalted types of butter from the same brand, there were significative differences (P-value <0.05) in work of shear: 7 out of 11 brands showed that salted butter had higher work of shear. Regarding spreadability, unsalted butter tended to be easier to spread than salted butter. Also, it's important to note, grass-fed butter was not necessarily more spreadable than conventional butter, which could indicate that there are other variables inherent to the product that influence its spreadability.

Key Words: butter texture, unsaturated fat

1388T Differential scanning calorimetry melting profile of butterfat in relation to spreadability.

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The melting profile of butterfat relates closely to how butter spreads at a given temperature. Spreadability (hardness) is generally considered an important attribute of butter or other dairy spreads and can be affected by a cow's diet. Previous work shows that a diet of palmitic acid-enriched supplement (PAES) on dairy cows increases milk, protein, and lactose yields and linearly increases fat yield and energy output ($P < 0.01$) compared with a control diet. Our objective was to measure the spreadability profiles of butterfat from the milk of cows' (n = 12) feed-controlled diets and correlate them with the melting profile of the aforementioned butterfat. Spreadability (g) was determined through texture profile analysis (TPA) using constant speed penetrometry at 10°C. Melting profiles (total enthalpy, J/g; peak temperature, g) were determined through differential scanning calorimetry (DSC) at 2°C/minute increase. The resulting TPA was grouped into high, medium, and low hardness, then compared with total enthalpy for each sample. A representative sample of high hardness was 32,579.0 g and 29.0 J/g (14.7°C). A representative sample of medium hardness was 24,914.4 g and 21.1 J/g (13.8°C). A representative sample of low hardness was 16,105.2 g and 14.1 J/g (11.8°C). A correlation (r > 0.96) between spreadability and melting characteristics of butterfat, with differences between each cow's production, was determined for all data. Statistical analysis (paired t-test; $P < 0.05$) shows definite patterns between the 2 attributes, and how changes in the diet fed to each cow affect each attribute. In conclusion, the spreadability of butterfat is related to its melting profile and peak temperature and decreases with increasing fat yields from diet. These findings demonstrating the impact of a cow's diet on butterfat properties may provide dairy producers more control when processing and developing new dairy products.

Key Words: spreadability, enthalpy, butterfat

1389T Impact of different molecular weight hyaluronic acid on the functional characteristics of process cheese products at different moisture levels.

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Hyaluronic acid (HA) a naturally derived polysaccharide has been shown to impact milk protein functionality. HA has been approved to use as a food additive in many countries. It is found that HA binds well with water and forms a highly viscous gel. However, it has been reported the functional properties of milk protein change based on the molecular weight (MW) of HA. The objective of the research was to evaluate the effect of different MW HA on process cheese products (PCP) functionality with different moisture content. PCP was manufactured without emulsifying salt using 0.15% of 8 kDa (T2), 320 kDa (T3), 980 kDa (T4), and 2,500 kDa (T5) HA. Each treatment was manufactured with 3 moisture levels (51%, 54%, and 57%). The PCP was formulated for 16% protein, 17% fat, and 1.8% salt. Control PCP (T1) was manufactured using emulsifying salt (sodium aluminum phosphate) with 3 moisture levels. PCP was analyzed for the modified Schreiber melt test, hot viscosity in a rapid visco analyzer, and penetration hardness test with a texture profile analyzer. The experiment was replicated twice. The statistical difference ($P < 0.05$) was determined by 2-way ANOVA using R software 4.2.2. As the MW of HA increased, the hardness increased significantly ($P < 0.05$) at 51% and 54% moisture. Hardness was similar for all samples at 57% moisture except T1. At all moisture levels, there was no significant difference found in the hardness of T4 and T5 whereas it was significantly lower in T1. As the MW increased, hot viscosity increased significantly ($P < 0.05$) at 51% moisture but reduced significantly at 54% moisture and no significant change was observed at 57% moisture. As the moisture increased, the hot viscosity decreased in all the samples. In the modified Schreiber melt test, both T4 and T5 showed the lowest melt area as compared with T2 and T3 at 51% and 54% moisture. At each moisture level, T1 had a significantly ($P < 0.05$) higher melt area. The MW of HA and moisture significantly impacted the functional properties of PCP.

Key Words: hyaluronic acid, process cheese product, functional property

1390T Effects of feeding cows a high omega-3 fatty acids diet in milk and dairy products quality. C. Licon*, D. Olmos, D. Hidalgo, and K. Thompson, *Fresno State University, Fresno, CA.*

Omega-3 fatty acids are known for their health benefits thus the interest to increase their content in dairy products. A few studies have explored the enrichment of dairy products with vegetable sources and animal sources of omega-3, however, the latter is known to produce a "fishy" taste. In this sense, the objective of this project is to evaluate the quality of milk and dairy products by feeding Holstein and Jersey cows with an omega-3 enriched diet. For this purpose, the treatments included control consisting of 20 Holstein (HC) and 20 Jersey cows (JC), enriched omega-3 feed at an 8% ratio to 20 Holstein Cows (H8%), and enriched omega-3 feed at a 6% ratio to 20 Jerseys (J6%) and 20 Holstein cows (H6%). The feeding trial was conducted over 90 d. At the end of the trial, the milk was collected and cheese, cream, and butter were obtained from the control milk, H8%, and J6% to identify potential off-flavors. For all samples, a fatty acid profile was completed using method AOAC 996.06 (2012). ANOVAs were used for statistical analysis. Results showed that no differences were found for the dry matter intake with each treatment, mean milk production significantly increased in J6% but not in H8%. No significant differences were found for the omega-3 fatty acid concentration in milk but the content was higher at the end of the trial for J6% and H8%. For dairy products, the content of omega-3 was higher in H8% and J6% cheeses compared with control by 2.16 and 1.71 times respectively, while cream and butter also showed an increase in the concentration of omega-3 for H8% and J6% compared with the control treatment. In conclusion, the results obtained from this study are promising as the mean milk production was significantly increased in Jerseys fed with LinPro-R at a rate of 6%, increasing pounds of fat, and protein. The omega-3 fatty acids were concentrated in cheese, cream, and butter.

Key Words: omega-3, milk, cheese

1391T Novel heteropolysaccharide-producing lactic acid bacteria with promising effects on the texture of model yogurts. C. Marchand*[1], S. Nahali[1], M.-H. Lessard[1], M. Lafantaisie[1], S. Fraud[2], D. Miller[3], S. L. Turgeon[1], and S. Labrie[1], *[1]STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Quebec, Canada, [2]Yoplait, Vienne Technical Center, Vienne, France, [3]General Mills, Minneapolis, MN.*

With the growing demand for clean label products, yogurt manufacturers search for alternatives to texturizing agents such as starch and gelatin. Lactic acid bacteria (LAB) are a promising solution because some naturally produce exopolysaccharides (EPS) with similar texturizing effects. However, their selection is laborious and requires the systematic screening of many LAB strains. Before this work, a bioinformatics pipeline was developed to cluster LABs with the potential to produce EPS. The aim of this project is to evaluate, based on their predicted potential for EPS production, the impact of adding adjunct LAB strains on the texture of set yogurt model. Eight strains from different genetic clusters were chosen based on the diversity of the EPS potentially produced. Preliminary screening of their ropiness was performed on MRS agar supplemented with 4% sucrose, incubated for 3 d at 37°C and 5 d at 15°C and 20°C. These 8 adjuncts were then inoculated individually at 10^5 cfu/mL in reconstituted skim milk (RSM) containing 4% sucrose. RSM was acidified using 1.4% glucono-delta-lactone (GDL) at 37°C for 7 d, without starter cultures. After 1 and 7 d of incubation, pH, viscosity, adhesivity and microbial population were measured. To better evaluate the effects of adjunct strains, uninoculated acidified RSM controls were done. *Lactiplantibacillus pentosus* LMA-1507 increased both the apparent viscosity at a shear rate of 100 s^{-1} (+0.18 Pa·s) and the adhesivity (+1.5 g·s) after 7 d, compared with the control, while *Lactobacillus helveticus* LMA-1509 increased the apparent viscosity at 100 s^{-1} as soon as d 1 (+0.08 Pa.s). *Lactobacillus delbrueckii* LMA-1497 showed a higher adhesivity after 7 d of fermentation (+2 g·s). Future works will consist of evaluating the biocompatibility of the selected strains with traditional yogurt starters, and to better characterize the EPS genomic clusters of the adjuncts. These results will help improve the efficiency of LAB selection producing texturizing EPS in yogurts.

Key Words: yogurt, exopolysaccharide, lactic acid bacteria

1392T Investigating relationship between composition and stickiness of different types of cheese slices by tack and wear measurements. S. B. Immadi*[1], N. Pace[1], A. Parhi[1], H. Esphari[2], and P. Sharma[1], *[1]Utah State University, Logan, UT, [2]Tillamook County Creamery Association, Portland, OR.*

Stickiness and broken edges are the most common issues related to cheese slicing operations. Cheese slices tend to stick to the moving blades, as well as between themselves, leading to various issues like food wastage and inconvenience for customers. In this study, we investigated whether measurement of the tackiness and the wear behavior is relevant to cheese sliceability and machinability. Tackiness is the sticking tendency of cheese to metal surfaces and wear behavior is the ability of cheese slices to withstand rotational shear and normal stresses. Five commercial varieties of sliced cheese were obtained (Mild Cheddar, Medium Cheddar, Mozzarella, Swiss, and Gouda) and examined in triplicate for fat, protein and moisture content using standard methods. Wear behavior of the samples was measured in terms of mass loss and penetration depth. These values were determined using a pin-on-disk wear attachment (PID 44) on a MCR 302 rheometer (Anton Paar, Graz, Austria) by applying a sliding velocity of 50 mm/s and 1 *N* normal force at 5°C. A parallel plate (PP 50) measuring system attached to the MCR 302 was used to assess the tackiness of the cheese slices. These tests were conducted by applying 10 N normal force for 60 s and using a separation speed of 5 mm/s from the cheese surface. The tack force was recorded as the maximum normal force needed to separate 2 surfaces that are in temporary contact, and the tack energy was calculated as the amount of work required to accomplish the separation. Significant differences between samples were determined using one-way ANOVA at 5% of level of significance. Significant differences ($P < 0.05$) were observed in composition and material properties of the 5 cheese types. Among the samples, Gouda (protein content 24.7%) exhibited the highest amount of tack force (12 *N*) and tack energy (353.46 mJ/m^2) while Swiss cheese (protein content 28.97%) exhibited lowest amount of tack force (6.35 *N*) and tack energy (180.37 mJ/m^2). Similar trends were observed for the wear measurements of the cheese slices. Penetration depth and mass loss results for Gouda and Swiss were 1.28 mm, 0.15 mm and 0.55 g, 0.06 g, respectively. For all cheese types, moisture content alone didn't correlate well with tack or wear measurements, but protein-to-moisture ratio correlated well (negatively, $r = -0.70$) with the mass loss. A strong positive correlation ($r = 0.94$) was found between tack energy and wear behavior of the cheese slices. Protein content of the slices was negatively correlated to the mass loss of the cheese slices ($r = -0.79$). Higher values of mass loss, penetration depth, and tack energy indicate poor slicing ability of cheese. These results suggest that evaluation of wear behavior and tackiness of cheese can be helpful in providing useful insights to cheese slicing operations. This could be used to improve the quality and consistency of cheese slices in dairy industry and may help to reduce the wastage by optimizing the cheese slice quality.

Key Words: cheese, tack energy, slicing

1393T Ice crystallization reduction strategies in ice cream: Use of cellulose-extract from agricultural biomass.

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Ice cream is a popular frozen dessert. The global ice cream market was worth $57 billion in 2017 and is projected to increase to $75 billion by 2024. The flavor and textural attributes of ice cream critically dictate consumer acceptance. Incidentally, temperature fluctuations cause detrimental effects on the quality, mainly due to the formation of ice crystals, causing a gritty and icy texture and affecting the mouthfeel and organoleptic properties. Stabilizers such as guar gum or locust bean gum could help ebb the ice crystal growth; however, high prices and sustainable supply are a couple of hindrances. To this viewpoint, cellulose extract from agricultural residues, agricultural byproducts, and other biowastes aids significantly. The study aimed to evaluate the effect of cellulose from corn stover, spent coffee grounds, lawn grass, and banana peel in ice cream. The ice cream was prepared using the standard protocol with 0.1, 0.2, and 0.5% cellulose extract. The microscopical ice cream studies were carried out using optical microscopy with temperature control and camera attachment, and crystal sizes were measured using image analysis protocol. The experiment was replicated thrice, and the data were subjected to a 2-factor ANOVA and mean comparisons using R software. Results reveal that ice crystal size decreases with an increase in cellulose. Thus, it appears that the cellulose's intrinsic ability to bind water molecules creates a gel-like structure, prevents ice crystals from growing, and, in turn, reduces the final product's gritty texture. Among the 4 extracts, cellulose from banana peel and lawn grass is more effective in reducing the ice crystals' growth. The outcome is deemed to aid in the designing and developing of novel ice creams and dairy products based on inexpensive and abundant agricultural biomass and agricultural byproducts.

Key Words: ice cream, ice crystals, cellulose

1394T Chlorinated water as a cause of chlorate contamination in farm bulk milk.

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Chlorine-based chemicals for cleaning milking equipment are viewed as the primary cause of chlorate contamination of bulk tank milk. In addition, chlorinated water supplies are also considered a potential cause of chlorate in milk. However, little is known if or how chlorinated water actually contaminates farm bulk milk. To address this a milking equipment rinsing trial was conducted at a 30 unit milking plant in Teagasc, Moorepark, Fermoy, Co. Cork, Ireland. Three rinse treatments were applied approximately one hour before milking, containing 0.10, 0.50, and 2.00 mg/L, respectively, of total available chlorine; levels typical to those found in a municipal water supply. Each treatment was repeated in triplicate. Chlorinated waters were sampled in triplicate before (rinse trough) and after they were used for rinsing (in-line tap). Milk was sampled in triplicate from each of the first 3 rows of cows milked.Statistical analysis was conducted using the Tukey, Hovtest, and Welch tests as part of the GLM procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, 2016) with the significance level set at $\alpha = 0.05$. Both milk and water were analyzed for chlorate using UPLC-MS/MS and milk was tested for extraneous water (freezing point; FPD) using a Milkoscan 7. Mean chlorate levels of chlorinated waters pre-rinsing were 0.079, 0.112, and 0.325 mg/L, respectively, for the 0.10, 0.50, and 2.00 mg/L treatments. Chlorate levels of these waters post rinsing displayed very similar levels to pre-rinsing levels at 0.081, 0.111, and 0.290 mg/L. Chlorate was detected in milks sampled from the first row of cows milked (0.015–0.024 mg/kg) but not from the second or third rows. Moreover, milks sampled from the second and third rows did not contain extraneous water but milks from the first row did. Milks with the higher levels of chlorate were not associated with rinse waters containing the higher levels of chlorate. Instead they were associated with the higher levels of extraneous water (<0.500°C FPD). Therefore, the outcomes of this study demonstrate that chlorate does not contaminate milking equipment and by extension milk independent of water as chlorate was only detected in milk with added water. Accordingly, to minimize the risk that chlorinated water poses to farm bulk milk it should be advised that as much water as possible be eliminated from the system in advance of milking.

Key Words: chlorate, residue, milk

1395T New dairy products with selected milk proteins and survival potential of bacteria *Lactobacillus acidophilus* LA-5.

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The aim of the study was to determine the effect of functional additives derived from milk on the survival of yogurt bacteria and probiotic strain *Lactobacillus acidophilus* LA-5. Six yogurt variants were produced with 2% addition of: skim milk powder (C), micellar casein concentrate (MCC), serum protein concentrate (SPC), whey protein isolate (WPI), whey protein concentrate (WPC) and native casein (CN). The standardized milk was pasteurized (90°C/2 min) and inoculated with starter cultures (FD-DVS YF-L811, Yo-Flex, Chr. Hansen) and probiotic strain (NU-TRISH, LA-5, Chr. Hansen). *Lactobacillus* cells (MRS [Merck]) and *Streptococcus thermophilus* (M17 [Merck]) were determined after incubation (37°C, 24 h) and storage (after 3, 14, 21 d) at 6°C. The number of *S. thermophilus*, independently of the additives used, were in the range from 7 to 10 log cfu/mL (after 21 d). Higher levels ($P < 0.05$) were observed for LA-5 (from log 6 to log 9 cfu/mL) in samples with C, WPC, and CN in comparison with samples with other additives (no more than level of log 7 cfu/mL). The storage time and protein content influenced the microstructure (SEM, QUANTA 200 [FEI]) of the yogurts. The addition of WPI had the biggest impact on protein matrix, resulting in bigger protein clusters and smaller pores within which serum phase was entrapped. Yogurt with CN addition after 21 d was characterized by dense protein network with small pores. The addition of protein preparations is very good prospect for the production of dairy fermented beverages, with high level of probiotic bacteria. Project financially supported by Minister of Education and Science in the range of the program entitled "Regional Initiative of Excellence" for the years 2019–2023, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

Key Words: microfiltration, probiotic, yogurt

1396T Protein ingredient quality within infant formulas impacts digestion and amino acid bioavailability: A combined *in vitro* and *in vivo* approach.

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Infant formula (IF), the only adequate substitute to human milk, is a complex matrix that requires numerous ingredients and processing steps. The objective was to understand how protein ingredient quality within IF modulates its structure, digestive kinetics, and plasma amino acid (AA) contents. Four isonitrogenous IFs were formulated following UE regulation, with a 60:40 whey protein (WP):casein ratio, using WP from different origins (cheese whey: IFs-A/B, vs. ideal whey: IFs-C/D) and denaturation levels (high: IF-A vs. low: IFs-B/C/D), and casein with different organizations (micellar: IFs-A/B/C, vs. non-micellar: IF-D). IFs were subjected to in vitro dynamic digestion at the infant stage. Structure and proteolysis of the digesta were followed during digestion. Ten mini-piglets (12- to 27-d-old), as an infant model, received each IF for 3 d according to a Williams Latin square, followed by a 2-d washout period. Jugular plasma was sampled from 10 min pre-prandial to 4 h post-prandial on the third day to measure free AA, urea, insulin and glucose contents. Data were analyzed with a linear mixed model for repeated measures. Before digestion, IFs microstructures were different. In vitro, IF-A had higher proteolysis degree at 180 min of digestion than IFs-C/D (+17%). Due to its different initial structure, IF-D caseins exerted greater hydrolysis resistance than other IFs, resulting in higher abundance of casein derived-peptides at 80 min of gastric phase. In vivo, IFs-A/B had significantly higher plasma total and essential AAs concentrations than IFs-C/D, regardless of the kinetic time. WP denaturation (IF-A) and casein supramolecular organization (IF-D) seemed to reduce AA catabolism. Most differences were present pre-prandially, indicating that a metabolic adjustment occurred during the dietary adaptation. Overall, this work highlights the importance of the quality of protein ingredients (WPs and caseins) within IFs. Consequences on gut maturation and microbiota, an important actor within the microbiota-gut-brain axis, are currently being investigated.

Key Words: infant formula, protein quality, digestion

1397T Withdrawn.

1398T Effect of the type of milk protein sources on the properties of low-fat dairy spreads. M. Baranowska*[1,4], J. Ziajka[1,4], J. Kowalik[1,4], B. Dec[1,4], J. Tarapata[1,4], K. Kielczewska[1,4], K. E. Przybylowicz[2,4], M. Darewicz[3,4], and J. Zulewska[1,4], [1]*Department of Dairy Science and Quality Management,* [2]*Department of Human Nutrition,* [3]*Department of Food Biochemistry,* [4]*University of Warmia and Mazury, Olsztyn, Poland.*

This study aimed to evaluate the effect of the type of milk protein carrier on the physicochemical characteristics of low-fat dairy spreads (LFDS). Six types of LFDS were produced using mix ingredients attached in Table 1 based on the patent EP 2869708. Reconstituted dry concentrates were incubated with transglutaminase (48°C/45 min), mixed with the other compounds (Table 1) and heated (84°C/2 min). Samples K were homogenized in 1-stage at 20 MPas, and K1, S1-S4 to 2-stage at 14/3 MPas. The study assessed the composition, pH value, firmness and spreadability (penetration test using TA.XT plus Texture Analyzer), and their sensory acceptability. Data were statistically processed in the ANOVA test. The protein content was 9.3–10.0%, except for the S4 sample which had significantly higher ($P < 0.05$) protein content (11.7%). The fat content in all samples (31.5–35.7%) was typical for LFDS containing less than 39% milk fat. The firmness (2.1–2.2 N) and spreadability (84.5–86.8 N) of K and K1 were significantly different ($P < 0.05$) from S1-S4 (0.53–1.53 N and 18.5–65.74 N, respectively). The values of these attributes between S1-S4 samples were also considerably different ($P < 0.05$). The method of homogenization has not affected the texture traits of K and K1 samples. The product S3 (Table 1) was the most

Table 1 (Abstr. 1398T). Ingredient list and their use in different types of LFDS

Components	K	K1	S1	S2	S3	S4
45% fat cream	+	+	+	+	+	+
Dry concentrates						
WPC 60	+	+	+			+
MPC 70	+	+	+			
MCC				+	+	+
SPC				+		
WPPC					+	+
Buttermilk powder			+	+	+	
Buttermilk microfiltration retentate						+
Liquid buttermilk			+	+	+	+
12% fat cream	+	+				

desirable and scored 4 points on a 5-point hedonic scale. Despite lower firmness and spreadability values noted for S1-S4, these samples were also desirable. The study concluded that the use of different milk protein carriers makes it possible to compose LFDS with similar nutrient content and different texture characteristics. Their firmness, spreadability, and sensory characteristics were depended on the type of milk protein sources. The research was financially supported by The National Centre for Research and Development, Project No. WPC1/DairyFunInn/2019, amount of funding 1.950.000,00 PLN.

Key Words: milk fat, buttermilk, firmness

1399T Python-based modelling of engineering and physico-chemical properties for prediction of paneer quality.

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Prediction modeling is a statistical technique using machine learning and data mining to analyze past data, identify patterns and relationships, and make predictions. These quality prediction models can help optimize processes, reduce defects, and improve overall quality. This research was conducted to evaluate the effect of fat, solids-not-fat, and ash on various properties- hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, resilience, L*, a*, b*, bulk density, porosity, and thermal conductivity for paneer quality prediction. This study was conducted on paneer samples collected from 35 different vendors across 2 cities in Punjab state. It was designed in a way that 3 samples were collected on 3 different days from each vendor. And further, each sample was replicated thrice for analysis to reduce the variation among the collected samples. The samples were analyzed for fat, moisture, and ash with instrumental texture properties, instrumental color properties, bulk density, porosity, and thermal conductivity. Data were analyzed using 2-way ANOVA to determine no significant differences amount the mean values at $P \geq 0.05$ using SPSS 27. Then the data were partitioned into 2 equal data sets for training and validation of the prediction model. Python programming language was used for the preparation of multiple regression models. The correlation shows good fit; R^2 value of hardness, adhesiveness, cohesiveness, gumminess, chewiness, a*, bulk density, and porosity was 0.99 while resilience, L*, thermal conductivity, b*, springiness had 0.83, 0.91, 0.97, 0.98, 0.98 value, respectively. The predicted values were found to be in good agreement with experimental values (error ranging from 1.42 to 8.68%). The results showed that the prepared model has the potential for accurate prediction of the quality properties of paneer.

Key Words: prediction modeling, paneer, quality

1400T Impact of seasonality on the microbiological and centesimal composition quality of bulk tank raw milk in northern Brazil.

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The northern region of Brazil has distinct rainy (October–May) and dry (June–September) seasons, with high temperatures (average of 32°C) year-round. These factors directly impact the quantity and quality of food provided to animals and may affect milk quality. This study compared the microbiological and centesimal composition qualities of refrigerated bulk tank raw milk in the northern region of Tocantins state, Brazil. A total of 92 bulk tanks of milk, which are shared by more than 250 small-scale milk producers (up to 100 L per day), were evaluated. In 2022, samples were collected from each tank once a month to determine the total bacterial count (TBC), somatic cell count (SCC), and centesimal composition (fat, protein, lactose, total solids, and nonfat solids). These analyses were performed using flow cytometry and Fourier-transform infrared spectroscopy in official laboratories of the Brazilian Milk Quality Network. The results from the rainy and dry seasons were then compared using non-parametric analysis (Pearson). During the rainy season, the mean TBC, SCC, fat, protein, lactose, total solid, and nonfat solid contents were 6.8×10^5 cfu/mL, 5.3×10^5 SC/mL, 3.39%, 3.13%, 4.56%, 12%, and 8.61%, respectively; during the dry season, the corresponding mean values were 9.3×10^5 cfu/mL, 5.3×10^5 SC/mL, 3.43%, 3.11%, 4.59%, 12.03%, and 8.59%, respectively. Comparison showed no significant differences ($P > 0.05$), although the TBC was 36.7% higher during the dry season. However, several samples were found to be non-compliant with the minimum composition standards set by the Brazilian legislation. This highlights the need for enhanced quality control measures and support for milk producers to improve the microbiological quality and composition of milk, thereby increasing its technological potential, regardless of seasonality.

Key Words: SCC, TBC, tropical climate

1401T Performances of skimmed milk crossflow microfiltration: Comparison of ceramic membrane configurations.

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Microfiltration is largely applied in the food sector for the separation and concentration of proteins. For example, skim milk 0.1 µm microfiltration is implemented in the industry to separate casein micelles (retentate) from serum proteins (filtrate) and to obtain high-purity serum protein fractions before further processing (ultrafiltration and spray-drying). The efficiency of the process, and especially the serum protein transmission, depends on the accumulation of casein micelles at the membrane surface. To overcome this problem, milk microfiltration is operated with specific ceramic tubular membrane configurations. The Uniform Transmembrane Pressure (UTP) configuration makes it possible to get a low transmembrane pressure (TP) along the membrane with an additional pump on the permeate side. The graded Permeability (GP) configuration uses a membrane designed to create uniform permeate flux along the membrane (higher hydraulic resistance at the inlet, when TP is higher). Unfortunately, the performances of these configurations have never been compared in a wide range of operating conditions. This study proposes a comparison of the performances (permeability selectivity, energy consumption) of the UTP and GP configurations in the case of milk 0.1 µm microfiltration operating in feed and bleed mode, at 50°C. Parametric study, that consisted in TP steps (0.8–2 bar), was performed in a large range of VRR, volume reduction ratio (1.0–3.5) and retentate pressure drop (2.0–2.6 bar). Duplicates of several experiments were shown to be reproducible (standard deviation <5%). Despite the fact that both configurations used membranes with similar filtering layers, the performances of the 2 ceramic membrane configurations show large discrepancies, especially at high VRR (3.0–3.5) where fouling is more severe: at a TP = 1 bar for instance, the transmission of serum proteins was higher with UTP (60%) compared with GP (44%) configuration. The results are discussed considering the difference in membrane configurations features. The optimal performances are not obtained under the industrially recommended conditions, which leaves room for significant improvements of existing filtration plants. The results allow the definition of optimal operating conditions for each volume reduction factor and each stage of an industrial skim milk plant using ceramic membranes.

Key Words: microfiltration, milk, protein

1402T Digestibility of ultra-pasteurized high-protein beverages: A comparative study. S. Bass*, K. Cashion, S. Vink, and C. K. Yeung, *Animal Science Department, California Polytechnic State University, San Luis Obispo, CA.*

The global market for extended shelf-life (ESL) food and beverage products is expected to grow at a compound annual rate of 10.8% in the next 5 years. With consumers leaning toward a higher protein content, ready-to-drink (RTD) protein shakes are trending in the health food sector. Many factors impact the digestibility of protein including processing conditions and protein source. The objective of this study was to compare the digestibility of RTD high-protein beverages made with milk and plant protein sources. Five commercial protein concentrate powders, namely milk protein concentrate (MPC), reduced-calcium milk protein concentrate (RCMPC), micellar casein (MC), soy protein isolate (SPI), and pea protein isolate (PPI), ranging from 70% to 88% protein, were included in this study. The protein powders were incorporated into a standard RTD beverage formulation containing the same amounts of added water, maltodextrin, sucrose, and food gums. The beverages were processed (143°C for 3 s) and homogenized (1st stage at 2,500 psi and 2nd stage at 500 psi) with a MicroThermics unit. Protein digestibility was assessed by first digesting the samples at 37°C with pepsin and HCl in a 1:12.5 pepsin/protein ratio (pH = 2) for 3 h, followed by pancreatin and phosphate buffer in a 1:62.5 pancreatin/protein ratio (pH = 8) at 37°C overnight. Total nitrogen (N) and nonprotein N (NPN) were measured before/after digestion by the Kjeldahl method to estimate the protein digestibility based on the ratio of (NPN after digestion − NPN before digestion)/(total N before digestion − NPN before digestion). The protein content of the beverages varied based on the protein source, ranging from 5.7% ± 0.06% (MPC) to 8.1% ± 0.07% (MC). PPI had the highest fat content (1.0% ± 0.02%) and the lowest moisture content (84.9% ± 0.4%). NPN of SPI before digestion (8.5% ± 0.2% of total N) was significantly higher ($P < 0.05$) than other protein sources (0.8%–1.7%). The RCMPC showed the highest protein digestibility at 96.3% ± 2.2%, and overall beverages with MPC and RCMPC were more digestible than the plant proteins ($P < 0.05$). Our results support the use of RCMPC in RTD beverage formulations. Further studies on storage stability of RCMPC beverages are warranted.

Key Words: digestibility, protein beverage, MPC

1403T Functional properties and flavor characteristics of milk by dietary supplementation with jujube powder. C. Zhang*, J. Mei, and H. Liu, *Zhejiang University, Hangzhou, Zhejiang, China.*

Jujube, an edible and delicious fruit, has various health benefits, which is extensively cultivating in tropical and subtropical regions, especially in East Asia (China, India), North Africa, and Middle Eastern countries. This study aimed to investigate the functional properties and flavor characteristics of milk by dietary supplementation of jujube powder (JP) to dairy cows. Here, milk volatile profiles and taste properties were analyzed by using an electronic nose, and headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS). Compared with the control (CT) group, the total antioxidant capacity ($P < 0.01$), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic (ABTS) free radical scavenging activity ($P = 0.02$), lactoferrin ($P < 0.01$), and IgG ($P < 0.01$) increased significantly in the JP group. The volatile flavor analysis indicated that the levels of ketones increased while acids abundances showed a significant decrease ($P < 0.05$), along with the significant elevation in the abundances of 2 specific volatile compounds of toluene ($P = 0.043$) and dimethyl sulfone ($P < 0.01$) in milks from the JP group. Taste profile analyses of complex mixtures in milk by electronic nose that can distinguish taste attributes of different volatile compounds demonstrated that supplementation of jujube altered multiple milk taste properties. In summary, the present data suggest that the dietary jujube powder supplementation improved the milk volatile flavor composition and aroma, as well as the content of bioactive components and antioxidant properties.

Key Words: *Ziziphus jujuba* Mill., milk flavor, bioactive component

1404T Ultrafiltration permeates as substrates in biogas production—Sustainable dairy processing. J. Tarapata[1], M. Zielinski[2], M. Baranowska[1], B. Dec[1], and J. Zulewska*[1], *[1]Department of Dairy Science and Quality Management, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, [2]Department of Environmental Engineering, Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland.*

The aim of the study was to compare the efficiency of biogas production (biochemical methane potential test, AMPTS II) from ultrafiltration (UF) and diafiltration (DF) permeates vs. lactose production. The following substrates were examined: UF and DF milk permeate, UF and DF milk serum permeate. The composition of the produced biogas was determined by gas chromatography. An attempt was made to estimate the relationship between the degree of lactose recovery and the amount of biogas obtained. In addition, the amount of electricity and heat generated in the cogeneration system was estimated assuming the use of UF and DF permeates in quantities generated by dairy plant at industrial scale (case study). A chemical oxygen demand (COD) of the permeates ($n = 3$) ranged from 7,610 ± 430 to 57,020 ± 510 mg O_2/L. The CH_4 production yield ranged from 149 ± 13 to 181 ± 3 NL per kg of added COD ($n = 3$). The process of anaerobic digestion (AD) of wastewater with higher COD (higher lactose content) was characterized by higher efficiency of CH_4 production. No significant differences ($P > 0.05$) were found in the methane production yields (NL of CH_4 per kg of added COD) between the substrates. The quality of obtained biogas (69 ± 1.7% CH_4, 31 ± 1.1% CO_2; $n = 3$) indicated that all samples subjected to AD were equally good substrates for biomethane production. The studies carried out using flow system (UASB reactor) confirmed the results of AMPTS tests. In addition, it was found that the use of UF and DF permeates as substrates in AD makes it possible to produce electricity and heat that can be used by the dairy plant. The permeates could also serve as raw material for powdered lactose production, giving another way of its management. However, in times of energy crisis and rising energy prices, solutions that build independence and energy security are of key importance. One of them may be the construction of on-site biogas plants. The research financially supported by The National Centre for Research and Development, Project No. WPC1/DairyFunInn/2019, amount of funding 1.950.000,00PLN.

Key Words: dairy waste, biomethane, membrane filtration

1405T Effect of raw milk pH on the finished texture of yogurt. P. Ankcorn*[1,2], C. Dibble[1], P. Beswetherick[2], C. Coggins[2], C. C. Fagan[1], and K. Niranjan[1], *[1]University of Reading, Reading, England, UK, [2]Yeo Valley Farms (Production) Ltd., Blagdon, England, UK.*

The objective was to demonstrate the pH impact of raw milk pH on yogurt production. Previous studies have demonstrated the pH mediated mechanism of whey protein denaturation using fractionation followed by PAGE. These studies mostly assessed the impact of this on milk acid gel models using glucono-delta lactone. This experiment used a pilot scale yogurt making process aiming to confirm these milk model studies. It aims to demonstrate, to manufacturers, the critical importance of pH and bacterial spoilage preventative controls before heat treatment. The firmness of yogurt produced from pH adjusted raw milk (6.5, 7.1) was compared with yogurt made from milk at a natural pH (6.87), under otherwise identical conditions. During processing, the milk was homogenized at 24 MPa and then pasteurized at 98°C. The milk composition determined after heat treatment using a Perkin Elmer FTIR Lactoscope was: 4.8 ± 0.0% fat, 3.3 ± 0.0% protein, and 13.4 ± 0.1% solids (wt/wt). Post homogenization, the fat particle size was measured using a Malvern Mastersizer S and the Sauter mean diameter, D [3,2], was 0.48 + 0.01 µm. The milk was then filled into pots, inoculated with a culture containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium* and fermented at 40°C for 14 h. It was then cooled overnight to 5°C, attaining a d 1 pH of 4.03 ± 0.04. Texture measurement was completed using a stable microsystems TAXT2 texture analyzer. The texture analyzer measured the work of penetration for a 25 mm diameter cylindrical plunger to penetrate the yogurt at 1 $mm\cdot s^{-1}$ for 30 s. The firmness of set yogurt obtained with milk with pH adjusted to a value of 7.1 was 7,118 ± 250 g·s, whereas the firmness of yogurt obtained with milk pH adjusted to 6.5 was 5,754 ± 200 g·s ($n = 10$); the natural milk with pH = 6.87 gave yogurt with firmness of 6,760 ± 150 g·s. The firmness of all 3 samples was significantly different ($P < 0.05$). A small pH drop, such as the drop observed in milk of poor bacterial quality, can lower yogurt's textural quality.

Key Words: yogurt, texture, quality

1406T Implementing environmental monitoring programs in small dairy processing facilities to control *Listeria*. S. Bolten*, T. Lott, R. Ralyea, A. Trmcic, A. Zuber Gianforte, N. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Small dairy processing facilities may face challenges in controlling *Listeria* in the processing environment due to limited resources and in-house expertise. The aim of this study was to implement environmental monitoring programs (EMPs) in 8 small dairy processing facilities located in the Northeastern US, and evaluate their effectiveness at controlling *Listeria*. Upon study initiation, environmental sponge samples of equipment surfaces were collected ~4 h into production (i.e., mid-operation) in an initial sampling event to obtain baseline *Listeria* prevalence, and individualized EMP sampling plans and site lists (representing zones 2 to 4) were developed and provided to each facility. Each facility was then followed for ~1 year; during this period the project team provided guidance for corrective actions to take to control *Listeria* as needed. A validation sampling event was performed to evaluate *Listeria* prevalence after ~1 year of EMP implementation, and the project team evaluated each facility's commitment to implementing their EMP in a 9-question survey. Environmental samples were processed according to the FDA-BAM standard method for *Listeria* detection in environmental samples, and presumptive positive colonies were confirmed as *Listeria* and speciated by PCR amplification and Sanger sequencing of a 660 bp fragment of *sigB*. Of the 1,117 swabs collected across both sampling events (i.e., initial and validation), 51 were positive for *L. monocytogenes* and 145 were positive for *Listeria* species other than *L. monocytogenes*. Across all facilities, *Listeria* prevalence in initial samplings (15%) was similar to the prevalence observed in validation samplings (16%). These findings suggest additional strategies (i.e., evaluating risk factors associated with *Listeria* persistence) may be needed to improve the ability of small dairy facilities to control *Listeria* in their processing environments. Additionally, mixed-effects logistic regression analysis showed that higher survey scores pertaining to a facility's commitment to implementing their EMP were significantly associated with reduced *Listeria* prevalence in the validation sampling event ($P < 0.05$), suggesting that a small dairy facility's commitment to carrying out an EMP is a predictor of the overall effectiveness of an EMP to control *Listeria*.

Key Words: *Listeria*, environmental monitoring, small dairy facility

1540T Understanding impact of particle size and relative humidity on the physicochemical and functional characteristics of milk protein isolate 90. K. Palmer[1], A. Parhi[1], S. Singh[1], A. Shetty[3], V. Sunkesula[2], and P. Sharma*[1], [1]*Utah State University, Logan, UT,* [2]*Idaho Milk Products, Jerome, ID,* [3]*Anton Paar USA, Ashland, VA.*

Dairy powders are produced by spray drying and the bulk product is pneumatically conveyed into storage silos. Improving product flowability is necessary to ensure sustainability during transportation, packaging and storage. Relative humidity and particle size can impact powder flowability and also influence the powder's overall functionality. This study aims to determine the impact of moisture content and particle size in relation to the physicochemical and functional characteristics of milk protein isolate 90 (MPI 90). The MPI 90 was mechanically sieved into 4 size ranges (µm) of < 50, 50–100, 100–150, and 150–250. An MCR302e Rheometer was used to conduct both shear cell and flow cell rheology tests. The shear failure test and Warren Springs test were conducted with pre-shear consolidation stresses of 1, 3, 6 kPa, and 3, 6, 9 kPa, respectively. Powder samples were equilibrated in relative humidity (RH) environments (10–55%) using saturated salts to attain specific moisture contents (4–8%). Shear failure testing was done in a RH chamber on the rheometer. Statistics were completed using a one ANOVA and Tukey's Post Hoc tests. Particle size had a significant difference on the flow characteristics between particle sizes of < 50 µm and 50–100 µm ($P < 0.05$). Smaller particles (<50 µm) had flow function coefficient (*ffc*) values > 2.0 and < 4.0 indicating *cohesive* nature. The size groups 50–150 µm displayed *easy flowing* behavior with *ffc* values between 4 and 10. The biggest particle size group (>150 µm) exhibited *cohesive* behavior, likely due to the presence of smaller particles in interstitial spaces, thus increasing friction and reducing overall mobility of the powder mass. The Warren Springs cohesion values followed a similar but inverse trend as the *ffc* value. With increasing moisture content, *ffc* values decreased and cohesion values increased, suggesting increased interactions between particles. Physical characteristics, including wettability and sink-ability, decreased as particle size increased. Smaller particles were also less soluble than larger particles. This analysis of the flow characteristics of milk protein isolate powders will assist industry professionals in increasing processing efficiency of dairy powders.

Key Words: rheology, powders, flowability

Growth and Development 1

1407T Effects of transition milk on growth performance and health of Holstein calves. C. Ostendorf*[1,2], M. H. Ghaffari[1], C. Koch[2], and H. Sauerwein[1], [1]*Institute of Animal Science, University of Bonn, Bonn, Germany,* [2]*Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler, Germany.*

Feeding transition milk may alter growth and health of calves; we therefore compared 2 groups, i.e., calves fed either transition milk (TRANS, n = 25, 15 females, 10 males) or milk replacer (MR, n = 25, 15 female, 10 male). After receiving 3.5–5 L colostrum milked from their during the first 12 h, the calves were fed according to their feeding group with 12 L/d maximal feed allowance until d 6 of life. Thereafter all calves received MR (12 L/d; 140 g/L) and were weaned at d 98 of life. Individual feed intake and body weight (BW) were monitored daily and weekly, respectively, until weaning. Calf health was checked daily and, if necessary, health disturbances were treated by veterinarians. Body weight and average daily gain (ADG) were analyzed by a linear mixed model, with group, time, and their interaction as fixed effects. The incidence of health disturbances was compared by chi-squared test. Colostrum quality and total protein concentrations were compared using Student's *t*-test. Colostrum quality did not differ between both groups, with average Brix values of 26.7%. Protein concentration in serum, 36–48 h after birth, measured by optical refractometer, was similar ($P = 0.94$) in both groups and thus confirmed an adequate intake of good quality colostrum. Feeding TRANS did not affect feed intake, ADG, and BW. In total, 60 cases of health disorders were detected. Out of the 25 calves per group, 76% of MR and 52% of TRANS were sick at least once until d 98 of life ($P = 0.07$) with diarrhea and respiratory disease being most prevalent: 7 and 5 TRANS, and 12 and 13 MR calves had diarrhea and respiratory disease, respectively. From the diseased calves, 58% in the MR and 30.5% in the TRANS group were sick twice or more often. Five out of 13 (38.5%) diseased TRANS calves and 8 out of 19 (42.1%) sick MR calves were treated with antibiotics. Repeated antibiotic treatment was required in 2 cases in MR out of 8 (25%), but in none of the TRANS calves. In conclusion, feeding transition milk until d 5 of life had no effect on growth; although insignificant, the numerical decrease in disease incidences in TRANS may indicate beneficial effects on health.

Key Words: calf, colostrum, transition milk

1408T The effects of extending colostrum feeding to Holstein calves on their metabolome 21 days after their first calving. M. Tortadès*[1], G. Elcoso[2], A. Bach[3], and M. Terré[1], [1]*IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Caldes de Montbui, Barcelona, Catalonia, Spain,* [2]*Blanca from the Pyrenees, Els Hostalets de Tost, Lleida, Catalonia,* [3]*ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Catalonia.*

Early nutritional strategies can influence the metabolism and performance of calves in the future and potentially improve farm economics. The objective of this study was to evaluate the impact of the amount of colostrum fed to calves on their metabolomic profile after their first parturition. Thirty-four female Holstein calves (n = 17) were fed 2.5 l of colostrum (≥25° Brix) either for 2 (Short) or 8 feedings (Long). During the 6 feedings in which Long calves were fed colostrum, Short calves were fed 2.5 l of milk replacer at 12.5% dilution (24.0% CP and 18.5% fat; MR). Then, all calves were fed the same feeding program, decreasing MR gradually from 3 l twice daily to 3 l once daily at 15% dilution until weaning (d 56). Concentrate feed, water, and straw were offered ad libitum during the preweaning period, and thereafter animals received different TMR according to their stage of development. Heifers were weighed at birth, weaning, and then bimonthly until first calving. When rearing health afflictions were recorded. Plasma samples were collected on d 21 after calving for a non-targeted metabolomics analysis using a quadrupole time-of-flight mass spectrometry. Performance data were analyzed with a mixed-effects model accounting for the random effects of animal and the fixed effect of treatment, time, and their interaction. Heath events were analyzed using a chi-squared test. Metabolic profiling was performed by principal component analysis and heatmap visualization. Comparisons of the observed metabolite abundances between treatments were executed by *t*-tests. No differences among treatments on ADG or health reports were observed between treatments. However, BW tended ($P = 0.06$) to be greater in Long than Short heifers at 14 mo of age. Principal component and heatmap analysis did not reveal distinct metabolic profiles between treatments. However, *t*-tests found differences in 22 plasma metabolites ($P = 0.01$), revealing a downregulation of 3-oxo-tetradecanoic acid, 5β-cholestane-3α,7α,27-triol, and lysoSM(d18:0), and an upregulation of succinyladenosine in Long compared with Short heifers. Results suggest modulatory effects of duration of colostrum feeding on insulin sensitivity (lysoSM(d18:0)) and liver metabolism (5β-cholestane-3α,7α,27-triol).

Key Words: colostrum, heifer, metabolome

1409T Evaluation of colostrum quantity, quality, and bioactive compounds from Jersey cows fed two concentrations of dietary cation-anion difference with or without nicotinic acid and its effect on calf performance. T. C. Stahl*, M.C. McBride, K. N. Klobucher, K. R. Johnston, T. Islam, and P. S. Erickson, *University of New Hampshire, Durham, NH.*

We studied 2 concentrations of dietary cation-anion difference (DCAD; −40 or −80 mEq/kg) ± 23 g/d unprotected nicotinic acid (NA) on Jersey colostrum yield, composition, and calf performance. Forty multiparous Jerseys housed on a bedded pack, blocked by calving date were assigned to treatments 4 wk prepartum. Colostrum was collected, weighed, and fed by 90 min after calving and sampled for nutrient composition, IgG, bioactive compounds and fatty acids. Calves were not enrolled if their dams produced less than 2 L colostrum. Calves were removed before suckling, weighed, and blood sampled (0 h IgG; 0.2, ± 0.4 g/L) by 30 min of birth, and if enrolled received maternal colostrum. Colostrum fed varied based on yield, calves received a minimum of 2 L and up to 3.78 L. Colostrum was fed at a maximum of 2.5 L at the first feeding, and the remainder at 6 h. The 31 calves (8 −40; 8 −40 +NA; 7 −80; 8 −80 +NA) were blocked by dams' treatments. Blood was sampled at 24 h for IgG (41.1 ± 16 g/L). Calves were fed 3.78 L milk (4.9% fat and 3.7% protein), starter, and water at d 1 until d 42. Calves had blood samples, weights, and skeletal measures taken weekly. A xylose challenge was performed on d 5 to evaluate small intestine development. Orthogonal comparisons compared DCAD, NA, and the interaction. Colostral-lactoferrin fed increased with NA ($P = 0.04$). There was an interaction for insulin-like growth factor-1 fed to calves ($P = 0.02$), with the highest amount from −40 + NA and the lowest from −40 − NA. For fatty acids, C18:0 was less ($P = 0.05$) and C18:1 tended to be less ($P = 0.09$) in −80. Omega-3 ($P = 0.04$) and omega-6 ($P = 0.03$) were less with NA. Fat % was less with NA ($P = 0.04$) and protein % tended to be more with NA ($P = 0.08$). There was an interaction for apparent efficiency of absorption ($P = 0.09$) with the greatest at −80 + NA and the lowest at −40 + NA. Xylose concentration was increased with NA ($P = 0.02$). These data suggest that supplemental NA may increase bioactive compounds and enhance calf intestinal development.

Key Words: nicotinic acid, DCAD, colostrum

1410T Effect of in utero choline exposure on Angus × Holstein carcass characteristics. W. E. Brown*[1,2], H. T. Holdorf[1], S. J. Johnson[1], S. J. Kendall[1], S. E. Green[1], and H. M. White[1], *[1]University of Wisconsin–Madison, Madison, WI, [2]Kansas State University, Manhattan, KS.*

Feeding rumen-protected choline (RPC) to late gestation dairy cows has been shown to enhance growth of offspring, including beef × dairy calves (BDC). It is unknown if maternal RPC supplementation may also affect carcass characteristics. Our objective was to investigate prepartum dam RPC dose and formulation on BDC offspring carcass characteristics. Multiparous Holstein cows with male (n = 17) or female (n = 30) Angus-sired calves were enrolled 21 d prepartum and randomly assigned to treatment (trt): 0 g (control; n = 12; CTL), 15 g (recommended dose; n = 10; RD), or 22 g (high dose; n = 12; HD) of choline ion from a concentrated RPC prototype (n = 13; RPC2; Balchem Corp.) or the RD of choline ion (RPC1; ReaShure, Balchem Corp.; positive control). Cattle were managed in 2 cohorts. At 311 ± 26 kg, BDC were stepped up to a complete finishing diet (12% CP; 1.34 Mcal/kg NE_g). At 415 ± 36 kg, BDC were switched to a self-feeder finishing diet (13.2% CP; 1.32 Mcal/kg NE_g) until harvest. Cattle were weighed 2 consecutive d immediately before delivery to a commercial harvest facility (JBS Foods, Green Bay, WI). Carcass weights were collected by the harvest facility, and USDA inspectors collected carcass quality data visually. Data were analyzed (PROC MIXED, SAS v 9.4) with the fixed effects of trt, sex, their interactions, and the random effect of animal (mean [95% CI]). Preplanned contrasts compared CTL vs. RPC exposure, and the linear effects of RPC. Sex and trt interacted for dressing percentage ($P = 0.01$). For males, $RPC2_{HD}$ increased dressing percentage (59.9% [58.9, 60.9]) compared with $RPC2_{RD}$ (57.7% [56.5, 58.8]), but there was no evidence of trt difference in females. Compared with CTL, any maternal RPC supplementation decreased dressing percentage ($P < 0.001$). Kidney, pelvic, and heart fat (KPH) was affected by trt, whereby $RPC1_{RD}$ increased KPH yield by 4.8 kg ($P = 0.04$) and percentage by 1.2% ($P = 0.01$) compared with CTL, but there was no other trt difference. Increasing RPC linearly increased KPH yield ($P = 0.05$), KPH % ($P = 0.03$), and marbling score ($P = 0.04$). The increase in marbling score could be beneficial and more research is needed to elucidate mechanisms of action.

Key Words: beef, developmental programing

1411T Butyrate metabolite hesperetin alleviates necrotizing enterocolitis by increasing tight junction proteins via inhibiting the PI3K-Akt pathway. Y. Liting[1,2], G. Yanan[1,2], Y. Qianqian[1,2], W. Jiaqi[1,2], and Z. Nan*[1,2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China, [2]Key Laboratory of Quality and Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Necrotizing enterocolitis (NEC) is one of the most common and serious intestinal illnesses in newborns. Butyrate is one of the short-chain fatty acids containing 4 carbon atoms, which can relieve intestinal inflammation, but its influence mechanism is unclear. In this study, butyrate was used as the research target to construct a rat model of NEC, and butyrate was given to rats by gavage. Rat feces were collected to screen key metabolites that can alleviate intestinal inflammation through metabolomics analysis, and then key metabolites were applied to cells to explore key pathways and genes for alleviating intestinal inflammation through transcriptomic analysis. Further elucidate the mechanism of butyrate alleviating NEC. All experimental data were presented as the mean of 3 independent experiments ± standard error of mean (SEM). Significant differences among groups were assessed using one-way ANOVA followed by Tukey's multiple comparisons. A P-value <0.05 indicated a significant difference. In vivo studies showed that butyrate could improve the body weight and histopathological injury of rats, reduce the expression of pro-inflammatory factors, and increase the expression of anti-inflammatory factors to alleviate NEC. Metabolomics analysis suggests hesperetin may be a key metabolite in alleviating intestinal inflammation. Transcriptomics was used to investigate the mechanism of hesperetin in an in vitro cell model to further investigate the mechanism of butyrate attenuating NEC. In vitro test results indicated that hesperidin significantly increased the expression of tight junction (TJ) proteins, reduced the ex-pression of pro-inflammatory factors, and increased the expression of anti-inflammatory factors by blocking the PI3K–Akt signaling pathway. In summary, this study was novel in clarifying that butyrate metabolite hesperetin alleviated necrotizing enterocolitis by modulating TJ proteins via inhibiting the PI3K-Akt pathway. By combining multiple omics analysis (metabolomics and transcriptomics), we revealed the mechanism of butyrate in alleviating NEC and provided a new idea for treating NEC.

Key Words: butyrate, necrotizing enterocolitis, PI3K–Akt pathway

1542T Longitudinal histological and ultrasound analysis of bovine mammary gland development. A. L. Vang*, W. S. Frizzarini, T. Bresolin, T. Cunha, G. L. Menezes, G. J. M. Rosa, L. L. Hernandez, and J. R. R. Dorea, *University of Wisconsin–Madison, Madison, WI.*

Although ultrasound (US) image has been explored in lactating animals, its association with internal structure during development directly measured through biopsies and longitudinal measures from the same animals is scarce. Therefore, the focus of this research was to investigate bovine mammary gland (MG) development using non-invasive and invasive tools to reveal the growth rate of the glandular tissue responsible for future milk production. To this end, MG US images, biopsy samples, and blood samples were collected from 36 heifer dairy calves at 10, 25, 39, and 52 weeks (W) of age. Whole-mount histological images were annotated, and features extracted using QuPath software to quantify and create morphological measurements of MG microstructure. Parenchyma (PAR) was quantified at 10W using US in addition to histological evaluation, and average echogenicity was utilized to quantify PAR after 10W. A linear mixed model was used to analyze body weight, average duct area, average duct max diameter, total duct area/whole tissue, total adipose area/whole tissue, and echogenicity average, standard deviation, minimum and maximum. The model included birthweight as a covariate, diet (high and low) and W (10, 26, 39 and 52) as fixed effects, and animal as random effect. Maximum echogenic value increased from 0.389 at 10W of age to 0.523 at 56W which is consistent with increase in adipose tissue seen in histological images ($P < 0.001$). There was a negative correlation between average echogenicity of PAR at 10W and total adipose as percent of whole tissue at 56W (r = 0.46, $P = 0.006$), suggesting higher PAR echogenicity at 10W is correlated with less overall adipose tissue at 56W. In addition, average daily gain (ADG) at

10W and 26W was negatively correlated with maximum echogenicity at 56W (r = −0.53, −0.68; *P* = 0.012, 0.001). These results suggest that US may be a useful noninvasive tool to track MG development. In addition, ADG before 39W is associated with subsequent development and could have lasting impacts on MG microstructure and ultimately future milk production.

Key Words: mammary gland, ultrasound, histology

Lactation Biology 2

1412T Venous blood composition of subclinically inflamed and healthy mammary glands. C. S. Gammariello*[1], J. Hanson[1], A. E. Relling[1], G. M. Canny[1], M. Oliveira[1], A. Sipka[2], K. M. Enger[1], and B. D. Enger[1], *[1]Ohio State University, Wooster, OH, [2]Cornell University, Ithaca, NY.*

Blood delivers all the precursors needed for milk synthesis to the mammary gland. Milk composition is recognized to be altered during mastitis, but it is unknown if mastitis can be characterized by changes in blood nutrient uptake by the mammary gland. This study's objective was to evaluate venous blood composition of healthy and inflamed mammary glands to determine if mammary gland inflammation significantly alters the composition of venous blood exiting the mammary gland. Mid-lactation primiparous Holstein cows (n = 4) acclimated to tie stalls had udder halves randomly allocated to treatments where quarters of 1 udder half were infused with 2 billion cfu of formalin fixed *Staphylococcus aureus* that was thrice washed with saline; the opposite udder half infused with saline. Following intramammary infusion, venous blood samples were taken from left and right subcutaneous abdominal veins every 2.6 h for 36 h. Blood metabolites were quantified using a hand-held blood analyzer (iStat Alinity) immediately after collection. Individual metabolites were analyzed in separate models using PROC GLIMMIX in SAS; fixed effects were treatment, time, and their interaction, and cow was a random effect and repeated measure across time. No quarters exhibited clinical mastitis. Mean milk somatic cell scores of challenged udder halves were greater than saline udder halves post-challenge (5.8 vs. 0.5 ± 0.4; $P < 0.001$). Venous hematocrit, hemoglobin, concentrations of creatine, ionized Ca, urea nitrogen, lactate, Na, K, Cl, total carbon dioxide, partial pressure of carbon dioxide, base equivalents, and bicarbonate did not differ between udder half treatments ($P \geq 0.2$). Challenged udder halves had marginally greater saturated ($P = 0.1$) and partial pressures ($P = 0.06$) of oxygen, greater anion gap values ($P = 0.02$), and concentrations of glucose ($P = 0.03$), but had a lower pH ($P = 0.1$) than saline udder halves. This study indicates that subclinical mastitis affects venous blood composition, particularly oxygen concentrations, pH, and the anion gap. Future studies could aim to incorporate total blood substrate uptake of the mammary gland in relation to the milk composition changes by mastitis.

Key Words: mastitis, synthesis

1413T Characterization of fatty acids that promote lipid synthesis in bovine mammary epithelial cells. M.-A. Guesthier*[1,2], T. Kustova[1], P. Piantoni[2], G. Shroeder[2], and S. A. Burgos[1], *[1]Department of Animal Science, McGill University, St-Anne-de-Bellevue, QC Canada, [2]Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN.*

The objective of this study was to characterize the effect on lipid synthesis of 3 fatty acids (FA1, FA2, and FA3) that were previously identified using a high-throughput screening method to measure lipid droplet (LD) accumulation in primary bovine mammary epithelial cells (BMEC). Isolated BMEC were induced to lactogenic differentiation for 4 d. During the last 16 h of d 4, BMEC were treated with 10 μ*M* of each FA conjugated to BSA. For the microscopy assays, BMEC were fixed, permeabilized, and stained with BODIPY 493/503, a specific fluorescent probe for neutral LD, as well as the nuclear (DAPI) and cytoskeletal (phalloidin-tetramethylrhodamine) counterstains. The BMEC were imaged using a high-content cell imaging microscope. Each plate contained vehicle control and the 3 FA treatments in triplicates. Data were analyzed by ANOVA using PROC MIXED in SAS. Validation assays showed an increase in LD of 75% for FA1 and FA2 and an increase of 66% for FA3 ($P \leq 0.05$) compared with vehicle control. MTT assay showed a 50% cytotoxic concentration at 190 μ*M*, 395 μ*M*, and 230 μ*M* for FA1, FA2, and FA3, respectively. In dose-response experiments, all treatments showed a linear increase in LD accumulation up to 125 μ*M* treatment. Pairwise combination did not show a synergistic or additive effect between compounds. Profiling of FA from total lipid BMEC extracts showed an increase of 36% and 21% ($P \leq 0.05$) in de novo (≤14 carbons) FA fraction for FA1 and FA3, respectively. FA2 did not impact the de novo FA fraction. To identify the potential mechanism of action influenced by our tested FA, we measured mRNA abundance of genes involved in lipogenesis by RT-qPCR for FA1 and FA3 treated BMEC. FA1 showed a 2.7-fold increase and FA3 showed a 1.8-fold increase (both $P \leq 0.05$) in *ACACA* mRNA abundance. FA1 and FA3 also increase *FABP3* mRNA abundance by 1.9- and 1.7-fold (both $P \leq 0.05$) respectively. The tested FA did not affect the expression of *SREBF1* or other lipogenic genes tested (*FASN*, *DGAT1*, and *SCD1*). These results indicate that FA2 did not influence lipogenic pathways evaluated whereas FA1 and FA3 had a positive impact on de novo FA synthesis and key genes involved in lipogenesis.

Key Words: gene expression, lipogenesis

1414T Cellular agriculture for colostrum and milk production. S. Purup*[1], Z. Sattari[2], J. Che[2], Y. Yue[1], R. Brødsgaard Kjærup[1], N. Aagaard Poulsen[2], S. Drud-Heydary Nielsen[2], T. Sørensen Dalgaard[1], and L. Bach Larsen[2], *[1]Aarhus University–Viborg, Dept. Animal and Veterinary Sciences, Tjele, Denmark, [2]Aarhus University, Dept. Food Science, Aarhus N, Denmark.*

The quality and quantity of colostrum and milk influence the survival and growth of the offspring. However, it is difficult for some species, e.g., sows, to produce enough colostrum. Therefore, cellular agriculture can be an alternative way to produce milk components in a sustainable way. Here we introduce a new biotechnology concept to produce milk secretomes by cultured mammary epithelial cells. Cells can be isolated either from the mammary gland by enzymatic digestion of mammary tissue or non-invasively from milk by centrifugation of milk of the target animal. Cell proliferation can be closely monitored by xCELLigence real-time cell technology (ACEA Biosciences) to ensure optimized culturing conditions and to investigate the longevity of the isolated cells. Cell differentiation and lactogenesis are achieved by the construction of 3D structures in Matrigel coated 6-well transwell system. When cells are stimulated with a lactogenic hormones complex containing a bovine pituitary extract (BPE; 0.1% wt/vol) or recombinant prolactin (5 mg/L), hydrocortisone (0.5 mg/L) and bovine insulin (1 mg/L), structures develops that mimic the alveoli in the mammary gland in vivo. Cell secretomes are collected after 4 d and the composition and functionality are analyzed to ensure the quality and explore the potential applications. To date, bovine mammary epithelial cells from both mammary glands at slaughter or milk samples are used to establish and optimize the cell culture system and to study milk synthesis in vitro. In a preliminary study, more than 500 proteins were identified in the secretomes by proteomics (MALDI-TOF MS and LC-TimsTOF MS/MS), and of these, 118 corresponded to proteins found in cow's milk, among these α-, β-, and kappa casein, α-lactalbumin and β-lactoglobulin, however in very low concentrations. Furthermore, gene expression of kappa-casein was significantly ($P < 0.001$) increased by treatment with BPE relative to recombinant prolactin and control. This biotechnology will provide insight into lactation physiology and synthesis and secretion of milk components in general, and lead to a potential future way of producing colostrum and milk, not only from cows but also from humans and other species including endangered animals.

Key Words: cellular agriculture, mammary epithelial cell, lactation physiology, milk secretion

1415T Associations between antepartum and parturitive serum mineral concentrations with colostrum composition and component yield.

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Our objective was to determine the relationship between antepartum and parturitive serum mineral concentration and colostrum composition and component yield. Serum was taken at 3 ± 0.2 d antepartum and 1 h postpartum from Simmental-Angus cattle (n = 152) to measure serum Ca, P, Na, K, Mg, and Cl concentrations. Within 1 h of calving, colostrum (50 mL) was collected, and a subset of cattle (n = 48) were administered 2 mL oxytocin and milked with a portable milking machine. Colostrum composition (fat, lactose, SCC, IgG, and insulin) was measured in all samples and component yield was calculated from the subset. Data were analyzed by nonparametric rank correlation (Spearman) using PROC CORR (SAS 9.4) and coefficients were considered weak ($\rho^s = [0.1]$ to $[0.29]$) or moderate ($\rho^s = [0.3]$ to $[0.59]$) when $P \leq 0.05$. Colostrum yield and fat concentration and yield were not correlated ($P \geq 0.09$) to serum minerals. Lactose concentration was weakly associated ($\rho^s = -0.26$; $P < 0.01$) with parturitive serum K, whereas lactose yield was moderately correlated ($\rho^s = -0.30$; $P < 0.05$) with antepartum serum K and parturitive P ($\rho^s = -0.32$; $P = 0.03$). Weak correlations were identified between antepartum serum P ($\rho^s = 0.21$; $P = 0.02$) and K ($\rho^s = 0.19$; $P = 0.03$) and parturitive Ca ($\rho^s = -0.21$; $P < 0.01$), but serum minerals were not ($P \geq 0.08$) correlated to IgG yield. Colostrum log(SCC) was moderately correlated ($\rho^s = 0.30$; $P < 0.04$) to antepartum, but not parturitive ($P = 0.58$) Mg. Rather, log(SCC) was weakly correlated ($\rho^s = -0.25$; $P < 0.01$) to parturitive Cl. Antepartum Mg ($\rho^s = 0.18$), Na ($\rho^s = -0.17$), Cl ($\rho^s = -0.24$), and K ($\rho^s = -0.31$) and parturitive Na ($\rho^s = 0.20$) and K ($\rho^s = -0.24$) were all correlated ($P \leq 0.05$) with colostrum insulin concentration. Correlations ($-0.30 \geq \rho^s \geq -0.40$; $P \leq 0.04$) were maintained between antepartum Na, K, and Cl and colostrum insulin yield, whereas a moderate correlation ($\rho^s = 0.32$; $P = 0.03$) was only found with parturitive Mg and colostrum insulin yield. These data demonstrate that associations are present between serum mineral concentrations and colostrum production that vary depending on the component.

Key Words: colostrum, mineral, cow

1416T The effects of stage of lactation and mastitis on peripheral blood mononuclear cell mitochondrial enzyme activity in Holsteins on 5 California commercial dairies.

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Mitochondria are central to metabolism and health, and the functionality of electron transport chain enzymes is associated with milk production in dairy cows. However, no research has explored the impacts of stage of lactation and mastitis on bovine mitochondrial function. The objective of this study was to determine if peripheral blood mononuclear cell (PBMC) mitochondrial enzymatic activity of complex I, complex IV, and complex V changed with stage of lactation across 5 California commercial dairies. Whole blood samples were collected from 110 Holstein cows (approximately 22 cows per dairy) at dry off (TPT1), one week post dry (TPT2) and 55–75 d (TPT3) into the subsequent lactation. Peripheral blood mononuclear cells were processed, and crude mitochondrial extracts isolated to test the enzyme activities of complex I, complex IV, and complex V using kits from Abcam (Cambridge, MA). Milk samples were collected from all individual quarters the week of dry off and sent to DHIA for testing. Low SCC cows were defined as <100,000 cells/mL in all quarters, and high defined as >200,000 cells/mL in one or more quarters. High and low SCC groups were not treated with intramammary antibiotics at dry off. Data were analyzed using the GLM and Mixed procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC). One dairy had increased complex V activity between low and high SCC groups, and the remaining 4 dairies had no difference in enzymatic activity. When comparing complex V enzymatic activity from TPT1 to TPT3, 2 dairies had increased complex V activity, one dairy had decreased activity, and 2 dairies showed no change in enzymatic activity across stage of lactation. For complex IV activity from TPT1 to TPT3, 3 dairies had decreased activity, one dairy had increased activity, and one dairy showed no change in activity by stage of lactation. These findings suggest that mastitis challenge and stage of lactation do not affect PBMC mitochondrial enzymatic activity of complex IV and complex V uniformly across commercial herds.

Key Words: mitochondria

Physiology and Endocrinology 2

1417T High body condition score at dry-off does not affect cyclicity, uterine health, or embryo quality in the subsequent lactation of Holstein cows. T. O. Cunha*[1], P. L. J. Monteiro Jr[1], E. A. Galvan[1], W. S. Frizzarini[1], N. N. Teixeira[1], T. Valdes-Arciniega[1,2], M. Z. Toledo[3], R. D. Shaver[1], J. P. N. Martins[1,2], M. C. Wiltbank[1], and L. L. Hernandez[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [2]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI, [3]Purina Animal Nutrition, Madison, WI.*

Our objective was to evaluate the effect of body condition score (BCS) at dry-off on embryo quality after parturition in Holstein cows. Beginning 75 d before dry-off multiparous cows (BCS 3.16 ± 0.03, 148 ± 0.5 d post-AI) were blocked by lactation and BCS and randomly assigned to one of 2 diets, CON (NE_L = 1.57 Mcal/kg; n = 44) and HighBCS (NE_L = 1.82 Mcal/kg; n = 45). Subsequently, all cows received a common ration during the dry and fresh periods. BCS was recorded weekly. Blood samples were collected on 28 ± 3 DIM for β-hydroxybutyrate (BHB) and on 18, 25, and 32 DIM for progesterone (P4) measurements. On 50 ± 3 DIM endometrial cytology was performed. Cows were synchronized, inseminated, and flushed 7 d post-AI twice for single embryo collection, on 62 ± 2 and 83 ± 3 DIM. Data were analyzed by GLIMMIX, MIXED, and FREQ procedure of SAS. At dry-off, HighBCS cows had a BCS of 3.72 ± 0.1 compared with 3.28 ± 0.1 in CON cows ($P < 0.01$). At 35 DIM of the new lactation, HighBCS and CON cows lost 0.66 ± 0.6 and 0.43 ± 0.6 of BCS, respectively ($P = 0.005$). Concentrations of BHB at 28 DIM differed between treatments (CON = 1.05 ± 0.13 vs. HighBCS = 1.47 ± 0.18 mmol/L, $P = 0.03$). Proportion of cows with P4 > 1 ng/mL at 32 DIM (53% vs. 65%, $P = 0.28$) and uterine polymorphonuclear leukocytes ≥ 6% ($P = 0.99$; 13%) did not differ between treatments. Fertilization rate was 75% (60/80) and was not affected by collection ($P = 0.57$) or treatment ($P = 0.85$). The number of transferrable embryos was not affected by collection ($P = 0.56$) or by treatment ($P = 0.24$). Treatment also did not affect proportion of degenerate or grade 3 embryos ($P = 0.11$). Cows were classified based on BCS differences from 0 to 35 DIM (C1 = BCS maintained or lost ≤ 0.25; C2 = lost 0.26 to 0.74; and C3 = lost ≥ 0.75). Classes had an effect on the proportion of fertilized oocytes ($P = 0.03$; C1:21/27; C2:15/27; C3:24/27) but not on the proportion of degenerated/grade 3 embryos ($P = 0.26$; C1:6/21; C2:6/15; C3:4/24). In conclusion, the manipulation and subsequent loss of BCS postpartum did not impact embryo quality classifications.

Key Words: BCS, cyclicity, embryo quality

1418T Differences in blood metabolites exist between feed-efficient cows differing in choline supplementation. M. J. Caputo*[1], S. J. Kendall[1], K. Estes[2], K. A. Weigel[1], and H. M. White[1], *[1]University of Wisconsin–Madison, Madison, WI, [2]Balchem Corp., New Hampton, NY.*

Post-absorptive nutrient metabolism has been identified as a source of variation in residual feed intake (RFI), but the contributing tissues and pathways are unknown. Our objective was to characterize blood metabolite differences in mid-lactation cows with divergent RFI. Multiparous Holstein cows (161 ± 3 DIM; n = 64) were assigned randomly to treatment (trt): rumen-protected choline (RPC; 17.7 g choline ion; Balchem Corp.) or control (CTL; 0 g choline ion). Tail vessel blood samples were collected from the highest and lowest 16 cows (n = 32) in the final week, as determined by a mid-point RFI calculation. RFI was determined as DMI regressed on milk energy, DIM, metabolic BW, ΔBW, and trt. Blood metabolites quantified included ALT, AST, albumin, BHB, creatinine, glucose, insulin, fatty acids, and triglycerides and fatty acid profile was determined by GS/MS. Final RFI was calculated and the lowest and highest 12 cows (n = 24) were retained as HFE (high feed efficient) and LFE (low feed efficient). The effect of RFI group on blood metabolites was determined using PROC GLIMMIX (SAS 9.4) with fixed effects of group, trt, and their interaction, accounting for heterogeneity of variance. Results are reported as (mean [95% CI]). The BHB tended to be greater in HFE compared with LFE ($P = 0.09$; 0.72 [0.60, 0.84] vs. 0.60 [0.53, 0.68]). A trt × RFI interaction ($P = 0.01$) of ALT (HFE-RPC: 24.7 [21.1, 28.4]; HFE-CTL: 31.4 [27.7, 35.1]; LFE-RPC: 36.0 [32.3, 39.6]; LFE-CTL: 33.0 [29.3, 36.6]) and C17:0 (HFE-RPC: 0.73 [0.66, 0.80]; HFE-CTL: 0.87 [0.78, 0.98]; LFE-RPC: 0.87 [0.79, 0.98]; LFE-CTL: 0.80 [0.72, 0.89]), and a tendency for an interaction of triglycerides ($P = 0.06$; HFE-RPC: 7.8 [6.9, 8.9]; HFE-CTL: 9.7 [8.4, 11.6]; LFE-RPC: 9.4 [8.2, 11.2]; LFE-CTL: 9.0 [7.9, 10.5) and C14:0 ($P = 0.08$; HFE-RPC: 0.47 [0.42, 0.50]; HFE-CTL: 0.42 [0.38, 0.46]; LFE-RPC: 0.43 [0.39, 0.47]; LFE-CTL: 0.46 [0.42, 0.50]) was detected. Differences in circulating metabolites among RFI group indicate that metabolites may contribute to, or serve as a marker for, feed efficiency status. Additionally, interactions in which HFE differed by trt suggest that choline supplementation may influence metabolism in high efficient cows.

Key Words: residual feed intake, choline

1419T Colostrum quality at calving can predict embryo viability in postpartum multiparous Holstein cows. A. H. Souza[1], T. O. Carneiro[2], D. Langwinski[1], M. Luchesi[1], B. O. Cardoso[1], R. O. Rodrigues*[3], and R. Sartori[4], *[1]Cargill Animal Nutrition, Campinas, SP, Brazil, [2]Independent Bovine Reproductive Veterinarian, Brotas, SP, Brazil, [3]Cargill Animal Nutrition, Lewisburg, OH, [4]ESALQ, University of Sao Paulo, Piracicaba, SP, Brazil.*

The objective of the study was to characterize the relationship between colostrum quality and embryo production and quality in postpartum multiparous Holstein cows. The study was performed on a commercial free-stall dairy herd located in the state of Sao Paulo, Brazil, from May to December 2022. All cows were grouped in the same pen during the pre-partum close-up period, where they were fed anionic diets for 3 to 4 weeks before calving. The final data set included 50 multiparous cows, presenting singletons (n = 48) or twins (n = 2), and no dystocia. A colostrum sample was taken from the first milking immediately after calving and quality was measured using a BRIX meter (PAL-1, ATAGO). Colostrum was classified as high quality when BRIX >22 (n = 24) or low quality when BRIX ≤22 (n = 26). Starting 16 d before uterine flushing, all cows underwent a standard 4-d FSH superovulation protocol, followed by AI performed twice at 12 and 24 h after induced ovulation using semen from a single fertility-proven sire (total of 2 sires used, balanced across colostrum quality groups; single batch per sire); uterine flushes occurred between 70 to 80 d in milk. Milk production was recorded daily, and 2-d AM/PM milk samples were collected weekly for component and somatic cell count analysis. Statistical analyses were performed with the GLIMMIX procedure (SAS 9.4), and significance declared at $P \leq 0.10$. Cows of high colostrum quality had greater proportion of viable embryos (59.7 vs. 39.1 ± 5.5%; $P < 0.05$) and less degenerated embryos (31.1 vs. 59.4 ± 5.6%; $P < 0.05$) compared with cows of low colostrum quality. Milk production from the 3rd to the 8th week of lactation was greater for cows with high compared with low colostrum quality (45.0 vs. 42.8 ± 1.1 kg/d; $P < 0.05$). Furthermore, no relationship between colostrum quality and milk components or somatic cell count was detected. In conclusion, colostrum quality measured at calving appeared to be associated with embryo quality and milk production in super-ovulated postpartum multiparous dairy cows.

Key Words: Brix, embryo quality, milk production

1420T Effect of over-conditioning around calving on the mRNA abundance of hepatic genes related to bile acid synthesis in dairy cows.

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Bile acids (BA) can be synthesized in the liver via 2 different pathways: the classical and the alternative pathway. In humans, liver dysfunctions such as non-alcoholic fatty liver disease, can alter the biosynthesis of BA; therefore, we hypothesized that the alternative pathway will be upregulated in over-conditioned dairy cows, being prone to metabolic dysfunctions when compared with normal conditioned cows. Fifteen weeks ante partum (ap), multiparous Holstein cows (n = 38) were divided into either a high BCS (>3.75; HBCS, n = 19) or a normal BCS (<3.5; NBCS, n = 19) group. Liver biopsies were obtained at wk 7 ap and wk 1, 3, and 12 postpartum (pp). The mRNA abundance of 8 target and 8 reference genes was measured by RT-qPCR using integrated fluidic circuit technology (Fluidigm, San Francisco, CA). The mRNA data were analyzed using a linear mixed model with repeated measures (SPSS 28). The mRNA abundances of genes involved in the classical BA pathway (*CYP7A1*, *CYP8B1*, and *HSD3B7*) were not different between the groups. A group × time interaction was observed for *STAR*, a key player in cholesterol transport into mitochondria, showing a 2.7-fold greater ($P = 0.02$) abundance in HBCS cows compared with NBCS cows at 3 wk pp. The mRNA abundance of *TSPO*, interacting with STAR to transport cholesterol into the mitochondria, was neither affected by group nor by group × time interaction. In HBCS cows, the mRNA abundance of *CYP27A1*, initiating the alternative pathway, tended to be 1.1-fold greater ($P = 0.07$) and the mRNA abundance of *CYP7B1*, the key enzyme within the alternative pathway, was 1.3-fold greater ($P = 0.01$) than in NBCS cows. Moreover, the mRNA abundance of *CH25H*, the key enzyme mediating hydroxylation of cholesterol, was 1.3-fold greater ($P = 0.01$) in NBCS compared with HBCS cows. Our results point to a predominance of the alternative BA pathway in the liver of HBCS cows. As suggested in humans, the prevalence of the alternative pathway in the liver may play a role for metabolic homeostasis and thus might attenuate liver dysfunctions.

Key Words: bile acid, over-condition, periparturient period

1421T Changes in plasma metabolome of newborn calves: Insights during the first 12 hours of life.

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The aim of this study was to describe the plasma metabolome of newborn calves during the first 12 h of life. German Holstein calves (n = 44, 30 females and 14 males) were randomly assigned to 2 treatment groups: either before or after colostrum feeding. All calves received their first colostrum (3.5 to 4 L, milked by their dams) by bottle within 2 h of birth, followed by 1.5–2 L of colostrum 11.5 h later. Blood samples were collected from the jugular vein 30 min before colostrum feeding and 12 h after birth. Targeted metabolomics was performed on the plasma samples using the MxP Quant 500 kit with liquid chromatography-tandem mass spectrometry. All metabolites with missing values above 20% were removed from the analyses and the remaining missing values of metabolites were calculated, with concentrations of 228 metabolites above the detection limit. Hexoses, lactic acid, Ala, Gln, Gly, Pro, Val, betaine, FA C18:1, taurine, FA C14:0, creatinine, Leu, His, and Glu were the top 15 metabolites with the highest concentrations. Statistical analysis of metabolite data was performed using MetaboAnalyst 5. Principal component analysis showed differences in plasma metabolome between 2 sampling time points. Volcano plots with log-transformed data identified significant metabolites with a fold change >1.5 and an FDR <0.05 while controlling for sex as a covariate. Plasma concentrations of 96 metabolites increased during the second sampling time compared with the first sampling time. In contrast, the concentrations of 14 metabolites, including trimethylamine-N-oxide, phenylacetylglycine, lysophosphatidylcholine, hippuric acid, hexoses, Gly, FA18:1, creatinine, cortisol, aconitic acid, and α-aminobutyric acid, decreased compared with the first sampling time. In addition, metabolic pathway enrichment analysis for significant metabolites identified by the Volcano plot showed that aminoacyl-tRNA biosynthesis, valine, leucine, and isoleucine biosynthesis, sphingolipid and phenylalanine metabolism, glyoxylate and dicarboxylate metabolism, and cysteine and methionine metabolism were the most enriched metabolic pathways. Overall, the results show that the plasma metabolome of newborn calves is altered primarily by colostrum intake and increasing calf age.

Key Words: calf, colostrum, metabolomics

1422T Effects of zinc-hydroxychloride on ketone metabolism in heat-stressed dairy cows.

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Objectives were to evaluate the effects of supplemental zinc (Zn)-hydroxychloride (HYD; Selko) on ketone clearance during and after heat stress (HS). Multiparous Holstein cows (710 ± 59 kg BW; 161 ± 15 DIM; n = 12) were randomly assigned to 1 of 2 dietary treatments: 1) control (CON; 75 mg/kg Zn from $ZnSO_4$; n = 6), or 2) HYD (75 mg/kg Zn from HYD; n = 6). Before study initiation, cows were fed their respective diets for 16 d and continued to receive them during the 3 experimental periods (P). P1 (5 d) served as a baseline and animals were in thermoneutral (TN) conditions, in P2 (5 d), cows were fitted with an electric heat blanket (EHB; Thermotex Therapy Systems Ltd.) to artificially induce HS, and during P3 (3 d), the EHB were removed, and animals recovered from HS. Ketone tolerance tests (KTT) were performed on d 5 of P1, d 3 and 5 of P2, and d 3 of P3 by administering BHB i.v. (1,400 μmol NaBHB/kg BW; KetoTech Inc.). Regardless of Zn source, HS markedly increased rectal and skin temperatures and respiration rate (2.0°C, 9.7°C, and 63 bpm, respectively; $P < 0.01$). DMI and milk yield progressively decreased during HS (56 and 33%, respectively; $P < 0.01$), but were unaffected by Zn source. Circulating glucose tended to decrease on d 5 of HS in CON (15%; $P = 0.07$) whereas it remained unchanged in HYD but did not differ by Zn source during P3. During HS, insulin levels decreased (25%; $P < 0.01$) relative to P1 regardless of Zn source, and in P3 insulin markedly increased (50%; *P*

< 0.01) relative to P1 and was decreased in HYD (42%; $P = 0.06$) on d 3 compared with CON cows. Basal NEFA and BHB gradually increased during HS in both diets, and HYD ameliorated the NEFA response but not the BHB response. Ketone clearance following the KTT was progressively reduced during HS and was decreased 77 and 160% in CON on d 3 and 5 of P2, but this was improved (26%; $P < 0.01$) in HYD on both days. On d 3 of recovery, both Zn sources had increased (34%) ketone clearance compared with d 5 of P2 but was still reduced relative to P1 (44%; $P < 0.01$). In summary, HS appears to reduce whole-body ketone clearance (and presumably oxidation) and Zn-HYD may benefit metabolic flexibility in heat-stressed dairy cows.

Key Words: ketone tolerance test, inflammation

1423T Muscle reserves in the prepartum period impact the response to pre- and postpartum intravenous glucose tolerance tests.

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Early lactation dairy cows are insulin resistant as they prioritize and partition glucose toward the mammary gland for milk production. Prepartum cows have higher circulating glucose concentrations than postpartum cows. This metabolic adaptation can be evaluated experimentally using intravenous glucose tolerance tests (IVGTT). The objective of this study was to determine if prepartum muscle reserves affected blood glucose and β-hydroxybutyrate (BHB) concentrations, on pre and postpartum dairy cows during IVGTT. To determine muscle reserve group, each cow had 3 ultrasound scans taken of their longissimus dorsi muscle at −42 d before expected calving and were assigned to either high (HM; > 4.6) or low (LM; < 4.6) muscle based on muscle depth. The IVGTT was performed −14 d before expected calving (n = 43; HM = 24 LM = 19) and 7 d after calving (n = 28; HM = 14 LM = 14) and cows were dosed with 250 mg/kg body weight of a 50% dextrose solution. Before sampling the cows were fasted for 1 h, and baseline blood samples were taken −15 and −5 min before dextrose administration, and blood was then collected at 12 time points over a 3 h time period. Prepartum, HM cows had a tendency ($P = 0.07$) to have greater baseline glucose concentrations, and there was a group by time interaction with HM cows having greater blood glucose concentrations immediately after dosing compared with LM cows ($P < 0.05$), but this effect was not seen in the postpartum period. Postpartum, HM cows had lower baseline BHB concentrations ($P = 0.01$) and this effect was maintained throughout the sampling period compared with LM cows ($P = 0.001$). These results suggest that the muscle reserves of the cows impact baseline glucose concentrations and response to IVGTT in the prepartum period, which was not demonstrated in the postpartum period potentially due to uptake of glucose by the mammary gland. Muscle reserve of cows may reduce BHB concentrations in the postpartum period for HM compared with LM cows, potentially positively impacting animal health.

Key Words: longissimus dorsi muscle, intravenous glucose tolerance test, transition period

1424T Glucose responses during glucose tolerance tests in transition Holstein cows with different body condition scores and their offspring.

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Early lactation of dairy cows and the first weeks of life of dairy calves are both marked by several changes in the energetic metabolism. The aim of this study was to evaluate the impact of the maternal prepartum body condition score (BCS) on the dynamics of blood glucose following a glucose tolerance test (GTT). Thirteen cows and 12 female calves were enrolled. Dams were categorized into 3 classes: class 1: BCS ≤3.0; class 2: 3.25 ≤ BCS ≤3.5; and class 3: BCS ≥3.75. The GTT was performed in Holstein cows between 7 and 9 d after calving, and in Holstein calves during the first week of life before the morning feeding. The GTT consisted of the infusion of 0.25 g of glucose/kg of BW infused in the jugular vein and blood samples were collected at −15, 0, 3, 6, 10, 15, 20, 30, 45, 60, 90, and 120 min relative to infusion. The data were analyzed using the GLM and MIXED procedures of SAS. We found an interaction between BCS and time for cows' glucose concentration. The BCS affected the cow's baseline blood glucose concentration (class 1 = 40.5 mg/dL; class 2 = 43.3 mg/dL; and class 3 = 52.3 mg/dL; SEM = 2.8; $P = 0.03$). The peak blood glucose concentration was higher (SEM = 15.9; $P = 0.03$) for cows in class 3 (217.8 mg/dL), followed by class 2 (180.8 mg/dL), than cows in class 1 (168.0 mg/dL). Blood glucose concentrations post glucose challenge, and area under the curve (AUC) were not affected by BCS. Maternal BCS had an effect ($P = 0.04$) in glucose levels during the test, however no interaction between glucose and time was present for calves. Baseline glucose was not affected (100.28 ± 7.7 mg/dL; $P = 0.40$), but the glucose peak was higher for the daughters of cows in class 3 (199.5 ± 7.6 mg/dL, $P = 0.04$) than classes 1 and 2. The AUC was higher for the class 2 daughters, followed by class 3 and class 1 in calves (18,466, 17,113, and 14,823 mg/dL per 120 min, respectively; SEM = 3,372; $P = 0.04$). The glucose uptake was higher in class 1 (lower BCS), for both mothers and their daughters, leading to a lower response in blood glucose after GGT compared with classes 2 and 3 (intermediate and higher BCS) animals.

Key Words: area under the curve, maternal effect

Production, Management, and the Environment 2

1425T Application of whole farm modelling to compare the emissions from regenerative and conventional dairy-beef production systems. S. Benitz*, C. Wand, J. Ellis, and M. Steele, *University of Guelph, Guelph, Ontario, Canada.*

Whole farm models (WFM) are a tool that allow for complete accounting and analysis of on-farm carbon dioxide equivalent (CO_2-eq) emissions and economics. Regenerative agriculture practices are a new farming system that impacts multiple facets of nutrient balance across farms. This work aims to compare CO_2-eq emissions from the finishing phase of conventional (CON) and regenerative (REG) dairy-beef production, using a WFM. Using Holos, a WFM software created by Agriculture and Agri-food Canada based on IPCC Tier 2 methodology, a REG and CON farm were simulated during the finishing phase on a yearlong basis. Simulations represented farms in Ontario with the cattle, manure management, and cropping system being the sole inputs. The REG cattle started at 428 kg and finished at 518 kg with an average daily gain (ADG) of 0.81 kg/d. The CON cattle started at 525 kg and finished at 606 kg with an ADG of 0.90 kg/d. The REG diet was supplied by crops grown on-farm using sustainable practices—reduced tillage, crop rotations, and no use of fertilizers. The CON diet was based on a corn-based finishing ration. Both systems simulated cattle housed in feedlots, with the REG system having the manure spread to the fields. Results show the REG system had higher enteric methane and lower manure methane (per kg of beef in CO_2-eq) compared with the CON system. This can be explained by a longer duration of days on feed/kg beef produced and the manure being utilized on the fields in the REG system. The CON system had higher direct and indirect N_2O emissions (per kg of beef in CO_2-eq). The use of nitrogen fertilizer in the CON system largely contributed to this increase in N_2O emissions. Although WFMs represent a useful tool to estimate sustainability outcomes, there are some limitations in assessing alternative systems due to their limited ability to represent alternative management strategies (e.g., housing and grazing options, manure storage systems). To properly consider the environmental impact of new system opportunities, such complexities, representations, and emission estimates should be built into the next generation of WFMs.

Key Words: dairy-beef, model, finishing

1426T Evolution of mature size, mature production, and the relative maturity and performance during the first two lactations of DHI registered Holsteins in Quebec. R. A. Molano*[1,2], R. K. Moore[1], and D. E. Santschi[1], [1]*Lactanet, Canadian Network for Dairy Excellence, Ste-Anne-de-Bellevue, QC, Canada,* [2]*Universitié Laval, Quebec, QC, Canada.*

Size and milk production of dairy cows have increased over time. However, the magnitude of this increase and its relationship with productivity across lactations has been little studied. The objective of this analysis was to describe the evolution over the last 2 decades of the mature BW (MBW), mature production and the relative maturity (RMAT) and performance (RPER) during the first 2 lactations of Holstein cows. Data from first (L1), second (L2) and third and more lactations (L3+) from 2002 to 2021 were extracted from the Quebec DHI database. Records of age at first calving (AFC, 1,413,772), BW (565,710; 713,668 and 1,152,530) and 305 d-adjusted milk and components yields (1,334,433; 1,0310,24 and 1,538,492) from L1, L2 and L3+ cows, respectively, were averaged per year. The L3+ cows were considered as mature and the reference to evaluate RMAT and RPER of L1 and L2 cows. Data from L3+ cows and AFC were regressed against time while RMAT and RPER were analyzed using a fixed effect model including year, parity and their interaction. The BW and milk (MY), fat (FY) and protein yields (PY) of L3+ cows increased since 2002 ($P < 0.01$) at rates of 3.7 ± 0.1, 109 ± 5, 5.7 ± 0.2 and 4.0 ± 0.2 kg per yr, respectively. In 2021 L3+ cows weighed 738 ± 1.2 kg and produced 11,184 ± 56, 447 ± 2.6 and 364 ± 2.8 kg of milk, fat and protein, respectively. The AFC decreased ($P < 0.01$) at a rate of 0.15 ± 0.01 mo per year, averaging 24.8 ± 0.13 mo in 2021. L1 cows' RMAT decreased ($P < 0.01$) at 0.09 ± 0.01% per year and was 87.4 ± 0.1% of MBW in 2021. Overall, L2 cows' RMAT was 94.46 ± 0.05% of MBW and did not change over time ($P = 0.61$). The RPER decreased over time ($P < 0.01$) but at a faster rate in L1 than in L2 cows ($P < 0.01$) for MY (0.22 ± 0.2 vs. 0.06 ± 0.2% per yr), FY (0.17 ± 0.2 vs. 0.04 ± 0.02% per yr) and PY (0.24 ± 0.01 vs. 0.07 ± 0.02% per yr). In 2021 MY, FT and PY relative to L3+ cows were 80.3, 81.5 and 81.5% for L1 and 95.0, 95.2 and 96.6% for L2 cows, respectively. Despite the significant progress in mature lactational performance and AFC, there has been a decline in the RPER of L1 and L2 cows that deserves to be addressed.

Key Words: maturity, lactation

1427T Combined effects of supplementing red algae (*Gracilaria* spp.) and lactic acid bacteria (*Lactobacillus pentosus*) on methane emissions and VFA production in vitro. A. Tiwari* and R. Kohn, *University of Maryland, College Park, MD.*

In ruminants, methanogenesis provides a sink for the disposal of hydrogen gas, contributing to methane production. Inoculating lactic acid bacteria (LAB) with red algae could provide an the alternative hydrogen sink to produce more volatile fatty acids (VFAs) from hydrogen and CO_2. However, information on the combined effect of gram-positive bacteria and anti-methanogenic properties of red algae has not been tested. The objective of this study was to evaluate the impact of adding lactic acid bacteria (*Lactobacillus pentosus*) and red algae into rumen fluid on VFA and methane emissions in vitro. Rumen fluid sample was taken from a fistulated nonlactating Holstein cow before early morning feeding. Rumen fluid samples (20 mL) in anaerobic glass bottles ($n = 20$) with 0.4 g timothy hay were incubated with or without algae (10% of DM), LAB (0.25 mL, 10^7 cfu/mL) or both for 48 h in a single batch. Treatments were as follows: 1) Control (no supplements), 2) LAB + Algae, 3) LAB, 4) Algae. Each treatment was replicated in 5 bottles, and samples of VFAs and gas were taken at 0, 4, 24, and 48 h. Data were analyzed using a completely randomized design in JMP 14.1 (SAS Institute Inc.). Similar to previous 2 runs (3% and 5% of DM as algae) there were no differences in methane emissions in the current level (10% of DM) of algae compared with control; however, acetate production was lower in Algae (48.2 ± 3.23 m*M*) than in LAB + Algae (55.8 ± 3.26 m*M*) ($P < 0.05$). Propionate was lower (21.9 ± 2.11 m*M*) and butyrate was higher (12.2 ± 1.18 m*M*) in Control, compared with Algae (30.6 ± 2.11 m*M*) and LAB (9.2 ± 1.18 m*M*) treatments ($P < 0.05$) respectively. A:P ratio was lower in Algae (1.9 ± 0.17) than in Control (2.4 ± 0.17) ($P < 0.05$). There were no interactions between the 2 supplements indicating these supplements have independent effects on VFA formation. This study suggests these supplements produce more end products without affecting methane emissions.

Key Words: greenhouse gas, methane inhibitors, probiotics

1428T Environmental performance of organic-certified grass-fed dairies stratified by production costs.

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Organic-certified grass-fed (OGF) is an emerging sector in the US characterized by dairy production systems exclusively based on forages. However, the interplay among greenhouse gas emissions, production practices, and economics of OGF dairies is not well understood. We aimed to evaluate the environmental performance of OGF dairies stratified by production costs using the Integrated Farm System Model (IFSM). The IFSM estimates the carbon footprint of dairies taking into account all farm activities, including land use, manure handling, and enteric gas emissions. The Dairy TRANS 4.4 tool was used to access the financial data of 16 northeastern dairies that had been certified OGF for at least 2 years to establish a cost of production benchmark. For the financial stratification analyses, only data from 2019 were used to avoid fluctuations in farm profitability due to the impact of the COVID-19 pandemic. Two farm groups were created based on total costs per hundredweight equivalent ($/cwt eq.): 1) Low-cost (n = 8, < $45/cwt eq.) with an average of 125 ha of land, 78 cows, 4,772 kg of fat and protein corrected milk (FPCM)/cow per year, and 2) High-cost (n = 8, > $60/cwt eq.) averaging 146 ha of land, 49 cows, and 3,639 kg of FPCM/cow per year. The IFSM simulations showed that the carbon footprint of High-cost OGF dairies was 15.3% greater than that of the Lost-cost OGF counterparts (Table 1). In brief, High-cost OGF dairy farms emit more carbon to the environment per kilogram of FPCM based on IFSM simulations, indicating that management strategies to increase milk production and reduce costs are needed to improve their environmental and economic sustainability.

Key Words: carbon footprint, grazing, whole-farm model

1429T Production performance, enteric gas emissions, and their associations with genomic predicted transmitting abilities of Holstein cattle.

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Enteric CH_4 from ruminants constitutes energy loss for the animal and contributes to anthropogenic greenhouse gas emissions. Genomic predicted transmitting abilities (gPTA) assess the genetic potential for productivity and health of Holstein dairy cattle. The objective of the current study was to evaluate the relationship between production variables and enteric CH_4 emissions as well as the association of gPTA with CH4 emissions using a database constructed from 20 experiments with a total of 420 cows conducted at the Pennsylvania State University. Production variables in the database included body weight (BW), dry matter intake (DMI), milk yield (MY), energy-corrected milk yield (ECM), and content of milk components [fat (CF), protein (TP), and lactose (LACT)]. Enteric emission variables included CH_4 (g/d), CH_4 yield (g/ kg DMI) and intensity (g/kg ECM), and CO_2 (g/d). Genomic PTA was available for 61 traits. Correlations and relationships among response and predictor variables were investigated using the CORR and MIXED procedures of SAS, respectively. Of the production variables, CH_4 had a weak positive correlation with BW ($P < 0.001$, r = 0.32), MY ($P < 0.001$, r = 0.31), and ECM (r = 0.36, $P < 0.001$), and a moderate positive correlation with DMI ($P < 0.001$, r = 0.48). Enteric CH_4 was weakly correlated with CF, TP, and LACT yields (r ≤ 0.35, $P < 0.001$). The dairy form gPTA had a weak negative correlation with CH_4 emission (r = −0.13, $P = 0.05$). Relationships of CH_4 emission with gPTA and production variables were weak ($R^2 \leq 0.13$) or non-existent, although many were statistically significant ($P \leq 0.04$). Overall, with the limited data set used in this study, none of the gPTA showed a strong correlation or relationship with CH_4 emissions, indicating that direct selection or non-genetic solutions may be required to help the selection of low CH_4-emitting animals by dairy producers.

Key Words: methane, milk production, genomic predicted transmitting ability

1430T Effects of wildfire-$PM_{2.5}$ on health and biomarkers of innate immunity in dairy cows.

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Wildfires are devastating natural disasters. Wildfire smoke inhalation causes respiratory disease and mortality in humans. Our group previously found that cows exposed to wildfire-particulate matter ($PM_{2.5}$) had lower milk production, changes in immune cells, and higher morbidity. The aim of this study was to determine relations between wildfire-$PM_{2.5}$ and biomarkers of innate immunity. Blood was collected from lactating, multiparous Holsteins (n = 13) before (baseline), during, and 3 weeks after exposure. Respiration rates (RR) were recorded 3X weekly. Rectal temperatures (RT), body weight (BW), and body condition scores (BCS) were measured weekly. A monitoring station near the farm recorded hourly $PM_{2.5}$, air temperature, and relative humidity data. Peripheral blood mononuclear cells (PBMC) and peripheral morphonuclear cells (PMN) were isolated and RNA was extracted for qRT-PCR to measure immune-related gene expression including interferon gamma (*INFγ*), interleukin (*IL*)-*1b*, *IL-8*, and *IL-6*. Data were analyzed with mixed

Table 1 (Abstr. 1428T). Environmental performance of organic grass-fed dairies stratified by production costs

	Low-cost (n = 8, <$45/cwt eq.)		High-cost (n = 8, >$60/cwt eq.)	
Greenhouse gas emissions, kg of CO_2eq^1/year	Mean	± SD	Mean	± SD
Animal emissions	366,418	1,683	244,242	1,001
Manure storage and handling	5,633	107	3,868	84
Direct and indirect land use	55,401	7,207	39,722	5,813
Carbon sequestration	−136,688	182	−78,285	136
Anthropogenic emissions	24,216	351	25,682	328
Carbon footprint, kg of CO_2eq/kg of FPCM[2]	1.22	0.02	1.44	0.03

[1] CO_2eq = carbon dioxide equivalent.

[2] FPCM = fat and protein corrected milk (4% fat and 3.3% protein).

model procedures in SAS. Fixed effects included daily average $PM_{2.5}$, THI, and their interaction. Separate models were run with lags of up to 7 d. The qRT-PCR data were analyzed using *t*-tests. The $PM_{2.5}$ ranged from 1.5 to 113.5 μg/m^3. The RR and RT increased or decreased, respectively, with greater combined temperature and humidity index (THI) and $PM_{2.5}$ up to lag d 2 ($P < 0.05$). The BW tended to decrease with combined elevated THI and $PM_{2.5}$ on lag d 0 ($P = 0.08$). There was a negative interacting effect of $PM_{2.5}$ and THI on BCS through lag d 2 ($P < 0.04$). Total white blood cells and lymphocytes tended to increase with $PM_{2.5}$ after a 1-d lag ($P = 0.07$ and 0.1, respectively). Relative to baseline, *IL-8* and *IL-6* were downregulated in PBMC during smoke exposure ($P < 0.01$). *IL-8* tended to remain downregulated 3 weeks after exposure ($P = 0.05$). In our study, gene expression was not altered in PMN. Exposure to wildfire-$PM_{2.5}$ induces an innate immune response characterized by changes in immune cell concentrations and gene expression of inflammatory biomarkers.

Key Words: smoke, inflammation, Holstein

1431T What measurable environmental factors impact rumination time in dairy cows at a pen level? A. A. McNeil* and B. J. Bradford, *Michigan State University, East Lansing, MI.*

Dairy technology has increased labor, feeding and milking efficiency, and aided in health management. Rumination monitoring has largely focused on individual cow health tracking; however, pen- and herd-level data could be capable of providing greater insight into herd-level disruptions. The purpose of this study was to investigate environmental and management variables that impacted pen-level rumination on a 6,000-cow dry lot dairy in the western United States. Retrospective rumination time data on a pen level from January 1 to June 29, 2022, were collected from a collar system (SCR, Merck Animal Health) and analyzed using statistical process control (JMP, SAS Institute). In this study, SPC was used to monitor farm-level mean rumination by day, as well as inter-pen variation over that time. Pen-level mean (m) and SD (s) for rumination time was determined for dry (m = 476 min/d, s = 41, n = 856 pen days), hospital (m = 349 min/d, s = 45, n = 170 pen days), lactating (m = 516 min/d, s = 28, n = 4,219 pen days), and fresh (<22 mean DIM; m = 498 min/d, s = 33, n = 229 pen days) pens. Control limits of dry pens, determined using the median moving range, were: upper control limit (UCL) = 525 min/d and lower control limit (LCL) = 431 min/d. Limits for lactating pens were UCL = 544 min/d and LCL = 487 min/d. Mean lactating pen rumination dropped below the LCL on 5 d (m = 477, s = 2.5, $P < 0.001$ vs. overall mean) and exceeded the UCL on 7 d. Stepwise logistic modeling was used to determine factors that influenced the risk of a negative deviation (below LCL); variables tested were pen count, mean DIM, precipitation (yes/no), temperature-humidity index (THI), and sea level pressure. Continuous variables retained were modeled as linear factors after testing higher-order functions. Precipitation (>2.5 mm) was associated with a 3.1-fold greater odds of a negative deviation ($P < 0.001$, 95% CI 1.98–5.08), while a 1-unit increase in THI was associated with a 6.5% lesser odds of a negative deviation ($P < 0.001$, 95% CI 0.92–0.95). Negative deviations in herd-level rumination were rare but were associated with rain and low THI on a western US dry lot dairy.

Key Words: statistical process control, health, management

1432T Effects of early lactation milking frequency in an automated milking system on cow performance. E. M. Kammann[1], N. S. Jozik[2], W. Li[3], E. A. French[3], and R. S. Pralle*[1,2], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*School of Agriculture, University of Wisconsin–Platteville, Platteville, WI,* [3]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Our study objective was to investigate the effects of early lactation milking frequency on cows milked via an automated milking system (AMS). Multiparous Holstein cows blocked by parity and due date were randomly assigned to fetch schedule treatments (TRT, n = 8 per TRT): 3 (3X) or 6 times (6X) per day. All cows had 3 milkings per day (MPD) from 0 to 3 d in milk (DIM). From 4 to 29 DIM (experiment phase, EXP), 3X cows were limited to 3 MPD while 6X cows were allowed 6 MPD. After EXP, all cows were allowed 6 MPD but fetching was reduced to twice per day from 30 to 90 DIM (carryover phase, COV). Body weight, milk yield, and feeding data was measured via the AMS. Weekly, composite milk samples from all milkings within a 24 h period were collected and analyzed by a commercial lab. Coccygeal blood was sampled at 3, 8 ± 1, and 13 ± 1 DIM; concentrations of glucose, β-hydroxybutyrate (BHB), and fatty acids (FA) were quantified. Data were analyzed by generalized linear mixed models (SAS 9.4, GLIMMIX) within the EXP or COV phase. Fixed effects included TRT, lactation group (LG, 2 vs. 3+ parity), DIM, and the interactions. Random effects were cow, block, and a repeated measure of cow across DIM. The 6X cows had greater milking frequency ($P < 0.01$) than 3X (4.9 ± 0.2 vs. 3.1 ± 0.1 MPD) during EXP; however, TRT had similar MPD ($P = 0.81$) during COV. Daily milk yield during EXP had non-separable TRT × DIM ($P = 0.10$) evidence. The 6X cows of 3+ parity had greater milk yield than 3X cows of 3+ parity during COV ($P = 0.01$, TRT × LG). During EXP, 6X cows of 3+ parity had greater ($P < 0.08$) milk fat content and 4% fat-corrected milk yield versus 3X 3+ parity cows. Pellet offered and the ratio of pellet offered per 45 kg milk was similar across TRT in both phases ($P > 0.87$). Total eating minutes in the AMS during EXP was greater ($P = 0.02$) for 3X than 6X cows. Body weight was similar across TRT in both phases ($P > 0.38$). Within the 3+ LG, 6X cows had greater blood FA ($P = 0.06$) and lower BHB ($P = 0.04$) than 3X cows. Glucose did not differ between TRT ($P = 0.90$). Greater early lactation MPD in AMS may shift cow nutrient partitioning to support greater production in 3+ parity cows.

Key Words: transition period, robotic milking, health

1433T Effects of alternative feeds on *in vitro* digestibility, rumen fermentation and gas production of lactating cow diets in semi-intensive Kenyan dairy systems. C. Arndt[1], Y. Li*[2], B. Habermann[1], C. Patino-Pinares[1], D. Korir[1], X. Sun[2], S. Yang[2], C. Kunz[2], L. Gichuki[1], and M. Niu[2], [1]*International Livestock Research Institute (ILRI), Nairobi, Kenya,* [2]*Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland.*

The objective was to evaluate effect of supplementing 10 different fodders on in vitro methane (CH_4), hydrogen (H_2), ammonia (NH_3), total fermentation gas, and volatile fatty acid (VFA) production as well as organic matter digestibility (OMD), and the correlations between dietary tannin content (total, condensed, and hydrolyzed tannins; TT, CT, HT) and CH_4 and NH_3 production. The basal ration was based on the lactating cow diet under semi-zero grazing conditions in semi-intensive dairy systems as reported by Kenya's greenhouse gas inventory. The diet contained 25% native grass, 25% Rhodes grass hay, 18% Napier grass, 5.5% maize stover, 5.5% bean residue, 8% dairy meal, 1% mineral mix, and 12% alternative feedstuff, on DM basis. The feed was partially replaced at 12%, the alternative feedstuff includes 10 fodders: *T. zebrina*, *D. intortum*, *C. calothyrsus* (*Cc*), *L. leucocephala* (*Ll*), *S. sesban*, *V. sativa*, *L. multiflorum* (*Lm*), *C. intybus*, *M. sativa*, or *I. batatas*. Rumen fluids were collected from 3 lactating cannulated Original Brown-Swiss cows before morning feeding, each representing a biological replicate. Hohenheim gas test method was performed according to Menke and Steingass (1988), each biological replicate contains 3 technical replicates per treatment. Data were analyzed using a mixed model including random effect of fistulated cows and fixed effect of treatments, where treatments were all the possible substitutions. Pearson correlations between CH_4 or NH_3 production and TT, CT, or HT, were calculated. Diets with *Cc* and *Ll* compared with *Lm* had similar OMD and produced a similar amount of total fermentation gases, but less CH_4 and more H_2. Further, they produced less isobutyrate potentially due to reduced amino acid fermentation due to higher dietary tannin content. Compared with all other feedstuff, *Ll* supplementation produced more isovalerate. TT, CT, and HT had a negative effect on NH_3 and CH_4 production ($P < 0.05$). The TT content of diets supplemented with *Cc* and *Ll* was 5 times greater than of the remaining diets (mean ± SD = 0.88 ± 0.04 vs. 0.16 ± 0.06 vs. % TT on a DM basis). *Cc* and *Ll* supplementation should be tested for their potential to reduce enteric CH_4 from semi-intensive Kenyan dairy systems.

Key Words: methane, batch culture, tannin

1434T Changes in the dietary phosphorus of dairy cows in Idaho: A case study. I. A. M. A. Teixeira*[1], S. A. Santos[2], A. Leytem[3], and M. Chahine[1], [1]*University of Idaho, Twin Falls, ID,* [2]*Universidade Federal da Bahia, Salvador, BA, Brazil,* [3]*USDA-Agricultural Research Service, Kimberly, ID.*

The objective of this case study was to evaluate the changes in dietary phosphorus (P) on Idaho dairy farms. Our database was comprised of actual diet formulations for lactating and dry cows in 2006 (n = 5 representative farms) and 2022 (n = 6 representative farms). The diet formulations from 2006 were assumed to represent the diets used in early 2000 (PAST) and the diets from 2022 were assumed to represent those currently used (CURR) on dairy farms in Southern Idaho. The variables collected were the P content in the formulated diets for lactating and dry cows, the dietary ingredients (forages and concentrates), and their percentage in the diets. Unpaired *t*-tests were applied to analyze the data. There was no difference ($P = 0.46$) in P content in lactating dairy cows diets (0.41%). The P content tended to decrease in the diets of dry cows (0.41% vs. 0.32%, $P = 0.07$). Alfalfa hay and corn silage were the primary forage source for lactating cows. In both time periods, all farms included alfalfa hay in the diets for lactating and dry cows. However, the inclusion of alfalfa hay decreased from 20.4% in PAST diets to 13.6% in CURR diets for lactating cows. More farms are including corn silage in the diets of lactating cows (67% vs. 100% of farms in 2006 and 2022, respectively). The inclusion of corn silage in the diets of lactating cows increased by 60% from early 2000 to 2022. The use of triticale and straw in the diets of dry cows has markedly increased. Currently, 80% of dairy farms use these ingredients for far-off-dry cows. We observed a wide variety of other ingredients being used in the diets of dairy cows in Southern Idaho and some of these show high levels of P and protein. This case study illustrates that P levels in diets of dry cows have changed since 2006. It might be related to the use of ingredients that are low in P (i.e., straw). On the hand, P levels did not change in the diets of lactating cows, even with changes in the ingredient composition (i.e., forage, by-products). Our findings also create an opportunity to raise a discussion of more holistic approaches to refine P nutrition in dairy, which ultimately would decrease the negative impact of P excretion on the environment. Further work seeks to improve these results by including additional data on past and current diet formulations of dairy cows.

Key Words: dairy cow, manure, phosphorus

1435T Improving cull cow carcass traits and meat quality. N. Berdusco*, T. F. Duffield, D. F. Kelton, K. M. Wood, and D. B. Haley, *University of Guelph, Guelph, Ontario, Canada.*

Despite a high volume of cull dairy cows entering the food chain every year, beef from cull dairy cows is largely considered insignificant when compared with beef cattle and is widely thought to be used for ground beef only. Our study aimed to evaluate the effects of feeding dairy cull cows on a lactating diet before slaughter on carcass and beef quality traits. 37 Holstein cull cows were randomly assigned into 2 treatments: FED or DIRECT. FED cows (n = 18) were dried off, then fed the lactating cow ration for 60 d whereas DIRECT cows (n = 19) were sent directly to slaughter. Hot carcass weight (HCW) was used to calculate the dressing percentage (DP) together with the animals' body weight recorded when animals left the farm to go to the abattoir. At 24 h post-mortem, the rib fat thickness (RFT) and rib eye area (REA) were measured by a certified grader. Afterward, a longissimus muscle sample was collected between the 12th–10th ribs from one side of the carcass and divided into 5 ribeye steaks. The first steak was used for intramuscular fat (IMF) content analysis by NIRS. The other 4 steaks were then aged for 7, 14, 21, or 28 d. Following aging, the steaks available were assessed for tenderness using the Warner-Bratzler Shear Force protocol. Data were analyzed by a *t*-test to compare experimental groups for continuous outcomes. The HCW of the FED cows was higher than the DIRECT cows, with a mean HCW of 402.52 kg and 332.01 kg, respectively ($P < 0.05$, SD = 59.73). Average DP in FED cows was 48.7%, compared with 43.6% in DIRECT cows ($P < 0.05$, SD = 4.83). The average percentage of intramuscular fat in DIRECT cows was 4.8%, and in FED cows was 8.4% ($P < 0.05$, SD = 2.83). No differences ($P > 0.05$) were found for REA or RFT. The steaks aged for 14 d resulted in meat with a mean shear force (SF) of 4.24 kg in FED cows, while DIRECT cows had a mean SF of 6.31 kg ($P < 0.05$, SD = 1.98). Feeding dairy cull cows for 60 d before slaughter enhanced carcass weight and yield, IMF content, and tenderness. These results indicate that 60 d of feeding can improve the quality of cull cow meat and may help improve cull cow value.

Key Words: meat quality, carcass trait, cull cow

1436T Identifying early indicators of subclinical mastitis via Raman spectroscopy. A. L. Ollinger*[1] and B. W. Jones[1,2], [1]*Tarleton State University, Stephenville, TX,* [2]*Texas A&M AgriLife Research, Stephenville, TX.*

Mastitis is one the biggest challenges dairy producers face as it is one of the most expensive diseases in the dairy industry. Somatic cell count (SCC) is used as an indicator of subclinical mastitis through diagnostics tests, but that data is gathered irregularly. Therefore, the objective of this study was to evaluate if Raman spectroscopy could determine subclinical mastitis in whole milk samples. Bi-weekly composite milk samples were taken from n = 400 dairy cattle at the Southwest Regional Dairy Center at Tarleton State University from June 2022 to October 2022. After collection of composite samples, one sample from each cow was immediately sent to the DHIA laboratory to determine SCC, fat, protein, lactose, solids nonfat, milk urea nitrogen, β-hydroxybutyrate, and acetone using a Bentley FTS/FCM Combi 600 (Bentley Instruments, Chaska, MN). Upon completion of the composite sample collection, the other sample from the same cow were immediately stored in a refrigerator at 1.6°C in the laboratory. The same composite milk samples were then evaluated for SCC via Raman spectroscopy. Samples were evaluated within 1 to 4 d of sampling. Samples were vortexed (Vortex Mixer, VWR, Radnor, PA) for 5 s before scanning. Each sample received 5 scans in rapid sequence. In total, n = 10,335 scans were taken via Raman spectroscopy. Data from the DHIA laboratory and the Raman spectroscopy were then compared with determine the accuracy in measuring SCC using a partial least square discriminant analysis model in MATLAB R2020b (Mathworks, Natick, MA). Scans used in the model were n = 5,576. Fifty-five percent of data warranted a true prediction rate. This true prediction rate is low, so further research is needed to evaluate if Raman spectroscopy can be a valuable tool in determining early indicators of mastitis.

Key Words: mastitis, Raman spectroscopy, milk quality

1437T Comparison of grazing perennial ryegrass and perennial ryegrass-white clover swards for enteric methane emissions and milk production in grazing dairy cows. C. Dwan*[1,2], L. Shalloo[1], F. Buckley[1,2], D. Hennessy[1], H. Irish[1,2], and B. Lahart[1], *[1]Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland, [2]School of Biological Earth and Environmental Science, Cork, Ireland.*

The incorporation of white clover (clover) in perennial ryegrass (PRG) swards has become increasingly popular in seasonal pasture based dairy systems in temperate regions such as Ireland. This is due to clover's ability reduce chemical fertiliser requirements through nitrogen fixation, improving economic and environmental sustainability. However, there is currently little information on the effect of clover inclusion on enteric methane emissions (CH_4). The current experiment consisted of Holstein-Friesian cows grazing one of 2 farmlets, PRG (GR; n = 19) receiving 225 kg N/ha/year or PRG with clover receiving 150 kg N/ha/year (CL; n = 17). Each farmlet was managed identically under an intensive rotational grazing system. Methane was measured using 2 GreenFeed systems over 4 fortnightly periods from June 20th to August 14th. Concentrate fed from the GreenFeed averaged 1.01 kg/cow/day (SD = 0.41), further supplementation in accordance with herbage availability brought the average to 1.75 kg/cow/day (SD = 0.94). The average proportion of clover in the CL swards per period was 30% (SD = 4.7%). Animal data were analyzed using the mixed procedure of SAS with period as a repeated measure and the fixed effects of treatment, parity, calving day of year, period, and the period by treatment interaction, and respective pre-experimental covariate. Clover had 16% higher CH_4 ($P = < 0.001$) and 7% higher CO_2 to CH_4 ratio ($P = 0.003$). There was no significant difference in CH_4 per kg of milk solids (MS; milk fat + protein). Clover had 7% higher MS yield ($P = 0.04$) with 6% higher fat ($P = 0.01$), but no significant difference in protein ($P = 0.41$). In conclusion, the addition of clover to PRG swards increased total CH_4 emissions but also increased MS yield in the mid grazing season, therefore having no effect on methane intensity within seasonal grazing dairy systems.

Key Words: methane, white clover, pasture-based dairy system

1438T Effects of supplementing a *Bacillus*-based direct-fed microbial on performance and digestibility of lactating dairy cows. D. Sousa*[1], O. Queiroz[2], B. Cappellozza[2], C. Alveblad[3], and B-O. Rustas[3], *[1]Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Skara, Sweden, [2]Chr. Hansen A/S, Hørsholm, Denmark, [3]Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden.*

The aim of this study was to evaluate the effects of a *Bacillus*-based direct-fed microbials (DFM) on milk production, composition, and production efficiency, as well as nutrient digestibility of dairy cows. We hypothesized that feeding a *Bacillus*-based DFM to lactating dairy cows would improve performance and nutrient digestibility. Sixty-eight Holstein and Swedish Red early-lactating cows (days in milk 50 ± 6) were blocked by calving date and parity in 1 of 2 treatment groups: (1) partial mixed ration (PMR) without DFM supplementation (n = 34; CON) or (2) PMR with the addition of 3 g/head per day of a *Bacillus*-based DFM (BOVACILLUS, Chr. Hansen A/S, Denmark; n = 34; DFM) for a 105-d experiment, including a 21-d reference period. The PMR contained clover grass silage, compound feed, and a mineral mixture. The nutrient composition (g/kg DM) of the PMR was NDF (322), CP (168), starch (115), and fat (25). Cows received a pelleted protein concentrate supplement separately, containing or not DFM. Milk yield and dry matter intake (DMI) were evaluated daily, whereas milk composition was analyzed every 14 d. Moreover, fecal samples were collected on wk 3, 9, and 15 of the experimental period. Data were analyzed with SAS (v. 9.4). A treatment effect was observed on milk yield ($P < 0.01$), as DFM-supplemented cows produced 1.2 kg more milk than CON (40.0 vs. 41.2 kg milk/day, respectively). No treatment effects were observed on DMI ($P = 0.73$), which benefited milk production efficiency upon DFM feeding (1.48 vs. 1.52 kg milk/kg DMI; $P = 0.01$). Milk lactose yield was greater ($P = 0.02$) and milk protein yield tended to be greater ($P = 0.09$) for DFM vs. CON, but no additional effects were observed on milk composition ($P > 0.16$). Lastly, nutrient digestibility did not differ between CON and DFM ($P > 0.55$). In summary, feeding a *Bacillus*-based DFM improved milk yield, milk production efficiency, and lactose yield, highlighting the potential of this technology to optimize milk production in dairy production settings.

Key Words: *Bacillus* spp., direct-fed microbial, milk production efficiency

1439T Relationships between birth and calving season on first-lactation performance of Holstein dairy cows in the Midwestern USA. K. N. Brost* and J. K. Drackley, *University of Illinois, Urbana, IL.*

Our purpose was to establish the effects of birth season on performance of first-lactation Holstein cows. Further analysis was conducted to determine if the impact of calving season was a more appropriate indicator. This study analyzed data from 2009 to 2022, and included 524 first parity Holstein cows from the University of Illinois Dairy Research Unit. Cows were managed per farm protocols. Seasons were assigned by birth and calving months: winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). The main comparison was winter vs. summer seasons of birth and calving, but summer vs. non-summer and winter vs. non-winter were also used. Data were analyzed using the MIXED procedure in SAS. Based on first lactation data, cows born in summer tended ($P = 0.091$) to leave the herd by the end of the first lactation more often than those born during non-summer months. On average, days pregnant was less ($P = 0.050$) for summer-born cows compared with non-summer. No difference was observed for the number of lactations or times bred. Summer-born cows produced less ($P = 0.026$) 305-d milk (kg) relative to winter. Average test day milk (kg) for d 5 to 35 was less for summer-born cows in comparison to winter ($P = 0.014$), but no differences were noted for d 5 to 21. Average test day % fat and % protein were less for summer-born cows compared with winter during d 5 to 35 ($P = 0.001$ and $P = 0.029$), and d 5 to 21 ($P = 0.027$ and $P = 0.061$). Summer-calving cows stayed in the herd fewer average lactations than winter ($P = 0.024$). Days pregnant was less for summer-calving compared with winter ($P = 0.006$), but no differences were detected in times bred, when cows left the herd, or 305-d milk. Summer-calving cows had less test day milk (kg; $P = 0.024$), % fat ($P = 0.001$), and % protein ($P = 0.002$), when compared with winter for d 5 to 35. The same pattern was seen for test day % fat ($P = 0.034$) and

% protein ($P = 0.006$) for summer-calving during d 5 to 21. Overall, an association between birth and calving seasons was observed, suggesting their influence on first-lactation performance.

Key Words: season, first lactation, performance

1440T Reproduction success of dairy cows associated with early lactation milk fatty acids. D. Warner*[1], L. Fadul-Pacheco[1], R. Gervais[2], and D. E. Santschi[1], [1]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*Université Laval, Quebec, QC, Canada.*

The metabolic challenges at the onset of lactation can influence the reproductive success of a dairy cow. Early lactation is typically characterized by important changes in milk yield and fatty acid (FA) profile. Routine milk analysis during early lactation could therefore be useful to predict the reproductive performance. First test milk recording samples from 236,827 Holstein cows of 5 to 35 DIM were collected over 3 years (February 2020 through January 2023) across 2,921 herds in Quebec, Canada, and analyzed using Fourier-transform infrared (FTIR) spectroscopy. Cows with similar characteristics in their first test milk FA profile (g/100 g of total FA), composition and yield were grouped using an iterated k-medoid clustering approach on subsamples after initial dimensionality reduction by principal component analysis, to account for the large data set. Production performance (305-d milk) was analyzed using a linear regression mixed effect model, reproduction performances using a binomial logistic regression mixed effect model (culling rate) and a Cox proportional-hazard model (first service to conception rate). Three main clusters were identified: Cluster 3 with low de novo FA (17.7%) and lactose (4.55%) but high preformed FA (55.8%), C18:1/C14:0 (4.56), BHB (0.16 m*M*), and fat/protein (1.45); Cluster 2 with high de novo FA (26.5%) and lactose (4.62%) but low preformed FA (38.3%), C18:1/C14:0 (1.94), BHB (0.08 m*M*), and fat/protein (1.17); and Cluster 1 with a milk profile mostly in between: de novo FA (22.7%), lactose (4.63%), preformed FA (46.7%), C18:1/C14:0 (2.83), BHB (0.10 m*M*), fat/protein (1.24). While Cluster 3 cows had the greatest production performance (+773 kg milk at 305 d relative to Cluster 2; $P \leq 0.001$), they also had lower reproductive performances with the highest likelihood for culling by 60 DIM (odds ratio of 1.88–2.93; $P \leq 0.001$), and the lowest first service to conception rate (odds ratio of 0.93–0.97; $P \leq 0.001$). The milk FA profile from routine screening of milk samples during early lactation via FTIR can be an early indicator of reproductive success and help improve the transition management for future lactations.

Key Words: milk recording, infrared, reproduction

1441T Effects of combining plant-based compounds with 3-nitrooxypropanol (3-NOP, Bovaer 10) on methane emissions and lactational performance of dairy cows. X. Ma*[1], S. E. Räisänen[1], K. Giller[1], M. Z. Islam[1], Y. Li[1], R. Peng[1], M. Reichenbach[1], X. Sun[1], I. Müller[2], and M. Niu[1], [1]*Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH, Lindau, Zurich, Switzerland,* [2]*Department of Animal Nutrition, DSM Nutritional Products, Kaiseraugst, Aargau, Switzerland.*

The compound 3-nitrooxypropanol (3-NOP) is one of the most consistent enteric methane (CH_4) inhibitors in ruminants. This pilot in vivo study aimed to investigate the effect of combining 3-NOP with 4 different plant-based compounds [tannin extract (TA), 2 essential oils (EO1 and EO2), and oilseed (OS)] compared with the CH_4 mitigating potential of 3-NOP alone. Twelve multiparous lactating Holstein and 12 Swiss Brown cows, [232 ± 71 d in milk, 29.5 ± 4.63 kg milk yield (MY)], were used in a randomized complete block design study with a 25-d experimental period preceded by an 8-d covariate period (with no supplementation). Cows were assigned to 1 of the 6 treatments: 1) positive control (+CON: 3-NOP alone), 2) 3-NOP + TA, 3) 3-NOP + EO1, 4) 3-NOP + EO2, 5) 3-NOP + OS1 (oilseed, 5% of TMR DM), and 6) 3-NOP + OS2 (oilseed, 10% of TMR DM). Dry matter intake (DMI) and MY were recorded daily, and CH_4 emission measured (with GreenFeed System) and milk samples collected during the last 3 d of the covariate and experimental periods. Data were analyzed in a mixed model using the *lmer* procedure of R statistical language with treatment, period, breed and baseline covariate as fixed effects, and cow as a random effect. Compared with 3-NOP alone (+CON), none of the tested combinations affected DMI (23.3 kg/d; SE = 0.60), MY (28.1 kg/d; SE = 1.15), or milk components. Milk urea N was 32.0 mg/dL vs. 24.6 mg/dL for +CON and 3-NOP + TA, respectively. Methane production, yield, and intensity were similar between the combinations vs. +CON, averaging 408.3 g/d, 17.6 g/kg DMI and 20.4 g/kg fat and protein-corrected milk (FPCM), respectively. For example, CH_4 yield was 16.3, 17.9 and 17.3 g/kg DMI, CH_4 intensity was 14.8, 14.4 and 13.7 g/kg FPCM for +CON, 3-NOP + OS1 and 3-NOP + TA, respectively. The results suggest that combining 3-NOP with TA or OS1 may enhance the efficacy of 3-NOP but warrants further investigation with a greater number of animals.

Key Words: methane mitigation, tannin, essential oil

1442T Combined effects of 3-nitrooxypropanol (3-NOP, Bovaer 10) and whole cottonseed on production and enteric methane emissions of dairy cows. X. Ma*[1], S. E. Räisänen[1], T. He[1], M. Z. Islam[1], Y. Li[1], R. Peng[1], M. Reichenbach[1], X. Sun[1], K. Wang[1], S. Yang[1], Z. Zeng[1], I. Müller[2], and M. Niu[1], [1]*Department of Environmental Systems Science, Institute of Agricultural Sciences, Lindau, Zurich, Switzerland,* [2]*Department of Animal Nutrition, DSM Nutritional Products, Kaiseraugst, Aargau, Switzerland.*

The objective was to determine potential combined effects of 3-NOP and whole cottonseed (WCS), through differing antimethanogenic mechanisms, on enteric methane (CH_4) emission and lactational performance of dairy cows. Sixteen cows (8 Holstein Friesian and 8 Brown Swiss; 224 ± 35.9 d in milk, 26 ± 3.7 kg/d milk yield), were used in a 4 × 4 Latin Square design with 2 × 2 factorial arrangement of treatments with 4, 24-d periods. The treatments were: 1) Control (basal TMR), 2) 3-NOP (formulated to 60 mg/kg DM), 3) WCS (5% DM), 4) 3-NOP + WCS. The diets were balanced for ether extract and NDF content (3.7% and 37% of DM) and fed 2×/day at 0800 and 1800 h. Milk yield and DMI were measured daily. Milk samples, and enteric gas emissions were measured (with GreenFeed system) during the last 3 d of each period. Data were analyzed in a mixed model using the *lmer* procedure of R statistical language including fixed effect of 3-NOP, WCS, 3-NOP × WCS interactions, period, and breed, and random effect of cow nested in sequence. There were 3-NOP × WCS interactions for DMI ($P = 0.04$), being greatest for cows fed 3-NOP + WCS. The WCS treatment increased ECM yield ($P = 0.01$), milk fat yield ($P < 0.01$) and tended to increase milk protein yield ($P = 0.09$). There were no interactions of 3-NOP and WCS for any of the enteric gas emission parameters, but 3-NOP decreased CH_4 production (g/d), CH_4 yield (g/kg DMI), and CH_4 intensity (g/kg ECM) by 12, 15 and 13%, respectively. Hydrogen production was increased by 185% in cows receiving 3-NOP ($P < 0.01$). The DMI and CH_4 data were further analyzed as hourly intake (% of daily DMI) and production (g/h), to characterize the diurnal intake and emission patterns. There were no treatment by time interactions for DMI, but 3-NOP by time interactions ($P < 0.01$) for CH_4 were observed; greatest reduction of CH_4 (20%) occurred after feeding, coinciding with the greatest DMI periods of the day. In conclusion, combining WCS with 3-NOP improved DMI, ECM, and milk fat and protein yields, but did not enhance CH_4 inhibition effect of 3-NOP of dairy cows.

Key Words: methane mitigation, whole cottonseed, 3-NOP

1443T Identifying gram-positive and gram-negative infections via differences in Raman spectroscopy spectra. L. A. Martin*[1] and B. W. Jones[1,2], [1]*Tarleton State University, Stephenville, TX,* [2]*Texas A&M AgriLife, Stephenville, TX.*

Mastitis is classified as inflammation of the mammary gland, which can lead to negatively impacting milk production and quality. The objective of this study was to identify gram-positive and gram-negative mastitis pathogens via differences in Raman spectroscopy spectra. Bi-weekly composite milk samples were obtained from n = 400 dairy cattle housed at the Southwest Regional Dairy Center in Stephenville, TX, and were collected from June 2022 to October 2022. Composite milk samples were analyzed at the DHIA laboratory using a Bentley FTS/FCM Combi 600 (Bentley Instruments, Chaska, MN). Any cows SCC > 200,000 cells/mL were aseptically sampled from each individual quarter. After teat prep for milking (fore-stripping, pre-dipping and removal of the pre-dip (0.15% iodine based germicide) with a clean towel) teat ends were scrubbed clean with cotton balls soaked in 70% ethyl alcohol. Approximately 5 mL of foremilk from each quarter was stripped into an individual sterile conical tube (VWR, Radnor, PA). Samples were frozen immediately and when thawed, 0.01 mL was plated onto a Bi-plate (Minnesota Veterinary Diagnostic Laboratory) and incubated at 37°C. After 48 h, plates were analyzed to determine if they were gram-negative, gram-positive, contaminated, or no growth. The composite milk samples were scanned via Raman spectroscopy. A total of n = 10,335 scans were used to identify spectroscopic signatures associated with mastitis. Spectroscopic signatures unique to the specific bacterial pathogen (gram-positive or gram-negative) were determined using a partial least square discriminant analysis in MATLAB R2020b (Mathworks, Natick, MA). The spectra were not specific from either gram-positive or gram-negative pathogens and cannot be classified as gram-positive or gram-negative. Further research is needed to evaluate if Raman spectroscopy can be a valuable tool in determining mastitis pathogens.

Key Words: mastitis, milk quality, Raman spectroscopy

1444T Study on categorization of factors affecting smallholder dairy production in Siltie zone, southern Ethiopia, applying multivariate analysis approaches. B. Tolasa Itafa*[1,2], [1]*Werabe University, Werabe, SNNP, Ethiopia,* [2]*Jimma University, Jimma, Oromiya, Ethiopia.*

Advanced understanding of the existing production systems plays an important role in developing appropriate and innovative technologies to alleviate challenges on dairy farms. The multivariate statistical approach is one of the most common techniques used in farm categorization to identify the affecting factors. Correspondingly, the aim of this study was to categorize the factors affecting dairy production of smallholder dairy farms in Siltie Zone, Southern Ethiopia. Survey data from 180 smallholder farms were subjected to categorical principal components analysis (CATPCA). Next, a two-step cluster method was used to classify the farms using object scores obtained from the CATPCA, resulting in 3 different principal components. These 3 components represented factors of environmental and genetic interaction, management and hygiene. The primary principal component (environment-genetic interaction factors) defined 17.056% of the entire variability within the data set, with 11 variables contributing to this dimension. It was strongly related to scarcity of feed, mastitis, abortion, crop residue, dystocia, uterine prolapse, poor genetic potential, adaptability, disease, forage and grazing. The second principal component (management factors), which defined an additional 12.431% of the overall variability, was definitely related to types of feeding, source of water and tools to offer water. However, it was negatively related to feeding, housing, land holding and land holding for crop production. The third principal component (hygienic factors) defined an extra 6.58% of general variability and was strongly related to 2 variables, which include cleaning and hygienic frequency. These variables had a negative impact. Indeed, hygiene is an essential component in ensuring an animal's fitness as well as milk quality. Following the CATPCA, a Two-Step cluster has produced 3 potential clusters. An evaluation across all production systems indicated that most farms in urban and peri-urban areas (76.4% and 49.2%, respectively) were in cluster 1. At the same time, most farms in rural areas (71%) fell into cluster 3. Overall, most farms fall within clusters 1 and 3 (45.00% and 32.22%, respectively). Key discriminating factors were feed scarcity and animal disease. Farmers in clusters 1 and 2 would need agricultural guidance that meets their needs with little land to allocate to dairy production. However, farmers in cluster 3 would require advice on how to feed animals using stall feeding and practicing improved forage. On the other hand, all clusters have homogeneity in regard to discriminating factors. Thus, training and forming farmer groups as a cooperative to offer services consisting of feed processing machines and health services is recommended to overcome these problems.

Key Words: cluster, dairy, eigenvalue

1445T Exploratory comparison between cows and bulk tank free fatty acids in Quebec dairy herds. L. Fadul*[1], D. Kelton[2], and D. E. Santschi[1], [1]*Lactanet, Sainte-Anne-de-Bellevue, Quebec, Canada,* [2]*University of Guelph, Guelph, Ontario, Canada.*

Free fatty acids (FFA) that exceed the sensory threshold of 1.2 mmol/100 g of fat can adversely affect milk quality, therefore, measuring FFA at the bulk tank and cow level can help identify issues and ultimately contribute to their prompt resolution. The aim of this study was to explore the relationship between FFA from individual cows and the bulk tank. Monthly cow and their closest bulk tank value (± 1 d) FFA data were obtained from the Lactanet (Canadian Network for Dairy Excellence) database from 3,205 herds for one year (May 2021 to May 2022), for a total of 315,253 cows and 2,103,641 records. Cows and bulk tank FFA average was 1.12 and 1.05 mmol/100 g of fat, respectively. To assess significant differences a one-way ANOVA followed by a Tukey HSD test ($\alpha = 0.05$) was performed. Results showed significant differences among breeds. Holstein cows had higher FFA than Ayrshires, Brown Swiss, and Jersey (1.12; 1.02; 1.01 and 0.99 mmol/100 g of fat; SEM = 0.70; $P < 0.001$). Also, there were significant differences ($P < 0.01$; SEM = 0.71) among parities (1, 2 and +3), where parity 1 had the lowest FFA concentration, parity 2 the highest and parity +3 was in between (1.1; 1.19; 1.08 mmol/100 g of fat, respectively). There were also significant differences among milking systems ($P < 0.01$; SEM = 0.32). The pipeline had the higher FFA (1.06) followed by the automatic milking systems (AMS; 1.04), and the lowest concentration of FFA was found in milking parlors (0.86). The absolute differences between bulk tank FFA and the cows weight average was significantly high for AMS (0.58) than for milking parlors (0.08) and pipeline (0.005; $P < 0.001$).

Results show that there was a high percentage of cows with FFA > 1.2 on AMS (53%), pipeline (31%) and milking parlors (22%; $P < 0.001$). There was a negative correlation ($R = -0.62$) between the difference of FFA among the bulk tank and the weighted average of the cows and the percentage of cows with high FFA > 1.2 mmol/100 g of fat in the herds. These preliminary results show that there are differences among milking systems and breed for the FFA. To have a better understanding of the implications of this results, more analyses and studies are needed.

Key Words: free fatty acid, bulk tank, milking system

1446T Exploring the genetic impact on box time indicators used to rank cows in automatic milking systems.

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Selecting cows based on their milking performance in the automatic milking systems (AMS) has been identified as a way to improve overall AMS efficiency. The aim of this study was to explore the effect of genetic traits, such as milking speed and udder conformation, on the kg milk/minute of box time (MMBT). Data was obtained from the Lactanet (Canadian Network for Dairy Excellence) database from May 2021 to February 2023 for 17 Lely AMS Holstein herds, for a total of 2,874 cows and 614,465 records. Data included milk production, MMBT, preparation time, milking speed (kg milk produced per minute of milking time), DIM, parity, and genetic traits for milking speed (MS), milk production and mammary system. Calculations were done based on the International Committee for Animal recording (ICAR) standards (i.e., 4-d average). Pearson correlation analysis was used to assess whether MMBT and some AMS variables were correlated with genetic traits. The correlation between the average MMBT (milk kg/min) and milking speed (kg milk/min) was $R = 0.83$, but it was lower between MMBT and preparation time (min; $R = 0.06$). The range of these correlations among herds was $R = 0.67$ to 0.92 and from $R = -0.02$ to 0.2 for MMBT and milking speed (kg milk/min) and MMBT and preparation time, respectively. The correlation between the actual milking speed (milk, kg/min) and MS genetic trait was $R = 0.24$ and it ranged from 0.12 to 0.43 among herds. Preparation time and mammary system trait were not much correlated ($R = 0.079$ with a range from $R = -0.049$ to 0.17 among herds). Finally, the correlation between MMBT and milk production trait was $R = 0.33$. Parities were significantly different for actual milking speed, where parity 2 was faster, followed by parity 3 and 1 (3.45, 3.30 and 3.16 kg milk/min, respectively; $P < 0.01$). However, the MS genetic trait was only significantly different for parity 2 ($P < 0.001$). These results suggest that udder conformation is not related to preparation time. Likewise, MS genetic trait does not seem to affect AMS efficiency. The next steps of this study are to explore other traits such as temperament to better understand management practices that can improve AMS milking efficiency.

Key Words: genetic, efficiency, AMS

1447T Characterization of antimicrobial resistance of *Mammaliicoccus fleurettii* isolated from cattle and humans on a Vermont dairy farm.

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Mammaliicoccus fleurettii is most frequently isolated from bovine associated environments and occasionally identified in milk samples from mastitis cases. Genetic analysis of the *Mammaliicoccus* genus has identified it as the probable source of the *mec*A gene and SCC*mec* element responsible for methicillin resistance in methicillin resistant *Staphylococcus aureus* (MRSA), which was likely transmitted from *Mammaliicoccus* to *S. aureus* of animal origin. This study aims to assess the antimicrobial resistance (AMR) genes present in *M. fleurettii* isolates collected from a Vermont dairy farm by whole genome sequencing, with a focus on methicillin resistance genes such as *mec*A and *mec*A homologs. We have a library of 32 *M. fleurettii* isolates collected from one Vermont organic dairy farm from sources including quarter milk of cows with subclinical mastitis, bulk tank milk, cow teat skin, cow perineal skin, cow nares, milking equipment, human hand skin, and human nares. Antimicrobial susceptibility testing of isolates was conducted following CLSI guidelines followed by PCR identification of *mec*A and *bla*Z gene fragments. Oxacillin resistance was identified in 25 (78.1%) of isolates, and cefoxitin resistance in one, however all isolates were positive for the *mec*A gene. Genomic DNA was extracted and submitted to UVM Core for whole genome sequencing. Sequenced genomes will be assembled with the established pipeline in our lab. AMR genes will be identified from assembled genomes using 'ABRicate' with the goal of analyzing agreement between AMR phenotype and AMR genes and identifying additional AMR genes in these isolates. To the best of our knowledge this is the first case report of *M. fleurettii* isolates collected from humans and cattle on the same dairy farm. We conclude from this case study that additional research may be warranted to assess the potential risks to animal and public health posed by the presence of a *mec*A carrying *Staphylococcus* species known to have potential for AMR gene transmission on dairy farms.

Key Words: *Mammaliicoccus fleurettii*, methicillin resistance, antimicrobial susceptibility

1448T Feeding increasing amounts of *Asparagopsis armata* decreases enteric methane yield in dairy cattle.

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Enteric methane (CH_4) is the main source of greenhouse gas emissions from ruminants. The red seaweed, *Asparagopsis armata* (AA), has shown promising results but the optimal feeding amount is unknown. The objectives were to determine the optimal feeding amount of AA to reduce CH_4 emissions without compromising production in lactating dairy cattle. Four multiparous Holstein cows (684 ± 56 kg of BW, 35 ± 2 kg/d milk yield, and 146 ± 12 DIM) were randomly assigned to a 4 × 4 Latin square design. Cows were randomly assigned to 1 of 4 treatments: 0, 15, 30, and 45 mg of AA/kg of feed DM (CTRL, 15AA, 30AA, and 45AA, respectively). The AA contained a 3 mg/g dry weight level of bromoform concentration. Each period included a 14-d adaption period followed by 7-d for the determination of DMI, MY, composition, and enteric CH_4 production. Enteric CH_4 emissions were measured during 3-d of each period at 4-time points relative to feeding (−1, 2, 8, 12 h) using the LASER (Crowcon LaserMethane Mini). Cows were fed TMR at 0730 h targeting 10% refusals. Data were analyzed with the MIXED procedure of SAS. Three single degree of freedom contrasts were tested that included linear, and quadratic effects of AA as well as the contrast of CTRL versus all other AA treatment amounts combined. Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Dry matter intake (26.4 kg/d), MY (31.5 kg/d), ECM (32.7 kg/d), BW (694 kg), and total protein (3.48%) did not differ among treatments. However, the concentration of fat decreased linearly with the addition of AA (3.72, 3.66, 3.67, and 3.55% for CTRL, 15AA, 30AA, and 45AA; respectively). Compared with CTRL (312 g of CH_4/d), feeding AA reduced linearly

($P < 0.01$) enteric CH_4 production by 15.1, 19.1, and 24.2% for AA15, AA30, and AA45, respectively. Enteric CH_4 yield (i.e., CH_4 per DMI) and intensity (i.e., CH_4 per MY or ECM) were linearly decreased with the addition of AA ($P < 0.001$). In summary, feeding cows *Asparagopsis armata* did not affect DMI, MY, or ECM but reduced daily CH_4 yield and intensity when consumed up to 45 mg AA/kg DM.

Key Words: seaweed, milk, methane

1449T Determining a practical sample size to assess heat stress using respiration rate.

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Elevated respiration rate (RR) is an early indicator of heat stress in lactating cows, with a threshold of ≥ 60 breaths/min commonly indicating need for heat abatement. However, taking measurements on all cows in large herds is infeasible. Therefore, our objective was to determine a practical sample size of cows to measure RR to assess the overall level of heat stress in a high-producing pen. From video recordings, we measured the RR in 56 Holstein cows (DIM: 152 ± 27 d; MY: 43.6 ± 8.6 kg/d) at 1400 h on 5 d with a range of temperature-humidity index (THI) values (68, 71, 74.6, 77, 80.6 at 1400 h). To simulate pens of varied sizes, bootstrap resampling methods were applied. The first resampling process (with replacement) created 6 simulated pens with 30, 50, 75, 100, 150, 200, and 300 cows. For each virtual pen on each of the 5 d, we calculated the mean RR and categorized it as < or ≥ 60 breaths/min, which would suggest the pen's heat abatement was adequate or inadequate, respectively. The second resampling process (without replacement) was performed for each day and pen size for sample sizes of 20, 25, 30, or 35 cows, with 100 iterations each. For each iteration, we calculated mean RR, categorized whether it was < or ≥ 60 breaths/min, and compared this to the classifications from the first resampling process to identify discrepancies (i.e., misclassification). A logistic model was fitted with misclassification (yes/no) as the dependent variable, and independent variables of pen size, number of cows sampled, and THI. We found that, at THI = 68, 71, and 80.6, the probability of misclassification was close to 0 for all sample sizes and pen sizes. At THI = 74.6 or 77, the probability of misclassification decreased as more cows were sampled, but was still relatively low across all pen sizes (range:1.6 to 8.2%). Our findings suggest that when maximum daily THI was relatively low or high (e.g., 68 vs. 80, respectively), as few as 20 cows can be sampled to represent RR of an entire pen. On days with intermediate THI (74–77), sampling more cows decreases the chance of mischaracterizing the heat stress status of high-producing cows.

Key Words: sampling, heat abatement, on-farm assessment

1450T Long term effect of the exposure of late pregnancy cows to heat stress on milk production of their daughters.

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Recent literature has reported changes in long-term milk production of dairy cows that were exposed to in utero heat stress. The aim of the study was to evaluate differences in milk production of 1st lactation Holstein cows undergoing different heat stress conditions during the last 2 mo of intrauterine development. A retrospective study was conducted to analyze data from a total of 15,181 first lactation cows born from November 2012 to May 2015 and calved in 2016, in 214 Argentinian dairies (4 to 883 registers). Average daily milk production during the first 5 test days of lactation was used for the analysis. We used the temperature-humidity index (THI) as an indicator of heat stress in all herds because no data on heat abatement was available. The THI was estimated from the agrometeorological station data of the Instituto Nacional de Tecnologia Agropecuaria (INTA) EEA Manfredi, Cordoba. The sum of 3 or more days with a THI > 68 was considered a heat stress cycle (HSC). Calving dates were grouped in seasons: winter (n = 5,256, June to August), spring (n = 3,003, September to November), summer (n = 2,127, December to February), and autumn (n = 4,795, March to May). Farms were categorized according to the average milk production using percentiles 25 and 75 as cut-points: < 23.0, 23.0–31.0, and > 31.0 kg/d, leading to 3 categories with 2,615, 10,057 and 2,509 lactations, respectively. Generalized Linear Mixed Models were used, considering the farm as a random factor within the model, and the farm production category, and the season of calving as adjustment factors. The number of HSC during the last bimester of gestation categorized as follows: 1) 0 HSC (n = 10,292), 2) 1–9 HSC (n = 1,775), and 3) > 9 HSC (n = 3,114). An interaction effect of season of calving and HSC category was found meaning the impact of HSC depends on the season of calving. We could not prove the long-term effect of the exposure of late pregnancy cows to high THI values on milk production of their daughters, probably because there are multiple factors (nutrition, health, management, comfort) during the rearing period and early lactation which can have a greater effect on milk production in dairy cows and were not considered in this study.

Key Words: heat stress, dry cow, performance

1451T Motivation of advisors and dairy farmers to adopt an improved replacement program.

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Although there are many recommended management practices to improve the replacement program, the application of that knowledge is still an issue in Argentina. Though, the understanding of the decision-making rationality involved in the application of those management practices is crucial. The aim of this study was to measure the preference of different motivating factors among farmers and advisors regarding the adoption of an improved replacement program in dairy farms. Two choice experiments (CE) were performed; one for dairy herd advisors (41) with at least 3 yr of experience in replacement management, and the other for farmers (48) of small and medium size farms (60–600 total cows) from Buenos Aires (Argentina). Each CE was built combining 3 motivating factors with 3 levels each, framed in a fractional factorial design in an orthogonal matrix. Each CE resulted in 9 cards, each of them containing a scenario with the 3 motivating factors and one specific level. Every participant assigned a unique rank from 1 to 9 to each card, where 1 was the most preferred and 9 the least preferred scenario. Such rank was used to estimate the average utility (U) considering the preferences of the participants. The model was as follows: $U = u(f_1) + u(f_2) + u(f_3)$, where U = total utility; f = factor; u(fi) = unit of change of U for u. A conjoint analysis was used to design and analyze the CE (IBM SPSS Conjoint v.20). The motivating factors evaluated showed similar relative importance either among advisors or farmers. For the advisors, the most motivating scenario was to work with a farmer able to build and manage work teams, to have the possibility of regular and frequent advice and well-disposed operators. And for farmers, the most motivating scenario included the goal of achieving optimal age at first calving, a feasible program to implement and the possibility to exchange opinions about the program with other farmers. In conclusion, the evaluated motivating factors for advisors and farmers should be considered to implement a replacement program in dairy farms under the current conditions in our country.

Key Words: heifer, decision-making, conjoint analysis

1452T Nitrogen losses of representative Colorado dairies and mitigation potential of selected beneficial management practices. A. Loudenback*[1], A. Rotz[2], S. Archibeque[1], C. Cramer[1], and J. Dillon[1], [1]*Colorado State University, Fort Collins, CO,* [2]*USDA ARS, College Station, PA.*

The objectives of this study were to (1) evaluate the effects of climate change on reactive nitrogen footprints (RnF) of representative Colorado dairies from 1990 to 2100 and (2) estimate the mitigation potential of selected Beneficial Management Practices (BMPs). The Integrated Farm System Model was used to simulate a 1100– (1100C) and 2000–head conventional (2000C) farm. Weather data from 8 general circulation models (GCMs) for 2 representative concentration pathway scenarios (RCP 4.5 and 8.5) were used. Model inputs were obtained from USDA databases, literature, and expert input. BMPs included a covered manure basin (CMB) for both farms, covered manure basin + flare (CMB+F) for the 1100C, and reduced dietary crude protein (RDCP) from the NRC recommendation of 16%, to 14%, with amino acid supplementation for 2000C. RnF was 11.3 ± 0.6 and 8.8 ± 0.6 g/kg fat and protein corrected milk for 1100C and 2000C, respectively, and increased over the century for both farms. CMB increased RnF by 1–3% from baseline for both farms. RDCP reduced RnF by 1–5% from baseline for 2000C. Direct ammonia (NH_3), nitrate (NO_3), and nitrous oxide (N_2O) losses contributed 75, 2, and 10% of the RnF, respectively. CMB effects on NH_3 were negligible (<1%) for both farms. CMB+F effects on NH_3 were negligible (<1%) for 1100C. CMB did not reduce NO_3 loss from baseline for 2000C or 1100C. CMB+F had negligible (<1%) effects on NO_3. CMB increased N_2O by 2 and <1% for 1100C and 2000C, respectively. CMB+F increased N_2O emission from baseline by 2%. RDCP decreased NH_3 emissions by up to 25% for 2000C. RDCP reduced N_2O < 1% for 2000C. Overall, CMB and RDCP were most effective in reducing RnF on Colorado dairy farms over the century, primarily through reduced NH_3 emissions.

Key Words: nitrogen loss, dairy

1453T Comparative evaluation of bulk tank milk cultures from Quebec dairy herds. E. Molgat*, L. Fadul, M.-H. Castonguay, and D. Santschi, *Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

Raw bulk tank milk microbial composition is complex, with a dynamic microbiota specific to each farm. It reflects the farm environment, management practices, animals' health status and the equipment used for milk harvesting and storing. The aim of this study was to characterize the microbiological population in bulk tank milk according to farm characteristics to improve milk quality relative to bacterial counts. For all 263 herds enrolled, a single aseptic bulk tank sample was analyzed using a culture-dependent approach. Herds were selected on a voluntary random basis to reflect the population of Quebec dairies. Bacteriological analysis was used to identify and enumerate specific microorganisms that serve as indicators of factors that are known to impact milk quality and udder health: total aerobic count (TAC); preliminary incubation count (PIC); lab pasteurization count (LPC); coliform counts (CC), non-coliform counts (NC); coagulase-negative staphylococci counts (CNS); streptococci and streptococci-like organisms counts (SSOC); *Staphylococcus aureus* (SA), *Streptococcus agalactiae*, *Mycoplasma* spp. and *Prototheca* spp. Herd sizes ranged from 22 to 191 milking cows. Most of the herds (63%) had pipeline milking systems, 26% of the herds had automatic milking systems (AMS) and 11% had milking parlors. Difference among milking system and type of bedding was done using least squares method and Tukey's test at significance level ($P = 0.05$). Results showed that AMS have significantly higher NC compared with pipeline (10,782 vs. 2,887 cfu/mL; $P < 0.001$), and higher than milking parlors (3,455 cfu/mL; $P = 0.01$). Comparable differences were found for SSOC, where AMS had significantly higher counts compared with pipeline (1,908 vs. 908 cfu/mL; $P < 0.001$), and milking parlors (857 cfu/mL; $P < 0.01$). There were no significant differences between pipeline and milking parlors. Neither for the other bacteria. In addition, there was no significant difference on the bedding type nor by the milking frequency (2 vs. 3×). These preliminary results may help in solving milk quality problems at the farm level with targeted intervention dependent on the milking system.

Key Words: bulk tank milk, milk culture, bacteria

1454T Associations between bulk tank milk de novo fatty acid concentration and management and nutrition parameters on Brazilian dairy farms. T. S. Silveira[1,2], P. C. Ribeiro[2], J. C. S. Lourenço[1], A. V. Siqueira[3], D. P. D. Lanna[4], and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Associação Brasileira de Criadores de Bovinos da Raça Holandesa, Castro, PR, Brazil,* [3]*Polinutri Alimentos S.A., São Paulo, SP, Brazil,* [4]*Universidade de São Paulo, Piracicaba, SP, Brazil.*

The goal of this study was to examine the relationship of farm management, dietary composition and milk composition with de novo fatty acid (FA) content in bulk tank milk from commercial dairy farms in Paraná State, Southern Brazil. Thirty dairy farms (386 ± 272 lactating cows; minimum 95 and maximum 1,424 cows) were enrolled in this study and they were visited 4 times in a year 2021, one herd visit each season. Milk samples were analyzed by gas chromatography to determine 52 FA profile. Statistical analyses were conducted using the GLIMMIX procedure of SAS. Across herds, milk yield averaged 36.62 ± 4.28 kg/d, with 3.68 ± 0.33% milk fat and 3.28 ± 0.11% total protein. Each farm visit was categorized as high de novo (HDN; 26.82 ± 0.69 g/100 g of FA; n = 40), medium de novo (MDN; 25.48 ± 0.40 g/100 g of FA; n = 39), or low de novo (LDN; 23.45 ± 1.73 g/100 g of FA; n = 40). No differences ($P > 0.05$) on milk yield were observed among de novo categories, but milk fat content was higher ($P = 0.02$) for HDN farms (Table 1). Low de novo herds showed more intense overcrowding, with lower ($P < 0.01$) values of m^2/cow. Ether extract content was lower ($P < 0.01$) and peNDF content was higher ($P = 0.01$) for HDN farms. This research confirms that the bulk tank FA profile, and particularly de novo FA, could be used as a tool to track and modify feed management practices and dietary composition on dairy farms.

Key Words: fatty acid profile, feeding management, milk fat

Table 1 (Abstr. 1454T). Results of study

Item	Low DN	Medium DN	High DN	SEM	*P*-value
Milk yield, kg/d	36.92	36.37	36.57	2.36	0.85
De novo FA, g/100 g FA	23.45^{c}	25.48^{b}	26.82^{a}	0.01	<0.01
Mixed FA, g/100 g FA	32.78	32.30	32.56	0.15	0.75
Preformed FA, g/100 g FA	39.29^{a}	37.50^{b}	35.76^{c}	0.14	<0.01
Odd and branched FA, g/100 g FA	3.63^{b}	3.86^{a}	4.00^{a}	0.01	<0.01
Milk fat, %	3.64^{b}	3.60^{b}	3.80^{a}	0.04	0.02
Milk protein, %	3.26	3.28	3.31	0.11	0.15
Meal frequency per day	1.70	1.56	1.63	0.10	0.46
Feed push-up frequency per day	5.25	5.18	5.50	0.40	0.71
Animal density, m^{2}/cow	9.17^{b}	10.64^{ab}	12.50^{a}	1.38	<0.01
NDF, % of DM	31.65	32.55	32.95	0.74	0.20
ADF, % of DM	16.89	18.07	18.23	0.59	0.06
Starch, % of DM	29.57	28.43	28.08	0.98	0.33
Ether extract, % of DM	4.22^{a}	3.96^{b}	3.76^{b}	0.16	<0.01
peNDF, % of DM	17.28^{c}	18.64^{b}	19.41^{a}	0.52	0.01

Reproduction 2

1455T Reproductive performance of Jersey heifers submitted for first insemination based on age vs. body weight.

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Our objective was to compare the reproductive performance of Jersey heifers submitted for first insemination (FAI) based on age vs. body weight (BW). We hypothesize that the reproductive performance of heifers moved to the breeding pen (BP) based on a minimum BW would not be inferior to those moved based on minimum age. The trial was performed on a commercial farm in California. Data were collected from Apr. 2022–Feb. 2023. An observational retrospective study was previously performed using heifer data collected at the farm from 2014 to 2020. The lowest quartile of BW at FAI (<480 lb) was associated with decreased milk yield (~4 lb/d; $P < 0.05$) in early lactation than other BW categories. This BW threshold was used to define a field trial to assess 2 management strategies. At 9 mo, animals were allocated to CON or TRT group. Allocation was blocked by dSort category (Animal Management execution tool; LG: light vs. HV: heavy) performed at 60–90 d. In all allocated heifers, eligibility for the FAI was assessed every 4 wk: TRT were weighed from allocation until eligible (≥480 lb); CON were moved to BP at a minimum of 12 mo. Animals entering the BP received one dose of PGF$_{2\alpha}$ and were bred to estrus (sexed semen). Logistic regression and survival analysis were used to assess FAI, pregnancy at FAI, and overall pregnancy. Full models included the effect of treatment, dSort, and their interaction. Of the 418 animals included, 36% were LG; 32% of the TRT (n = 217) were moved before 12 mo. The odds of being bred, pregnant at FAI, and overall pregnancy ($P \geq 0.4$) were not different between groups. An interaction of treatment × dSort was detected in time to move to BP and to FAI ($P < 0.001$), with significant differences observed in the HV group. In the HV group, time to move to BP (HR 1.7 [1.3–2.2]; median time: CON = 379 d, TRT = 350 d), to FAI (HR 2.4 [1.9–3.1]; CON = 387 d, TRT = 351 d) and time to pregnancy (HR 1.5 [1.1–1.9]; CON = 393 d, TRT = 370 d) was reduced in the TRT vs. CON ($P \leq 0.004$). Submitting heifers to first AI based on BW reduced time to pregnancy, their rearing period, and associated costs. Future performance should be assessed before adopting change.

Key Words: first AI, management, first breeding

1456T Induction of luteinizing hormone release after gonadotropin releasing hormone delivery with the e-Synch system.

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Our objective was to evaluate LH concentrations after intravaginal instillation of GnRH with the e-Synch system, an intravaginal electronically controlled hormone delivery and sensing device. Lactating Holstein cows (n = 29) at 48 h after induction of luteolysis with prostaglandin F2α were randomized to receive 100 µg of GnRH (Gonadorelin diacetate) by intramuscular injection (IM) in 2 mL, or an intravaginal treatment with e-Synch consisting of 100 (LoD) or 1,000 (HiD) µg of a GnRH analog (Gonadorelin diacetate) in 2 (LoV) or 10 (HiV) mL of solution. All solutions contained 10% citric acid as an absorption enhancer. Solution release was set for 1,000 or 2,000 s for the 2 mL and 10 mL dispensed volume, respectively, and was confirmed by visual observation in all cows at device removal 8 h post-insertion. Blood was collected at 0, 1, 2, 2.5, 3, 4, 6, and 8 h after IM injection or e-Synch device start of GnRH release and analyzed for plasma LH and progesterone. All cows had < 0.5 ng/mL of progesterone at time 0. Data were analyzed with linear mixed models with or without repeated measurements. The 1,000 µg dose of GnRH elicited more LH release than the 100 µg dose, regardless of solution quantity (Table 1). The overall LH response, as determined by area under the curve (AUC), mean (1 to 6 h after treatment; treatment by time $P < 0.001$), and maximum LH concentrations, was similar between cows receiving 1,000 µg of GnRH given by e-Synch and 100 µg of GnRH IM (Table 1). Increasing solution volume for delivering the same dose of GnRH partially increased LH release only for the 100 µg dose of GnRH (Table 1). To conclude, a 1000 µg of GnRH delivered with e-Synch induced a surge of LH of similar magnitude than after IM injection of 100 µg of GnRH independently of the volume of solution in lactating Holstein cows with low circulating concentrations of progesterone. The GnRH dose delivered by e-Synch was more critical than the volume of solution.

Key Words: automation, internet of things, synchronization of ovulation

1457T Factors affecting the adoption and success of AI by Bangladeshi dairy farmers.

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Studies on the socioeconomic impact of genetic improvement of cattle via conventional Artificial Insemination (AI) programs are very limited in Bangladesh. Therefore, the study was undertaken to determine the factors affecting the adoption of AI using different bull semen by farmers and the reproductive efficiency along with daily milk production of 3 generations of cattle population (grandmother, mother and daughter). A total of 971 farms were purposively surveyed from milk-producing areas in 10 different districts of Bangladesh using closed questionnaires to collect data on the socioeconomic characteristics of the dairy cow owners. Information on the reproductive efficiency in terms of sexual maturity, estrous cycle length, estrus duration, service per conception, parity and calving interval of cows and milk production was studied from 117 farms and was compared among generations. A binary logit model was used to report the socio-economic characteristics of the respondents, where the variance inflation factor (VIF) was also calculated to detect multicollinearity. A one-way ANOVA was used to compare the reproductive parameters in the corresponding generation. In this study, all the explanatory variables were free from multicollinearity (VIF < 10).

Table 1 (Abstr. 1456T). Luteinizing hormone responses after IM injection or intravaginal release of GnRH with e-Synch

Treatment	n	LH AUC	Maximum LH (ng/mL)
IM	7	3,503 ± 520^{a}	19.1 ± 3.2^{a}
LoD-LoV	6	525 ± 142^{c}	2.4 ± 0.8^{b}
LoD-HiV	6	1,046 ± 21^{b}	4.3 ± 1.1^{b}
HiD-LoV	6	5,128 ± 842^{a}	26.8 ± 4.7^{a}
HiD-HiV	4	5,340 ± 1,698^{a}	27.4 ± 9.5^{a}
P-value		<0.001	<0.001

$^{a–c}$Different superscripts in a column indicate $P < 0.05$.

The parameter estimated from logistic regression showed that farmers' tertiary education (1.4156 ± 0.4809; $P = 0.003$) and the presence of Frisian crossbreed bull related to more milk production (2.2409 ± 0.4408; $P = 0.000$) affected the adoption of AI significantly ($P < 0.001$) in this study. Binary logistic regression also showed that most of the surveyed farmer's main occupation was only dairy farming (1.6392 ± 0.4474; $P = 0.000$) (1 if the main occupation of the household head is only dairy farming; 0 otherwise). Findings also revealed that earnings from the Frisian crossbred daughter's milk were insignificantly ($P > 0.05$) higher in comparison to that from mothers' milk in 8 districts of Bangladesh. In addition, results showed that there were significant ($P < 0.05$) differences in parameters like the time needed for sexual maturity (639.33 ± 4.32 vs. 707.28 ± 5.05 vs. 722.76 ± 4.97 d), service per conception (1.44 ± 0.07 vs. 1.51 ± 0.04 vs. 2.61 ± 0.03) and parity (7.48 ± 0.08 vs. 5.43 ± 0.09 vs. 2.84 ± 0.07) among grandmother, mother and daughter, respectively. Furthermore, changes in milk production between generations were not detected in this study($P > 0.05$). By presenting the existing cattle farming scenario focusing on increased milk production from the increased use of AI technology using high-yielding exotic Frisian breed semen this study may help breeding policymakers in designing proper breeding policy in Bangladesh.

Key Words: adoption of AI, Bangladeshi dairy farmers

1458T Association of the age at first calving with survivability, milk yield, and fertility up to the third lactation in one herd of Holstein dairy cows in Japan.

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Accelerating the time of the first calving is one strategy for sustainable dairy farming. However, little information about the association between the first calving age (AFC) and survivability, subsequent fertility, and milk yield has been available. This study aimed to clarify the association of the AFC with survivability, fertility, and milk yield up to the third lactation. In this study, the calving records of 169 Holstein Friesian heifers that calved between 1999 and 2012 in one Japanese experimental herd were used. Their AFC ranged from 20.8 to 28.7 mo (from 1 to 2 years old). Based on our previous study, the heifers were categorized according to the AFC as young (<22.5 mo, n = 23), moderate (22.5 to <24.0 mo, n = 67), old (24.0 to <25.5 mo, n = 52), and very old (≥25.5 mo, n = 27). The survival rate of 169 cows at the end of the first, second, and third lactation was 61, 40, and 20%, respectively, and the cows categorized as the young AFC had a numerically higher survival rate compared with the cows categorized as the others (78 vs. 50 to 66%, 61 vs. 33 to 42%, and 35 vs. 11 to 24%, respectively). The average interval from calving to the first service and conception at the end of the third lactation was similar between the AFC groups. Multivariate analysis using a generalized linear model was performed to evaluate the association of AFC, year-period, and calving season, with the outcome variables of lifetime daily milk yield at the end of the third lactation. Data from 129 animals that had more than a total of 200 d in milk were included in this analysis. There was a significant association between the year-period and lifetime daily milk yield ($P < 0.05$), while not with AFC and calving season. The cows categorized as the young AFC had numerically higher lifetime daily milk yield than that as moderate, old, and very old (15.0 vs. 13.0, 12.2, and 11.9 kg/day, respectively). In conclusion, the younger AFC by <22.5 mo could have an advantage on survivability up to the third lactation in this experimental herd.

Key Words: first calving age, Holstein Friesian heifer, lifetime productivity

1459T Association of body condition loss with hepatic and ovarian function of lactating dairy cows.

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The severe loss of body condition score (BCS) during early lactation period has been associated with infertility in cows. However, the mechanisms are not fully understood. The aim of this study was to examine the association among BCS loss, liver health, and ovarian functions in cows during early lactation. Retrospectively multiparous cows from 2 farms (farm A = 14 and farm B = 6) housed in a tie-stall were categorized based on units of BCS (1–5 scale) lost as Moderate (MOD, <0.75 units; n = 11) or Severe (SEV, ≥0.75 units; n = 9) loss groups. From week −3 to 7 relative to calving, MOD and SEV cows lost on average 0.4 and 1.0 unit BCS, respectively. Blood samples were taken on −3, 0, +1, and +7 week relative to calving. All data except hepatic transcriptomes were analyzed with PROC MIXED procedure of SAS. The plasma levels of nonesterified fatty acids at week 0 and 1 and γ-glutamyl transferase at week 1 and 7 relative to calving were higher in SEV cows. Hepatic transcriptome analysis showed that 1186 genes were differentially expressed in SEV (n = 3) compared with MOD (n = 3) cows at week 7 after calving. Pathway analysis revealed that pathways associated with lipid metabolism, type I diabetes, fatty acid biosynthesis, apoptosis and cellular defense were enriched among differentially expressed genes, in SEV cows. The impaired liver function in SEV cows was associated with 1.5-fold reduction of hepatic insulin like-growth factor 1 (*IGF1*) gene expression and lower plasma IGF1 concentrations. At the ovarian level, SEV cows had lower IGF1 concentration in the follicular fluid of the dominant follicle of the synchronized follicular wave in comparison with that of MOD cows at 7 weeks after calving. Further, the follicular fluid concentration of estradiol-17β was lower in SEV cows along with lower transcript abundance of genes from granulosa cells associated with dominant follicle competence including *CYP19A1*, *NR5A2*, *IGF1R*, and *LHCGR*. These data show that SEV loss of BCS during early lactation is associated with liver dysfunction, including lower IGF1 secretion, and impaired function of the dominant follicle in the ovary.

Key Words: body condition score, dairy cow, ovarian function

1460T Association of transition cow health with pregnancy per AI and pregnancy loss in cows receiving AI using a Double-Ovsynch protocol.

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This observational study was conducted to evaluate the effect of transition cow health on pregnancy per AI (P/AI) and pregnancy loss (PL) in cows synchronized with a Double-Ovsynch protocol (DO) for first service. Lactating Holstein cows (n = 15,041) from one commercial dairy farm in Northern Germany between January 2015 to December 2021 were enrolled into a modified Double-Ovsynch protocol (GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH, 7 d later GnRH, 7 d later $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16 to 18 h later timed AI) for first service at 72 ± 3 DIM. Parity was classified as 1st, 2nd, and ≥3rd lactations. At d 32 and d 60 post-AI, cows underwent pregnancy diagnosis through transrectal ultrasound. Pregnancy loss was defined as the proportion of pregnant cows on 32 d post-AI that were found nonpregnant on 60 d post-AI. Health related events (i.e., milk fever, ketosis, retained fetal membranes, metritis, mastitis, displaced abomasum) were assessed by farm personnel using standard operating procedures. Multivariable logistic regression was used for testing potential associations between disease occurrence and outcome variables, including P/AI and PL. There were 20.0% (885/4,430), 34.9% (1,391/3,989), and 53.9% (3,570/6,622) of cows with at least one disease event for 1st, 2nd and ≥3rd lactations, respectively. In 1st lactation cows the most prevalent disease was metritis (10.7%; [473/4,430]). Cows in 2nd lactation suffered mostly from mastitis (16.6%; [664/3,989] and ketosis (16.6%; [661/3,989]). Cows in ≥3rd lactations were mostly affected by ketosis (33.2%; [2,198/6,622]). We observed a negative association of inflammatory disorders (i.e., retained fetal membranes, metritis, mastitis) and P/AI in all cows irrespective of parity. Metabolic disorders (i.e., hypocalcemia, ketosis and displaced abomasum) were negatively associated with P/AI only in multiparous cows. Irrespective of parity, only uterine diseases (i.e., retained fetal membranes, metritis) were significantly associated with PL. These results highlight the importance of optimizing transition cow health as a prerequisite for achieving high fertility in a DO protocol.

Key Words: Double-Ovsynch, transition cow health, pregnancy per AI

1461T Effect of time and magnitude of the nadir body condition score in early lactation on fertility of Holstein cows. C. Hernandez-Gotelli*[1], D. Manríquez[1], J. Azocar[3], A. De Vries[2], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*DeLaval Inc., Madison, WI.*

The objective was to identify the effects of the time and magnitude of nadir BCS (nBCS) on the subsequent pregnancy at first artificial insemination (P/AI1) and pregnancy loss of Holstein cows. This retrospective observational study used data from 12,042 lactations in 7,626 Holstein cows calving between 2019 and 2022 in a commercial dairy in Colorado, USA. Scores generated by an automated BCS camera system at calving (BCS1) and nadir (nBCS; the lowest daily BCS from calving to 100 DIM) were selected for the analyses and subsequently categorized into quartiles (Q1 = lower BCS). Changes in BCS were calculated from calving to nadir and assigned into quartile categories (Q1 = greater loss of BCS). Data were examined using logistic regression and ANOVA. Multivariable models considered parity category, calving season, BCS1, and average milk yield up to 30 DIM (M30) as covariables. Mean DIM to AI1 for primiparous and multiparous cows following a double OvSynch protocol were 90.7 d and 69.9 d ($P < 0.0001$), respectively. Pregnancy at AI1 was 53.4% (primiparous = 63.2%; multiparous = 47.8%; $P < 0.0001$) and DIM at conception was 113.3 ± 0.4 d (primiparous = 118.3 d; multiparous = 110.3 d; $P < 0.0001$). The overall incidence of early pregnancy loss was 9.1%, with primiparous cows evidencing lower pregnancy loss than multiparous cows (5.4% vs. 11.4%; $P < 0.0001$). Cows that conceived at AI1 had their nBCS earlier (45.1 ± 0.32 d) than cows that remained open after AI1 (46.4 ± 0.35 d). On the contrary, DIM at nBCS did not differ by pregnancy loss status. Nadir BCS was greater in cows that conceived at AI1 (2.86) compared with cows that did not conceive at first AI (2.82; $P = 0.004$). Similarly, cows non-pregnant after AI1 had greater loss in BCS between calving and nadir (−0.48) than cows pregnant after first AI (−0.44; $P < 0.001$). Although cows that lost their pregnancy had lower nBCS, this association was not consistent across the analyses. This study identified small differences in time to and magnitude of nBCS, as well as in the change in BCS from calving to nadir between cows that conceived or failed to conceive at first AI.

Key Words: nadir body condition, automated, fertility

1462T Associations of transition metabolism with subsequent estrous activity. C. Chantel[1], G. Madureira*[1], B. Mion[1], O. Chiu[1], A. Madureira[2], T. Burnett[2], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Ridgetown Campus, University of Guelph, Ridgetown, ON, Canada.*

Our objective was to investigate associations of transition metabolism with subsequent estrous activity. Cows (n = 273) were enrolled 45 d before expected calving and followed until 70 DIM. Data of BW, BCS, DMI, milk yield, and energy balance were summarized weekly. Blood samples were collected on d −21, −10, −3, 0, 3, 7, 10, 14, 21, and 65 relative to calving to measure target metabolites. Cows received injections of $PGF_{2\alpha}$ at 40 and 54 ± 3 DIM and were then monitored for estrous behavior for a period of 11 d using automatic activity monitors (DeLaval DelPro). Duration of estrus, relative peak of activity during estrus, and area under the curve (AUC) for the relative increase in activity were calculated using the raw activity data. Within parity, cows that had an estrus were ranked according to the AUC and classified as below (low activity; n = 68) or above (high activity; n = 68) the median. Cows that did not express estrus were classified as 'no estrus' (n = 90), and the remaining 47 cows were excluded because of culling or anovulation. Data were analyzed using the GLIMMIX procedure of SAS, and considered estrus classification, parity, season, and the random effect of cow. By design, AUC was 2.5-fold greater, the relative peak was 67% higher, and the duration of estrus was 74% longer in high vs. low activity cows. Except for NEFA during transition, which was higher ($P = 0.02$) in high activity cows (0.46 vs. 0.40 ± 0.02 mmol/L), no other differences were observed between high vs. low activity cows. Concentrations of NEFA (0.49 vs. 0.43 ± 0.02 mmol/L; $P < 0.01$), ceruloplasmin (53 vs. 50 ± 1 mg/L; $P = 0.03$), and superoxide dismutase (2.3 vs. 2.1 ± 0.5 U/mL; $P = 0.03$) in serum were higher in cows that subsequently did not express estrus than in those that expressed estrus. Similarly, peak of serum haptoglobin on d 3 was higher in cows that did not express estrus (0.9 vs. 0.7 ± 0.04 g/L; $P = 0.01$). BCS tended to be lower ($P = 0.06$) in cows that did not express estrus (3.27 vs. 3.33 ± 0.03). At 65 DIM, concentrations of NEFA in serum was lower in cows that did not express estrus (0.14 vs. 0.17 ± 0.01; $P = 0.02$). No differences in postpartum BW (667 ± 7 kg), DMI (21 ± 0.2 kg/d), milk yield (40 ± 0.7 kg/d), and energy balance (−3.1 ± 0.4 Mcal/d) were observed. In conclusion, expression of estrus as a binary trait was moderately associated with blood metabolites during transition, while intensity of estrus was not.

Key Words: behavior, reproduction, estrus

1541T Peripartum omega-3 fatty acids affect endocannabinoid system components in granulosa cells of preovulatory follicles and in uterus of dairy cows. B. Mualem[1], G. Kra[1,2], J. R. Daddam[1], U. Moallem[1], and M. Zachut*[1], [1]*Agriculture Research Organization, Volcani Center, Rishon Lezion, Israel,* [2]*Faculty of Agriculture, the Hebrew University in Jerusalem, Rehovot, Israel.*

The endocannabinoid system (ECS) has an important role in modulating central and peripheral functions including reproduction in mammals. Endocannabinoids, mainly derived from the omega-6 (n-6) fatty acid (FA) C20:4n-6, regulate oocyte and follicle maturation, embryo transport through the oviduct and blastocyst implantation. Dietary omega-3 (n-3) FA reduce the activity of the ECS, thus we examined the effects of n-3 on ECS components in granulosa cells from pre-ovulatory follicles and uterine biopsies. Twenty-four 256-d pregnant multiparous cows were individually fed: i) CTL – prepartum and postpartum (PP) a TMR rich in n-6 FA (n-6/n-3 ratio = 9); ii) FLX – prepartum a diet containing 700 g/d/cow and PP supplemented at 6.4% of diet (DM basis) an extruded flaxseed supplement containing C18:3n-3 (n-6/n-3 ratio = 1.3; Valomega

160, Valorex, France). From 30 d PP, cows with a corpus luteum were injected with PG; others were injected with GnRH followed by PG. Seven d following behavioral estrus, PG was injected and 44 h later follicular fluid (FFL) aspirations and uterine biopsies were performed at 47–66 DIM. Concentrations of estradiol (E_2) and progesterone (P_4) were determined in FFL, and follicles with $E_2/P_4 > 1$ were considered as pre-ovulatory follicles. Abundances of ECS and inflammatory mRNA and proteins were examined by RT-PCR and immunoblots. Data was analyzed with GLM (SAS). In granulosa cells (n = 5 per treatment), mRNA abundances of the cannabinoid receptor 2 (*CNR2*), the ECS receptor G-protein-coupled receptor 55 (*GPR55*), nuclear factor kappa-light-chain-enhancer of activated B cells(*NFKB*), the ECS-enzyme N-acyl phosphatidylethanolamine-phospholipase-D(*NAPEPLD*) and tumor-necrosis-factor-α (*TNFA)* were decreased in FLX compared with CTL. In uterus (n = 6 per treatment), average mRNA abundances of cannabinoid-receptor-1 (*CNR1/*CB1*)*, *CNR2*, and ECS-enzymes monoglyceride-lipase (*MGLL*) and *NAPEPLD* were decreased in FLX vs. CTL. Uterine protein abundances of CB1 and of the ECS receptor transient-receptor-potential-cation-channel-subfamily-V-member-1 (TRPV1) were decreased in FLX vs. CTL. Peripartum omega-3 reduced ECS and inflammatory components in pre-ovulatory follicles and uterus, which could affect the physiological and immune state of reproductive tissues in dairy cows.

Key Words: endocannabinoid, reproduction, omega-3

Ruminant Nutrition: Calves and Heifers 2

1463T Medium quality colostrum enriched with colostrum powder, associated or not to transition milk: Effect on the health and performance of dairy calves. A. P. Silva, A. M. Cezar, A. F. Toledo, C. R. Tomaluski, M. G. Coelho, I. M. Nascimento, G. H. B. Silva, and C. M. M. Bittar*, *Dept. Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil.*

The present study aimed to evaluate how the correction of medium-quality colostrum using colostrum powder (CP), associated or not with transition milk, affects the passive transfer, health, and performance of dairy calves. Fifty Holstein calves were allocated in randomized blocks according to sex, birth weight (34.2 kg), and date of birth and distributed in a 2 × 2 factorial arrangement (2 Ig sources and 2 transition milk protocols). Newborn calves were fed the volume corresponding to 12% of BW within 3 h after birth: 1) Bovine colostrum (BC) with 25% Brix; 2) Enriched colostrum (EC): medium quality BC (20%) enriched with CP to reach 25% Brix. After the first colostrum meal calves were fed 4 L/d for 3 d one of the following: 1) whole milk (WM); or 2) FTM: WM enriched with 70 g/L of CP (totaling 280 g/d). From the 4th day of age, calves received starter and water free-choice and were fed 6 L/d of WM until weaning at 56 d of age. All treatments reached excellent levels of passive transfer (average of 10.1% Brix) because of the high IgG intake (517.5 g) and there were no differences in apparent efficiency absorption of IgG with a mean of 30.4%. There were no differences ($P > 0.05$) in ADG (577 g/d) or weaning weight (67 kg). Health was also not affected by the colostrum and transition protocols with low average fecal scores (0.72), but with 8.7 d with diarrhea and 3.8 d with fever. The enrichment of medium-quality colostrum with CP is an alternative to high-quality maternal colostrum shortage; however, the supply of 4 L/d of FTM with 70 g/L of colostrum powder for 3d does not present additional benefits for the health and performance of calves in the short term.

Key Words: Brix, fecal score, newborn

1464T Withdrawn.

1465T A rumen-protected blend of B vitamins improved the pre-weaning growth rate of Jersey female calves fed ryegrass pasture. R. Balogun*[1] and O. AlZahal[2], [1]*Jefo Australia Pty Ltd., Toowoomba, Qld, Australia,* [2]*AlZahal Innovation and Nutrition, Kitchener, Ontario, Canada.*

The objective of this study was to evaluate the effect of supplementing pre-weaning female calves with a rumen-protected blend of B vitamins (RPBV) on average daily gain (ADG) and body weight (BW) during the pre-weaning period. One hundred twenty-eight female calves (27 ± 3 kg of BW and 8.4 ± 4.8 d of age, mean ± SD) were randomly allocated to 8 pens and 2 experimental treatments in a staggered manner as they were born. Each pen contained 16 calves and received either a starter concentrate (SC) without (Control) or an SC with 3 g/head per d of RPBV (folic acid, thiamine, biotin, pyridoxine, and pantothenic acid) until weaning (d 90). The SC contained 21% CP, 33% starch, 21% neutral detergent fiber, and 2.1% fat. Calves received colostrum within the first 12 to 24 h of their lives followed by a milk replacer feeding program until weaning (4 L in 2 feedings from wk 1 to wk 5 of age, 4 L in one feeding from wk 6 to wk 12 of age, and a gradual decrease to weaning on d 90 of age). At 5 wk of age, calves were transferred to paddock pens and grazed ryegrass pasture in addition to SC until weaning. All other management practices on-farm were the same for all calves. Calves were weighed individually at the start of feeding and every month until weaning to calculate ADG. Statistical analyses of final BW and ADG were conducted using Proc Mixed procedure (SAS) with treatment and pen(treatment) as the fixed effects and initial BW as a covariate. Compared with the Control, calves that received RPBV achieved 10% greater ADG (0.59 vs. 0.54 ± 0.01 kg/d; $P < 0.05$). The final BW for RPBV calves was 7% greater than that of the Control calves (84.0 vs. 78.5 ± 1.1 kg; $P < 0.05$). This study demonstrated that supplementation with a rumen-protected B vitamin blend has the potential to improve the growth performance of pre-weaning Jersey female calves grazing ryegrass pasture.

Key Words: B vitamin, pasture, pre-weaning calf

1466T The effects of probiotic supplementation on the prevention of diarrhea in pre-weaned Holstein dairy calves. L. Widmer*[1], E. Meissner[2], D. Ledgerwood[3], D. Vagnoni[2], and H. Rossow[1], [1]*University of California, Davis, Davis, CA,* [2]*California Polytechnic University, San Luis Obispo, San Luis Obispo, CA,* [3]*Chr. Hansen, Milwaukee, WI.*

Feeding probiotics can improve health and mitigate adverse effects caused by pathogens entering a calf's gastrointestinal tract. The objective of this study was to evaluate the effectiveness of a probiotic fed daily, on fecal consistency and fecal pathogen shedding. Holstein heifers were enrolled within 48 h of birth into 2 treatment groups: 1) Control (CON: n = 173) 0.5 g of lactose, and 2) Probiotic (PRO: n = 151) 0.5 g of a multi-strain probiotic. Treatments were administered 1/d in milk from 48 h to weaning at 60 d. The probiotic was a combination of *B. subtilis*, *B. lichenformis*, *L. animalis*, and *P. freudenreichii* (1.1×10^{10} cfu/g). Fecal scoring for fecal consistency was performed daily using a scale from 1 to 3, with 1 being normal and 3 being loose. Feces were collected from 50 calves per treatment at 7, 14, 21, and 42 d via digital stimulation to assess fecal pathogen shedding. A Kaplan-Meier analysis was used to compare age at first diarrhea event using the LIFETEST procedure (SAS Institute v. 9.4, 2021). All other data were analyzed using the general linear models procedure in SAS. No differences were observed between treatment groups for age at first diarrhea event, length of first diarrhea event, total days with a 2 fecal score, and risk of having a diarrhea episode during the prewean period. However, total days with a 3 fecal score were higher in the PRO than in the CON (3.46 ± 0.35 vs. 2.85 ± 0.31 d; $P < 0.05$) during the first 4 wk after birth. There were no differences in episode length, age at first episode, and risk of diarrhea. No differences were observed between treatment groups for fecal shedding of *E. coli* and *E. coli* O157:H7 at all time points and *C. perfringens* at 7 and 14 d. However, fecal shedding for *C. perfringens* was higher in the PRO group than in the CON group at 21 d (4.38×10^5 vs. 2.34×10^5, $P < 0.05$) and at 42 d (1.29×10^6 vs. 6.11×10^5, $P < 0.05$).

Key Words: calf, probiotic, diarrhea

1467T Enriching whole milk with a vitamin and mineral supplement: Effect on hemoglobin status, milk intake, and calf growth. A. Zupan, L. Yanch, and A. Kerr*, *Grober Nutrition Inc., Cambridge, Ontario, Canada.*

Whole milk provides many essential nutrients, however, is deficient in certain micronutrients required for calf growth (e.g., iron). It is of interest to consider a vitamin and mineral supplement to meet micronutrient requirements of calves fed whole milk to support preweaning growth and welfare. Following colostrum feeding, Holstein calves ($n = 62$) were assigned to one of 2 treatment groups: unsupplemented whole milk (CON; $n = 32$) or whole milk with a vitamin and mineral supplement (5 g per L; WMS; $n = 30$; Promiks, Grober Nutrition Inc., ON). Whole milk was offered up to 9 L per day until weaning at wk 7. Calf starter and water was offered *ad libitum*. Milk refusals and treatments were recorded daily, body weight recorded weekly, and jugular blood samples for hemoglobin (HGB) analysis taken at birth, d 14, 28, and 42 following morning milk feeding. HGB was used as an indicator of iron status. Data was analyzed using a multiple comparison model with treatment and time as fixed effects. Body weight and HGB status at birth did not differ by treatment ($P > 0.3$). Average daily gain (ADG) was greater for WMS calves (0.99 kg/d) compared with CON calves (0.7 kg/d) in wk 4 ($P = 0.002$). The WMS calves had greater milk intake for the first 5 weeks than CON calves (8.05 L/d, 7.8 L/d, respectively, $P = 0.02$). In addition, WMS calves had improved HGB status at d 14, 28 and 42 (110 g/L, 110 g/L, 116 g/L, respectively, $P < 0.001$), compared with CON calves (95 g/L, 89 g/L, 94 g/L, respectively). Calves with low HGB status (<84 g/L) at d 14 had lower ADG during wk 2 ($P = 0.04$) and a tendency during wk 7 ($P = 0.08$) compared with normal HGB calves. In addition, low HGB status at d 14 resulted in significantly lower milk intakes at wk 3 ($P = 0.01$) and tended to have lower milk intake on average during the first 5 weeks (7.5 L/d, 8.2 L/d, respectively, $P = 0.06$) than normal HBG calves. This study suggests supplementing whole milk fed to calves provides required micronutrients as supported by improved HGB status and milk intake for WMS group.

Key Words: dairy calf, whole milk, iron

1468T Performance and health of cold-stressed dairy calves fed waste milk enriched with transition milk or milk replacer. I. R. R. Castro[1], B. Moradi[2], S. Kargar[2], M. Kanani[2], I. F. Carrari[1], and M. I. Marcondes*[1], [1]*Department of Animal Sciences, Washington State University, Pullman, WA,* [2]*Department of Animal Science, School of Agriculture, Shiraz University, Shiraz, Fars, Iran.*

Young calves are more vulnerable to cold than older animals and may have difficulty consuming the energy required to cope with cold stress, which can be detrimental to health and performance. To overcome this problem, we can increase milk's energy by adding total solids. We aimed to evaluate the effects of increasing total solids of waste milk (WM) via milk replacer (MR) or transition milk (TM) on performance and health of cold-stressed Holstein dairy calves. Fifty-one calves were used for 3 groups of calves fed (1) WM; (2) MR (WM + MR); and (3) TM (WM + TM) [$n = 17$ per group; 4-d of age; BW = 40.0 ± 0.63 kg]. The average maximum, mean, and minimum temperature during the study were 12.4, 4.2, and −3.8°C, respectively. Animals were randomly housed in individual pens and had access to fresh water and starter feed freely. Calves were weaned on d-61 trial and kept until d 101. For a comparison of treatment means, P-values were specified for contrasts (1) WM vs. the average of MR and TM (CON vs. SUP) and (2) MR vs. TM, using the Contrast statement of SAS. We found that supplementation was responsible for a greater dry matter, crude protein, and ether extract intake ($P = < 0.01$) during preweaning. Further, we observed an overall greater body development, body weight ($P = 0.03$), withers height ($P = 0.02$), and heart girth ($P = 0.04$) in MR and TM groups (Table 1). Average daily gain in WM group was smaller compared with the supplemented group during overall period ($P = < 0.01$). We observed no difference in diarrhea ($P = 0.77$), but pneumonia in WM was higher in comparison with MR and TM ($P = 0.02$). Our findings showed that adding MR or TM to waste milk is an alternative to improve intake, performance, and health of young calves under cold stress.

Table 1 (Abstr. 1468T). Intake, performance, and health in calves fed waste milk (WM) and a blend of WM with milk replacer (MR) or transition milk (TM)

	Treatment				*P*-value	
Item	WM	MR	TM	SEM	CON vs. SUP	MR vs. TM
DMI, g/d	1,985	2,139	2,029	28.41	<0.01	<0.01
BW, kg	79.2	80.3	80.8	0.53	0.03	0.50
ADG, kg/d	0.93	1.01	0.99	0.01	<0.01	0.34
Diarrhea, d	7.8	7.2	7.2	0.09	0.77	0.55
Pneumonia, d	1.3	0.4	0.5	0.34	0.02	0.15

Key Words: calf nutrition, calf supplementation, total solids

1469T The effects of probiotic supplementation on serum metabolite concentrations in young Holstein heifers. E. G. Meissner*[1], H. Rossow[2], L. Widmer[2], D. Ledgerwood[3], K. Bryan[3], and D. B. Vagnoni[1], [1]*California Polytechnic State University, San Luis Obispo, CA,* [2]*University of California, Davis, Davis, CA,* [3]*Chr. Hansen, Milwaukee, WI.*

The objective of this trial was to evaluate the effect of a multi-strain probiotic on serum concentrations of selected metabolites in young calves. Holstein heifers ($n = 112$) from a commercial dairy were enrolled within 48 h of birth and placed into individual hutches. Calves were weaned at 60 d of age and remained in their hutches until 90 d of age, when they were moved to group housing. Calves were placed into 2 treatment groups: 1) control and 2) *B. subtilis*, *B. lichenformis*, *L. animalis*, and *P. freudenreichii* probiotic (Bovamine Dairy Plus, Chr. Hansen, Milwaukee, WI). Control calves received 0.5 g of lactose in milk once daily until weaning and 0.75 g in grain thereafter. Treatment calves received 0.5 g (1.1×10^{10} cfu/g) probiotic in milk once daily until weaning and 0.75 g (1.65×10^{10} cfu/g) probiotic in grain thereafter. Jugular blood samples (6 mL) were collected at 14, 25, 45, 63, 77, 120, and 180 d of age for the determination of serum β hydroxy-butyrate (BHB) and total free AA (TFAA) and plasma glucose. Data were analyzed as a mixed linear model to determine the effect of treatment, with day as a repeated factor. Single degree of freedom contrasts were used to evaluate the effect of phase (pre- vs. post-weaning) and the interaction of treatment with phase. Glucose concentrations were greater ($P < 0.001$) for the pre-weaning (88.0 mg/dL) vs. post-weaning (81.1 mg/dL) phase and greater for calves receiving probiotics in the pre-weaning phase only (Treatment × phase, $P < 0.01$). Concentrations of BHB increased throughout the 180 d of the study and were greater ($P < 0.001$) in the post-weaning (661 μ*M*) vs. pre-weaning (240 μ*M*) phase and greater for the probiotic-treated calves in the post-weaning phase only (Treatment × phase, $P < 0.001$). Serum TFAA concentrations were greater ($P < 0.001$) in the pre-weaning (3.37 m*M*) vs. post-weaning (3.03 m*M*) phase and greater for probiotic-treated vs. control calves (3.22 m*M* vs. 3.11 m*M*; $P < 0.001$). These data suggest that nutrient metabolism and ruminal development were enhanced by feeding a multi-strain probiotic.

Key Words: calf, probiotic, rumen development

1470T Serum profiles of dairy calves fed a milk replacer or whole milk at two levels of supply. T. Chapelain*, J. B. Daniel, J. N. Wilms, J. Martín-Tereso, and L. N. Leal, *Trouw Nutrition R&D, Amersfoort, the Netherlands.*

The objective of this study was to compare the effect of feeding fresh whole milk and a milk replacer at 2 different levels of supply on calf metabolism. Forty-eight Holstein calves (2 ± 1.0 d of age) were enrolled and blocked by age and arrival date and randomly assigned to one of 4 treatments at: 9.0 L/d of milk replacer (MR-H) or whole milk (WM-H), and 4.5 L/d of MR (MR-L) or WM (WM-L). Dry matter (DM), protein, fat and lactose content (% of DM) in MR and WM were: 16.2 vs. 13.9, 23.7 vs. 25.8, 17.5 vs. 32.7, and 48.8 vs. 32.9, respectively. Starter feed and straw were introduced at wk 6. Calves were gradually weaned from wk 6 to 10 and studied up to wk 13. Blood samples were taken weekly, and data were analyzed using PROC MIXED (SAS 9.4) for testing source and level effects and their interaction. This last was not significant. Calves fed MR had higher blood glucose concentrations preweaning ($P = 0.01$), but lower during weaning ($P = 0.02$), compared with WM-fed calves. Preweaning blood concentrations of β-hydroxybutyrate (BHB), nonesterified fatty-acids (NEFA), triglycerides (TG) and cholesterol ($P \leq 0.06$) were greater for WM-fed calves. However, during weaning and postweaning, only TG remained greater ($P \leq 0.04$). Concentrations of total protein, urea, albumin, and globulin were greater for WM-fed calves during preweaning ($P < 0.01$). Total protein and albumin remained greater during weaning ($P \leq 0.01$). Calves fed high milk allowance had greater glucose ($P = 0.02$) and lowered cholesterol ($P < 0.01$) concentrations in the blood during preweaning. During weaning, blood concentrations of glucose, NEFA, total protein, albumin ($P < 0.01$) and TG ($P = 0.04$) were greater for calves on high milk supply, whereas BHB ($P < 0.01$) and urea ($P = 0.06$) were lower. Postweaning, calves fed high milk allowances had greater NEFA ($P = 0.04$), total protein and albumin ($P < 0.01$) concentrations and lower TG concentrations ($P < 0.01$). The differences observed in blood metabolites during preweaning and weaning confirm the significant influence of feeding WM or MR and their supply on calf metabolism. However, most metabolites did not differ with the milk source provided during postweaning.

Key Words: calf, metabolism, milk source

1471T Effects of supplementing colostrum to preweaned heifers for 14 days on metabolism and gut permeability.

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Supplementing colostrum to calves beyond the first day of life has been shown to increase average daily gains (ADG) and serum IgG concentrations, as well as decrease the risks of mortality and diarrhea. However, the mechanism behind these observed benefits is not understood and must be explored. This study aimed to explore the effects of supplementing colostrum beyond the first day of life on metabolism and gut health in preweaning calves. Holstein heifers (n = 32; 16/TRT) were enrolled at birth, fed colostrum replacer (CR; 150 g IgG/feeding) at 0 h and assigned to 1 of 2 feeding schemes for d 1–14: Control (CON; 100% milk replacer; MR) or extended (EXT; 90% MR + 10% CR). Both treatments were fed 3 L twice daily from d 1–14, after which calves received 4 L MR twice daily from d 15–49, before weaning occurred from d 50–56. Calves were blocked by birth weight and dam parity. Body weight was recorded at birth and weekly until d 56. Blood samples were taken at 0, 24, and 48 h relative to birth, and weekly until d 56. The calves underwent postprandial insulin and glucose analysis on d 13 of life and gut permeability was assessed using on d 14 using lactulose, mannitol, and chromium-EDTA as markers. All data were analyzed using the GLIMMIX procedure in SAS (v. 9.4). No treatment effects ($P > 0.10$) were observed on serum IgG concentrations and ADG. In looking at the postprandial response, no treatment effects ($P > 0.10$) were seen on glucose or insulin to glucose ratios, insulin concentrations were greater ($P = 0.01$) in the EXT calves. However, there were no treatment differences ($P > 0.10$) seen in weekly insulin and glucose concentrations. For gut permeability, there were no treatment differences ($P > 0.10$) observed in concentrations of the markers. While the postprandial insulin differences suggest an effect on metabolism, more research is needed to determine the mechanism of action behind the improvements seen during long-term colostrum supplementation.

Key Words: dairy calf, colostrum supplementation, metabolism

1472T Performance and health of calves fed texturized calf starter with oats or an oat alternative from birth to 8 weeks of age.

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Inconsistent ingredient availability and global supply chain issues have created difficulty predicting availability and price of common feedstuffs, such as oats, which are commonly used in texturized calf starters (TCS). Feeding TCS containing high-fiber oat alternatives such as beet pulp, cottonseed hulls and barley may be a cost-effective way to feed calves during times of high input cost variability. The objective of this study was to examine effects of feeding conventional TCS formulated with oats or a combination of high-fiber alternative ingredients (ALT) on growth, feed efficiency, and health in dairy calves. Holstein heifer calves (n = 102) were randomly assigned to 1 of 4 treatments in a randomized complete block design from birth to d 56 of age. Treatments included TCS with 1) high fiber (17.6% aNDFom) and oats (OAT-HF); 2) high fiber and ALT (ALT-HF); 3) low fiber and oats (16.2% aNDFom) (OAT-LF); and 4) low fiber and ALT (ALT-LF). TCS was fed ad libitum throughout the study. Calves received 340 g of 24% protein, 20% fat milk replacer (MR) 2×/d from d 1–42, and 1×/d from d 43–49. Body weight (BW) was measured at d 0, 14, 28, 42, 49 and 56. Fecal scores were measured weekly. Feed intake was measured daily and calculated bi-weekly. Data were analyzed as a 2 × 2 factorial design using the PROC MIXED procedure of SAS, with fixed effects of fiber and oat inclusion and random effects of source herd and barn. Initial BW was used as a covariate for BW, gain and feed intake. During the post-weaning period (d 50 to 56), BW gain was impacted by interaction of fiber concentration and oat inclusion ($P = 0.04$), with ALT-HF causing a 23% greater gain than ALT-LF. This coincided with a tendency for greater feed intake in ALT-HF compared with ALT-LF ($P = 0.10$). No effects of treatment on BW gain or intake were observed in the pre-weaning period ($P > 0.05$). Hip height gain tended to be increased by ALT-HF compared with OAT-HF ($P = 0.07$). Neither feed efficiency nor fecal scores were affected by treatment. Overall, increasing fiber concentration of starter increased post-weaning growth and feed intake of calves fed oat alternatives.

Key Words: calf starter, oats

1473T Performance and health of calves fed milk replacer supplemented with increasing levels of choline from birth to 8 weeks of age.

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Supplementing choline (CH), specifically during the transition period, supports energy balance and helps to prevent fatty liver syndrome and ketosis in lactating cows. NASEM (2021) currently recommends supplementing CH at 1,000 mg/kg DM for calves but limited in vivo research has been conducted to support these recommendations. The objective of this study was to examine the effects of increasing concentrations of CH in milk replacer (MR) on growth, intake, and health of dairy calves. Holstein heifer calves (n = 100) were randomly assigned to 1 of 4 treatments in a randomized complete block design from birth to d 56 of age. Treatments included MR supplemented with 0 (C0), 900 (C9), 1800 (C18) or 2700 (C27) mg/d CH. The basal 24% CP, 20% fat MR was fed at 583.4 g (12.5% wt/vol in water) 2×/d from d 1 to 7, and 340.13 g 2×/d from d 8 to 42, 1×/d from d 43–49. Body weight (BW) was measured at d 0, 14, 28, 42, 49 and 56. Fecal scores were measured weekly. Feed intake was measured daily and calculated bi-weekly. The experiment was conducted from Oct 2021 to Feb 2022. Data were analyzed using the MIXED procedure of SAS with fixed effects of treatment, and random effects source herd and barn. Initial BW was included as a covariate for BW, average daily gain, and feed intake. Linear and quadratic orthogonal contrasts of increasing CH were determined. MR intake during d 1 to 14 ($P = 0.03$) and the entire pre-weaning period ($P = 0.05$) linearly decreased with increasing CH, and starter intake was decreased by C18 compared with C9 and C27 ($P < 0.05$). Post-wean (d 50–56) BW gain was decreased in C18 and C27 compared with C0 and C9 ($P < 0.05$). No effects were observed for gain to feed or fecal scores. Overall, modest negative effects on intake and growth were observed with increased CH supplementation. Results may have been because concentrations provided were well over established recommendations and may provide support to suggest that NASEM requirements are appropriate.

Key Words: calf performance, choline, milk replacer

1474T Fecal dry matter analysis of calves fed high solids milk replacer diets.

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The objective of this study was to determine if fecal dry matter (DM) corresponded to fecal score and if fecal DM could be manipulated by nutrition. Holstein calves were fed milk replacer (MR) at high solids concentrations (18%) with various nutritional additives. On all trials, calves were housed in individual outdoor hutches. Fecal scores were recorded twice daily. Fecal scoring was conducted using a 4-level scoring scale: 1 = normal, firm; 2 = semi-formed, pasty; 3 = loose but not watery; and 4 = watery, sifts through bedding. Approximately 50 g of feces were collected daily from each calf via rectal palpation during sampling periods for all trials. A Latin Square design with 7-d periods was utilized for each trial. Data were analyzed using MIXED procedures in SAS. In the preliminary study, 8 calves were assigned 1 of 4 treatments: low concentration and low DM amount (CON), low concentration and high DM (LH), high concentration and low DM (HL), or high concentration and high DM (HH). Fecal DM% tended to be greater for CON compared with LH ($P = 0.083$). In experiment 1, 10 calves were assigned 1 of 5 treatments: low solids % MR (PCON1), high solids % MR (NCON1), high solids % MR with sodium chloride (SC), high solids % MR with psyllium (P1), or high solids % MR with casein (C1). There was a tendency for greater fecal DM% for SC compared with NCON1 ($P = 0.074$). In experiment 2, 10 calves were assigned 1 of 5 treatments: low solids % MR, high solids % MR, high solids % MR with mineral mix, high solids % MR with psyllium, or high solids % MR with guar gum. Fecal DM events did not differ by treatment for this study. In experiment 3, 21 calves were assigned 1 of 5 treatments: low solids % MR (PCON3), high solids % MR (NCON3), high solids % MR with casein (C3), high solids % MR with psyllium (P3), or high solids % MR with casein plus psyllium (CP). Fecal DM% was greater for C3 compared with NCON3 ($P = 0.032$). There was a negative correlation between fecal DM% and fecal score across all trials combined ($P < 0.0001$). In all, sodium chloride and casein have potential to improved fecal DM% when feeding a higher solids diet but further research is needed.

Key Words: calf, feces, dry matter

1475T Impact of fortifying maternal colostrum with colostrum replacers with and without fat for an initial colostrum meal on achievement of transfer of passive immunity in newborn male dairy calves.

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Previous work has shown supplementing low-quality maternal colostrum (MC) with colostrum replacer (CR) improves serum IgG levels and apparent efficiency of absorption (AEA) of IgG. However, differing IgG intakes made interpretation of data challenging. Further, little to no data exist to support the superiority of colostral fat over other fat sources. The primary objective of this study was to assess the impact of adding additional IgG to low-quality MC on AEA compared with a high-quality MC providing similar levels of IgG and to determine the impact of fat source and amount consumed on achievement of transfer of passive immunity (TPI) in newborn calves. Sixty newborn male Holstein calves were weighed and assigned to one of 4 colostrum treatments at birth: 1) High-quality MC (HQMC; 92 g IgG/L), 2) Low-quality MC (LQMC; 28 g IgG/L); 3) LQMC with 850 g of added CR containing 20% fat from animal fat (CRF+; Genesis, Cargill Animal Nutrition, Lewisburg, OH), or 4) LQMC with 644 g of added CR containing < 2% fat from colostral fat (CFF−; Premolac Plus, Zinpro, Eden Prairie, MN). IgG intake between HQMC, CRF+, and CRF− were the same. All treatments were fed at a rate of 3.6 L within 2 h of birth. Fat intake between HQMC and CRF+ were the same, and 60% of the fat in treatment CRF+ came from animal fat. Treatment CRF− calves consumed 50% less fat than calves fed HQMC or CRF+. Blood samples were taken at birth and 24 h post-colostrum feeding and analyzed for serum IgG, total protein, and Brix. Data were analyzed using Proc MIXED in SAS 9.4. Serum IgG at 24 h was lowest LQMC calves, similar between calves fed HQMC and CRF+, and greatest for CRF− ($P < 0.01$). Similarly, AEA was higher for calves fed CRF− compared with calves fed HQMC and CRF+, which did not differ ($P < 0.01$). Serum total protein and serum Brix were poor predictors of serum IgG in calves fed treatments other than HQMC. Boosting MC with CR does not seem to compromise TPI or AEA in newborn calves and colostral fat does not appear to provide advantages compared with other fat sources.

Key Words: immunoglobulin G, calf, whey

1476T Effects of reducing total solids in colostrum replacer on IgG absorption in newborn Holstein calves.

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Colostral IgG absorption could be compromised with decreased abomasal emptying rates and, possibly, by high total solids (TS). This could be avoided by diluting colostrum replacer (CR). The objective of this study was to determine if reducing TS of CR by increased dilution has an effect on IgG absorption, abomasal emptying rate per h (AB), or health scoring. Male Holstein calves (n = 48; 12/TRT) with birth body weights

(BW) of 40–52 kg were randomly assigned to be fed 949 g (150 g IgG) of CR with different final volumes (FL). The total solids (TS; g/L) were equally spaced and treatments were defined as a dose-response with 4 levels: Control (C) = 1.79 kg water, FL: 2.64 L, 360 g/L CR; Dilution 1 (D-1) = 2.08 kg water, FL: 2.97 L, 320 g/L CR, Dilution 2 (D-2) = 2.57 kg water, FL: 3.39 L, 280 g/L CR, or Dilution 3 (D-3) = 3.11 kg water, FL: 3.96 L, 240 g/L CR within 1 h after birth. The Saskatoon Colostrum Company Ltd. (SCCL) provided the CR used (15.8% IgG, 16.4% fat, 47.1% protein). A subset of calves (6/TRT) had a catheter placed to estimate the AB and determine gut permeability (GP). Baseline blood samples were taken followed by samples at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 26, 28, 30, 32, 36, and 48 h relative to colostrum feeding. Data were analyzed using the GLIMMIX, MIXED, NLMIXED, and GENMOD procedures in SAS (University Edition). Unless otherwise stated, all results are presented in the following order: C, D-1, D-2, and D-3. The osmolality of TRTs was significantly reduced with additional water: 835.1, 714.8, 514.0, and 410.3 +23.32 mOsm/kg ($P < 0.01$). Serum IgG concentrations at 24 h tended to linearly increase: 15.5, 12.2, 17.3, and 18.4 +1.75 g/L ($P = 0.10$). The apparent efficiency of absorption tended to linearly increase with reduced TS levels: 43.9, 35.1, 49.5, and 51.2 + 4.77% ($P = 0.09$). The AB linearly increased with decreased TS levels: 0.09, 0.11, 0.13, and 0.13 + 0.017 ($P = 0.03$). The lactulose, mannitol, and Cr-EDTA concentrations during the GP test did not differ ($P > 0.05$). Abnormal fecal scores or respiratory scores until d 7 did not differ ($P > 0.05$). These results suggest that reduced TS could benefit IgG and increase AB.

Key Words: colostrum replacer, IgG absorption, total solids

Ruminant Nutrition: Carbohydrates and Lipids 2

1477T Impact of Valopro NRJ on primiparous dairy cow's milk production. L. Drouet* and C. Guyvarch, *MiXscience, Bruz, France.*

Essential oils have an impact on ruminal microorganisms and can improve carbohydrate digestibility, health status, and therefore production. The objective of this experiment was to determine the effects of a blend of phenol essential oils and spices (Valopro NRJ, MiXscience, Bruz, France) on post-peak lactating Holstein cows performance (n = 30; 60% primiparous cows; DIM 184 ± 64; 35.6 ± 6.8 kg of milk/day; 723 ± 67 kg body weight). Cows were housed in MiXscience Research Center (Saint-Symphorien, France) and received during 6 weeks a diet made up of 44.5% corn silage, 14.5% grass silage, 36.7% concentrates (2 kg corn, 2 kg wheat), 0.5% calcium soap of fatty acid, 2.8% liquid feed, 1% mineral (16.2% protein, 34.8% NDF, 1.65 Mcal/kg). Cows were blocked by lactation number, days in milk (DIM), milk yield, protein and fat contents and were assigned to one of the 2 groups with 0 g/cow/d (control group, CTL) or 30 g/cow/d of Valopro NRJ (VN group). Individual milk yield and composition (protein, fat, urea, SCC) was monitored daily and weekly, respectively. Individual intake and body weight were assessed every day. Data were analyzed using a generalized linear mixed model (R software) with random effects on cows (production level on first trial day and specific evolution during the trial). Parity, group (VN vs. CTL) and interactions (parity:group, parity:trialDays, group:trialDays) were included as fixed effects in the model. Milk production was more stable for cows fed VN during the trial and the difference between the 2 groups at the end of the trial was +1.7 kg for VN compared with CTL (VN: 35.6 kg vs. CTL: 33.9, $P = 0.002$). At the end of the trial, milk composition was similar between the 2 groups (+1.7 g protein/kg and +1.8 g protein/kg for VN and CTL groups, respectively, and +1.1 g fat/kg for both VN and CTL groups) so that ECM was improved during the trial for VN cows (+2.1 kg at the end of the trial, $P = 0.015$). There was no difference between groups for milk urea, intake and body weight. Results indicates that 30 g/cow/d of Valopro NRJ can significantly improve milk yield and ECM and is a suitable solution to increase profitability in dairy cows system.

Key Words: essential oils, dairy cow, milk yield

1478T Comparison of neutral detergent fiber methods. M. B. Hall*[1] and D. R. Mertens[2], [1]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI,* [2]*Mertens Innovation and Research LLC, Belleville, WI.*

Neutral detergent fiber (aNDF) is the most commonly reported fiber metric in dairy cattle nutrition. An empirical analyte, aNDF is defined by the method (M) used. The definitive method is AOAC Official Method 2002.04, performed on samples ground to pass the 1-mm screen of a cutting mill (1CM) with refluxing and then filtration through Gooch crucibles without (AOAC−; definitive M) or with (AOAC+) a glass fiber filter. Other M in use include grinding materials through the 1-mm screen of an abrasion mill (1AM), filtration through a Buchner funnel with a glass fiber filter (Buch), and the ANKOM filter bag system (ANKOM Technology, Macedon, NY) using filter bags with 25 µm (F57) or 6–9 µm (F58) particle size retentions. Our objective was to compare AOAC and alternative M using feeds ground with 1CM and 1AM. Dry ground and high moisture corn grains, 2 alfalfa silages, 2 corn silages, grass hay, ryegrass silage, soybean hulls, calf starter, and sugar beet pulp were analyzed in duplicate in replicate analytical runs. Data were analyzed as a completely randomized design with a 2 × 3 factorial arrangement of treatments. Significance declared at $P \leq 0.05$, and tendency at $0.05 < P \leq 0.10$. The aNDF% of dry matter (aNDF%) from 1AM as compared with 1CM ground feeds were or tended to be lower for 8 of 11 feeds. Method affected aNDF% results for all feeds, with M × grind interactions for 6 of 11 feeds. For ash-free aNDF% of 1CM ground feeds, a priori contrasts showed that the number of feeds for which M differed or tended to differ from AOAC M were Buch: 4, F57: 8, and F58: 3; and 3 for AOAC− versus AOAC+. Number of aNDF% values that were outside of 2 standard deviations from AOAC− reference means for 1CM and 1AM ground feeds, respectively, were AOAC−: 0, 4; AOAC+: 0, 2; Buch: 2, 2; F57: 8, 10; and F58: 4, 7. We conclude: 1) 1CM grinds or equivalent are necessary for the aNDF M tested to agree with the AOAC reference M; 2) with 1CM grinds, M in order of agreement with AOAC are Buch, F58, and F57, which often produced lower values; and 3) as an allowed modification of AOAC−, AOAC+ is worthy of consideration for general use. Further evaluation with an expanded sample set is needed.

Key Words: fiber, neutral detergent fiber, method

1479T Effects of forage level and branched short-chain fatty acids on lactation performance of dairy cows. M. Vedovatto[1], K. F. Kalscheur[2], and J. Kraft*[1], [1]*The University of Vermont, Burlington, VT,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

The objective of this study was to evaluate the inclusion of forage and branched short-chain fatty acids (BSCFAs) on lactation performance of dairy cows. Using a randomized complete block design, 56 lactating cows (48 Holsteins and 8 Jerseys; 151 ± 47 DIM) were assigned to one of 4 treatments in a 2 × 2 factorial arrangement evaluating forage level [low forage (LF, 50% of the diet on a DM basis) and high forage (HF, 65% of the diet on DM basis)] with or without supplementation of BSCFAs (2.4 g/kg of DM of a BSCFA mix containing isobutyrate, 2-methylbutyrate, and isovalerate in a ratio of 2:1:1, respectively). The forage portion of all diets consisted of 70% corn silage and 30% alfalfa silage on a DM basis. The experiment included a 2-wk covariate period where all cows were fed a common diet, followed by a 9-wk treatment period. Data were analyzed using the MIXED procedure of SAS with week as the repeated measure. Cows fed LF compared with HF resulted in greater dry matter intake (DMI; 28.4 vs. 24.9 ± 0.35 kg/d; $P < 0.01$), milk yield (41.8 vs. 39.1 ± 0.56 kg/d; $P < 0.01$), energy-corrected milk yield (ECM; 43.5 vs. 41.3 ± 0.69 kg/d; $P < 0.01$), and yield of protein, lactose, and total solids ($P < 0.01$). Furthermore, cows fed LF diets resulted in lower milk fat percentage (3.79 vs. 3.93 ± 0.03%; $P < 0.01$) compared with HF-fed cows but no differences in protein, lactose, and total solids percentage were observed. Accordingly, feed conversion efficiency (ECM/DMI; 1.54 vs. 1.66 ± 0.02 kg/kg, $P < 0.01$) was lower in LF-fed cows compared with HF-fed cows. The addition of BSCFAs did not affect BW, BCS, DMI, milk yield, ECM yield, or milk components ($P > 0.05$), nor were any forage × BSCFA × wk interactions detected for any variable ($P > 0.05$). In summary, cows fed LF diets increased milk yield and milk components yield, however, cows fed HF diets resulted in greater feed conversion efficiency. The inclusion of BSCFAs, regardless of forage level, did not affect lactation performance of dairy cows in this experiment.

Key Words: ketoacids, forage inclusion, feed conversion efficiency

1480T Supplementation of rumen-protected sugar decreases blood β-hydroxy butyric acid concentration and improves reproduction in fresh lactating dairy cows.

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The objective of the study was to supplement rumen-protected sugar (RPS) to transition cows to decrease blood β-hydroxy butyric acid (BHB) concentration in early lactation. Sixty-two Holstein cows were separated into either control (CON), or on-top supplementation of RPS (LipoAktiv Glu 60, Berg + Schmidt GmbH & Co. KG, Hamburg). Supplementation started - 3 wk before estimated calving date with 150 g/cow/d RPS, after calving until 21 d in milk (DIM) RPS was fed at 200 g/cow/d. Blood glucose level was measured at calving, 7 and 14 DIM. Blood BHB level was measured at 7 DIM. Daily milk yield was measured until 63 DIM. Reproduction data were measured. Daily milk yield and blood glucose concentration were analyzed using statistical program R version 4.1.1 (2021). Treatment, time, interaction of treatment and time were included as independent variables, cow nested within treatment as random effect in a linear mixed model. Reproduction data and blood BHB level were compared by *t*-test statistics. Supplementation of RPS during transition period had no effect on blood glucose level (P = 0.88). Cows fed RPS tended to have lower blood BHB concentration compared with CON at 7 DIM (0.72 mM CON vs. 0.62 mM RPS; $P <$ 0.1). Subclinical ketosis (SCK) was detected 12.5% in CON and 0% in RPS ($P < 0.05$), where SCK was defined as blood BHB concentration ≥1.2 mM. Blood BHB level ≤0.8 mM are associated with higher migration of circulating polymorphonuclear leucocytes (PMN). The rate of animals with blood BHB level ≤0.8 mM was higher in RPS (90.9%) than in CON (77.5%) ($P < 0.1$). There was no difference in milk yield by treatment ($P = 0.98$). Average days from calving to estrus tended to be reduced in RPS compared with CON (52.4 CON vs. 45.4 RPS; $P <$ 0.1). Average days to first insemination were 129 for CON and 120 for RPS ($P < 0.1$). In conclusion, supplementation of RPS in transition dairy cows decreased blood BHB concentration which can be associated with reduced subclinical ketosis and improved migration of circulating PMN.

Key Words: rumen-protected sugar, subclinical ketosis, reproduction

1481T Interaction of digestible fiber and acetate supplementation on milk fat yield in dairy cows.

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Milk fat is an important and desirable component of milk because of its economic value and the demand for processed dairy products. Milk fat is sensitive to nutritional changes and acetate supplementation has been reported to increase milk fat in lactating cows. The objective of this experiment was to test the interaction of sodium acetate and diet digestible fiber level. Our hypothesis was that acetate would increase milk fat more in diets with lower digestible fiber as they are expected to also have lower acetate yield. Twelve multiparous Holstein cows were arranged in 4 × 4 Latin square design with 21 d period and a 2 × 2 factorial arrangement of treatments. Treatments were lower digestible fiber with a diet based mainly on forage fiber and high digestible fiber diet that substituted soyhulls and citrus pulp for corn silage (13.1% diet DM) with or without acetate (ACET) supplementation. The soyhull and citrus pulp substitution was balanced to decrease dietary starch 4 percentage units and forage NDF 1.3 percentage units with only a 0.4 percentage unit increase in total NDF. Sodium acetate was mixed in the TMR targeting 600 g/d. Data were analyzed by using JMP Pro 16 with a model that included the random effect of cow and period and the fixed effect of fiber digestibility, acetate, and their interaction. There was no effect of fiber digestibility, acetate, or interactions of fiber digestibility and acetate on milk yield. Acetate increased milk fat yield 103 g/d ($P <$ 0.02) but there was no interaction of fiber digestibility and acetate ($P >$ 0.36). There was no effect of fiber digestibility or interaction of digestibility and acetate on milk fat percentage but acetate tended to increase fat concentration 0.14 percentage units ($P = 0.08$). There also was no interaction of fiber digestibility and ACET for milk protein yield and concentration, but acetate tended to increase milk protein concentration ($P = 0.06$). There were no effects on milk urea nitrogen. In conclusion, acetate increased milk fat yield regardless of fiber digestibility of the basal diet.

Key Words: milk fat, sodium acetate, fiber digestibility

1482T Effect of organic solvent emulsifiers on *in vitro* rumen fermentation and gas production.

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Performing in vitro incubations of lipophilic supplements in rumen fluid requires the utilization of emulsifiers. When selecting an emulsifier, it is important to ensure the absence or at least minimization of emulsifier effects on fermentation parameters to minimize potential biases to the lipophilic treatments in the in vitro experiments. This study aimed to evaluate the effect of 7 emulsifiers, 2 concentrations each, on in vitro gas production and rumen fermentation, to identify the most inert emulsifier. In vitro incubations of emulsifiers were performed using the Hohenheim Gas Test. Individual rumen fluids were collected immediately before the morning feeding from 3 nonlactating cannulated Original Brown-Swiss cows and incubated for 24 h with TMR (40% grass silage, 40% maize silage, 15% hay and 5% concentrate, dry matter). Ethanol (E), ethyl acetate (EA), propylene glycol (PG), glycerol (G), ethylene glycol (EG), soy lecithin (SL), and Tween 80 (T80) were added in dosages of 0.5% or 1% vol/vol. The untreated diet served as control (CON). Compared with CON, the 24-h methane production was higher ($P < 0.05$) for E (36.9, 40.8%), EG (47.5, 48.2%), and G (63.8, 90.1%) for 0.5 and 1% dosage respectively. The acetate was 20.6 mM and 48.9 mM higher for EA in 0.5 and 1% respectively, and 13.5 mM higher ($P < 0.05$) in 0.5% EG compared with the CON. The propionate was 13.2 mM and 20.6 mM higher for 0.5 and 1% G, respectively, and 10.7 mM and 10.8 mM higher for 0.5 and 1% PG respectively, when compared with the CON. In comparison to the CON, the in vitro organic matter digestibility increased ($P < 0.05$) by EA (30.7, 41.5%), EG (18.4, 18.0%), G (42.5, 50.1%) and SL (17.7, 24.0%) at 0.5 and 1% dosage respectively. In summary, T80 was the only emulsifier that did not significantly affect in vitro rumen fermentation parameters and can therefore be considered most suitable for in vitro incubations of lipophilic substances in rumen fluid.

Key Words: Tween 80, methane, volatile fatty acid

1483T Relationship between pre-trial milk fat concentration and milk fat response to marine oil diets that caused milk fat depression in sheep.

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Biohydrogenation (BH)-induced milk fat depression (MFD) results from ruminal alterations when sheep are fed marine oils. However, there is no available information on the relationship between pre-trial milk fat concentration and response to the MFD diet. The objective of this study was to conduct a meta-analysis to characterize whether pre-trial milk fat concentration may determine subsequent changes in milk fat synthesis when ewes were fed diets containing marine lipids. Data was used from 10 experiments in which 160 Assaf ewes were fed 16 MFD diets (including fish oil or marine algae, alone or in combination with sunflower oil) for 3 to 5 wks. The decrease in milk fat concentration was calculated using 3 different methods: the absolute change (final − initial), change as a percent of pre-trial value, and potential change relative to the maximal expected MFD (assuming a maximal decrease to 3% milk fat). The statistical model included the random effect of the experiment and interaction of experiment and pre-trial milk fat concentration, and the linear and quadratic effect of this latter variable. There was a linear relationship between initial milk fat content and the absolute ($P < 0.001$; $R^2 = 0.46$; partial R^2 of 0.08), relative ($P < 0.01$; $R^2 = 0.39$; partial R^2 of 0.06), and "potential" change in milk fat concentration ($P = 0.04$, $R^2 = 0.17$; partial $R^2 = 0.03$), with higher milk fat ewes experiencing greater reductions. It can be hypothesized that MFD severity in more responsive ewes may be related to some rumen function alterations (which in turn, might result in higher concentrations of certain antilipogenic metabolites). Overall, there is a linear inverse relationship between pre-trial milk fat concentration and the magnitude of MFD when sheep are fed marine oils, with higher reductions in ewes with higher milk fat percentage. Supported by PID2020–113441RB-I00 (MCIN/AEI) and PRE2018–086174 (MCIU/AEI/FSE, UE).

Key Words: ewe, biohydrogenation, marine lipid

1484T Do dairy sheep display Δ^{13}-desaturase activity? An in vivo study using ^{13}C-labeled fatty acids.

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The fatty acid desaturase 3 (FADS3) enzyme introduces a Δ^{13} double bond in fatty acids (FA). Using isotopic tracers, the activity of FADS3 has been identified in the mammary tissue of goats, catalyzing the desaturation of *trans*-11 18:1 to *trans*-11 *cis*-13 CLA. However, we have not found any similar research on other ruminant species in the literature. Neither does there seem to be any publication on the possible Δ^{13} desaturation of 18:0 in dairy animals. Thus, this study was conducted to examine the presence of FADS3 products in ewe milk, using ^{13}C-labeled FA. In a first trial, 5 Assaf sheep received an intravenous injection of 200 mg of [1-^{13}C]*trans*-11 18:1, and milk samples were collected at −24, −15, 0, 9, 24, 33, 48, 57, 72, 81, and 96 h postinjection (p.i.). In a second trial, 6 ewes received an intravenous injection of 2 g of [1-^{13}C]18:0, and milk samplings were conducted at −24, −15, 0, 4, 8, 12, 16, 20, 24, 36, 48, 60 and 72 h p.i. Compound-specific isotope analysis of milk FA were conducted by gas chromatography-combustion isotope ratio mass spectrometry. Despite this methodology offers high accuracy and precision to quantity isotope distribution at low abundance levels, in trial 1 we failed to detect increases in the ^{13}C % of *trans*-11 *cis*-13 CLA above basal levels. However, the presence of [1-^{13}C]*cis*-13 18:1 was detected in trial 2, with a maximum ^{13}C enrichment of 0.36% at 24 p.i. On average, the proportion of *cis*-13 18:1 being synthesized endogenously represented 51.4% of the amount secreted in milk, which corresponded to Δ^{13}-desaturation of 0.35% of the 18:0. These results would confirm the activity of FADS3 in sheep, but the question remains as to whether the lack of *trans*-11 18:1 desaturation was due to a specificity in this ruminant species or to the low concentration of its putative product (*trans*-11 *cis*-13 CLA) in trial 1 (on average, 0.007% of milk FA). Further research would be advisable using diets that favor the presence of this CLA isomer in sheep milk (e. g., linseed oil).

Key Words: conjugated linoleic acid, desaturation, isotopic tracer

Ruminant Nutrition: General 2

1485T Rumination is associated with transition period health indicators and performance outcomes. T. M. Nelson* and T. R. Overton, *Cornell University, Ithaca, NY.*

We hypothesized that rumination was positively related to transition period outcomes. Holstein cows (n = 256) entering 2+ lactations were retrospectively categorized into low or high groups based on the median for explanatory variables collected −1 or +1 wk relative to parturition [milk yield, dry matter intake (DMI), rumination, and concentrations of nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHB)] and variables were averaged weekly for the outcome of interest. Variability of rumination in the first 10 d in milk was also calculated using a coefficient of variation to further elucidate how rumination variability (RV) is related to transition period outcomes. Outcomes were evaluated using ANOVA with repeated measures, when applicable (SAS v. 9.4), with rumination as the outcome or explanatory variable of interest. Prepartum outcomes were not associated with prepartum rumination, whereas high postpartum rumination cows had higher DMI (17.0 vs. 15.4 ± 0.2 kg/d, $P = 0.002$) and higher milk yield (33.8 vs. 31.9 ± 0.6 kg/d, $P = 0.01$) in the first wk postpartum compared with low rumination cows. Postpartum rumination group was not associated with NEFA or BHB concentrations. High postpartum DMI cows tended to have higher rumination in the prepartum period (537 vs. 525 ± 5 min/d, $P = 0.09$) and lower rumination in the postpartum period (598 vs. 604 ± 6, $P = 0.10$). Categorized milk yield and BHB was not associated with postpartum rumination, but high NEFA cows tended to have more rumination than low NEFA (581 vs. 564 ± 6 min/d, $P = 0.06$). Cows with high RV had higher milk yields than low RV (35.7 vs. 33.7 ± 0.4 kg/d, $P = 0.008$), whereas high RV cows had lower DMI than low RV cows (18.3 vs. 19.4 ± 0.2 kg/d, $P < 0.001$). Cows with high RV also had higher NEFA (588 vs. 447 ± 48 mEq/L, $P < 0.001$) than low RV cows. High postpartum rumination was associated with favorable results during the first wk postpartum; however, rumination during the first wk postpartum may not be a good indicator of early lactation performance. High RV was likely related to a greater absolute change in rumination and should be further investigated.

Key Words: transition period, rumination, performance

1486T Supplemental branched-chain volatile fatty acids (BCVFA) interact for lactation performance by Holstein cows fed low protein diets. A. White*[1], J. Copelin[1], C. Lee[1], D. Kleinschmit[2], M. Socha[2], and J. Firkins[1], [1]*The Ohio State University, Columbus, OH,* [2]*Zinpro Corporation, Eden Prairie, MN.*

We previously documented that 2-methylbutyrate (2MB) stimulated NDF degradability (NDFD) most in vitro; isobutyrate (Ibu) and isovalerate (Ival) also increased NDFD but interchange in bacterial lipid metabolism. Ival was hypothesized to be sufficient in dairy diets, so Ival supplementation would increase Ibu needed to maintain a balance of BCVFA to improve milk fat production and efficiency. Holstein cows were blocked by parity (n = 72; 30 primiparous) and DIM (103 ± 32) and enrolled in 3 phases. Treatments were low (LCP, 15.0%) or high (HCP, 16.5%) crude protein controls without BCVFA and 4 LCP diets with 0X or 1X Ival and 1X or 2X Ibu in which X was equimolar at 20 g/d 2MB or Ival or 17.2 g/d of Ibu. After 2 wk on the HCP diet, cows were randomly assigned within block to the 6 diets for 8 wk. Data were analyzed with PROC MIXED in SAS (v. 9.4, SAS Institute 2015) with random effects of phase and block(phase) and fixed effects of parity, diet, week, and their interactions. Two orthogonal contrasts were either the LCP or HCP controls vs. the BCVFA treatments, and main effects and interaction of Ival and Ibu within the 4 BCVFA treatments. Supplemental BCVFA were within 10% of target, but CP was 14.7 and 16.1%, averaged over 3 phases for LCP and HCP. Compared with LCP, BCVFA treatments increased ($P = 0.05$) ADG and decreased ($P = 0.09$) ECM, primarily due to lower ($P = 0.07$) fat yield without change in milk protein or DMI. Adding Ival decreased ECM ($P = 0.05$) and tended to decrease fat ($P = 0.10$) yields within BCVFA, but 2X Ibu enhanced ($P = 0.08$) the shift toward ADG for multiparous cows. Compared with HCP, BCVFA cows ate less ($P = 0.02$) and yielded less protein ($P < 0.01$) and ECM ($P = 0.01$) but not less fat. Parity affected ($P = 0.10$) DMI; increasing Ibu decreased ($P = 0.01$) DMI but not ADG for primiparous cows. For LCP and BCVFA diets, MUN were <8.0 mg/dL. Residuals of milk fat yield were inversely associated with decreasing MUN, especially when Ibu was increased without added Ival. LCP diets must provide adequate rumen NH_3 when supplementing BCVFA to optimize NDFD to support milk fat production.

Key Words: isoacid, milk fat production, lactating dairy cow

1487T Effect of dietary citrus pulp on milk production and milk composition in dairy cows: A meta-analysis and meta-regression. E. E. Corea-Guillen*[1,2], J. M. Castro-Montoya[3], A. Lizarazo[1], G. A. Flores[2], M. Benaouda[4], R. Vieyra-Alberto[5], L. Hernandez-Trapala[5], Y. D. Zamora-Raygadas[5], L. Guevara[6], and J. C. Ángeles-Hernandez[5], [1]*Programa de Maestría y Doctorado en Ciencias de la Salud y Producción Animal, Universidad Nacional Autónoma de México, México DF, México,* [2]*Departamento de Zootecnia, Facultad de Ciencias Agronómicas, Universidad de El Salvador, San Salvador City, San Salvador, El Salvador,* [3]*Programa de posgrado y educación continua, Facultad de Ciencias Agronómicas, Universidad de El Salvador, San Salvador City, San Salvador, El Salvador,* [4]*L'Institut Agro Dijon, Dijon, Bourgogne, France,* [5]*Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Tulancingo, Hidalgo, México,* [6]*Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Río de Janeiro, Brasil.*

Citrus by-products have been used as low-cost nutritional supplements in ruminant rations to support growth and lactation. The aim of the current study was to evaluate the effect of diet inclusion of citrus pulp (Cp) on dairy cow performance through a meta-analysis. A systematic search was based on the question: What is the effect of Cp feeding on dairy cow performance compared with the control group? The search was conducted using the scientific databases: PubMed, Google Scholar, Scopus, and PRIMO-UAEH. The outcome variables considered were milk yield (MY), fat, protein, lactose content, dry matter intake (DMI), and DM apparent total-tract digestibility (ATTD). The effect size was measured through the raw (RMD) and standardized mean difference (SMD). The meta-analysis was conducted using the "Meta" package in R. The meta-regression included the covariates: breed (Holstein, crossbreed, and Jersey), inclusion level (IL; g/kg DM), Cp type (dry, ensiled, and molasses), and milk production level (MPL; low <24, medium 24–30, and high >30 kg/d). The inclusion of Cp in dairy cow rations had a negative effect on MY (−0.71 kg), protein (−0.09 g/100 g), lactose (−0.08 g/100 g), and DMI (−1.91 kg/d) (Table 1). The MY of high-producing cows was more affected by Cp feeding. The negative effect of Cp on protein, lactose content, and DMI was higher in Holstein cows. A negative relationship between MY and DMI with the IL was observed. The inclusion of Cp as a source of energy must consider fac-

Table 1 (Abstr. 1487T). Effect of dietary citrus pulp on dairy cow performance

Item	Trials	Control mean (SD)[1]	RMD	SMD	I^2	Significant covariates
MY (kg/d)	50	27.2 (4.85)	−0.71***	−0.27**	73.9	MPL, type and IL
Fat (g/100 g)	49	3.64 (0.49)	+0.01	+0.04	64.8	
Protein (g/100 g)	49	3.13 (0.46)	−0.09**	−0.51*	75.1	MPL and breed
Lactose (g/100 g)	33	4.69 (0.17)	−0.08**	−0.49**	77.1	MPL and breed
DMI (kg DM/d)	40	20.8 (2.35)	−1.91*	−0.74**	96.5	MPL, type, IL and breed
ATTD (g/100 g)	16	68.2 (3.18)	−0.34	−0.21	80.6	

[1] Mean and standard deviation of unsupplemented group; I^2, measure of heterogeneity.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

tors such as MPL, breed, type, and IL to minimize the adverse effects on dairy cow performance.

Key Words: citrus pulp, dairy cow, milk

1488T Feed hygiene survey of total mixed rations and fermented feeds from farms across the United States.

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Escherichia coli, coliforms, *Salmonella*, *Clostridium*, yeasts and molds are commonly found in various feed types on farms across the United States. When ingested in high amounts, these organisms can cause negative health issues in cattle such as septicemia, enteritis and diarrhea. Consuming these organisms may also lead to reduced feed intake and decreased milk production and quality. Additionally, yeasts and molds negatively impact silage quality by decreasing its nutritive value. To maintain healthy cattle and high producing milk cows, good feed hygiene maintenance is important to reduce the abundance of these organisms. The objective of this survey was to determine bacterial and fungal levels and prevalence in total mixed rations (TMR) and various fermented feeds from farms across the US. From February 2016 to January 2023, 8,942 samples of 4 different feed types (TMR [n = 5,446], corn silage [CS] [n = 1,922], alfalfa haylage [AH] [n = 1,252] and high moisture corn [HMC] [n = 215]) were collected from farms across 35 states. Feed samples were diluted 1:10 with peptone, *E. coli* and coliforms were enumerated on CHROMagar, clostridia were enumerated on tryptose sulfite cycloserine agar, and yeasts and molds were enumerated on potato dextrose agar. The feed samples were also analyzed for *Salmonella*, where black colonies on XLT-4 agar were considered presumptive *Salmonella.* Of the feed samples that were collected, *E. coli*, coliforms and clostridia were detected at higher levels and were more prevalent in TMR than in CS, AH or HMC ($P < 0.05$). *Salmonella* was also detected at a higher prevalence in TMR (11.9%) compared with CS (0.01%), AH (0.03%) or HMC (0.02%) ($P < 0.05$). Yeasts and molds were more prevalent in TMR (93.9% yeasts and 81.6% molds) compared with CS (54.8% and 33.1%) or AH (50.8% and 28.0%) ($P < 0.05$). This data displays that the levels of spoilage organisms and potential pathogens are higher in TMR than in the fermented feed components of the diet. Additionally, this data can be used to emphasize the importance of proper feed management practices that need to be ensured from the time of aerobic exposure to mixing the TMR to reduce the loads of these organisms.

Key Words: feed hygiene, TMR

1489T Comparison of three nutritional models on predicting dry matter intake for commercial pens of lactating dairy cows.

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The objective of this study was to compare different dry matter intake (DMI) prediction models for groups of lactating cows on commercial herds. The DMI prediction equations compared were the one proposed by NRC (2001) 7th Edition (NRC2001), and the 2 updated equations proposed by NASEM (2021) 8th Edition; AnimalNASEM and RationNASEM. AnimalNASEM equation has only animal factors, whereas the RationNASEM equation has factors related to the filling effect of rations. Data from 48 high-producing commercial pens of lactating Holstein cows (17 primiparous, 25 multiparous, and 6 mixed pens) from Paraná State, Southern Brazil, were used. The models were evaluated for both accuracy and precision using the significance of the mean bias and slope bias, the goodness of fit (R^2), and the root mean square error (RMSE). On average, cows produced 41.8 ± 8.6 kg/d, weighed 687 ± 46 kg, and had 165 ± 77 DIM. Observed DMI (ObsDMI) mean was 25.7 ± 3.2 kg/d, while prediction by NRC2001 was on average 0.81 kg/d higher, AnimalNASEM was 0.36 kg/d lower, and RationNASEM was 0.73 kg/d lower than ObsDMI. AnimalNASEM had the lowest RMSE value compared with the NRC2001 and RationNASEM models (1.33 vs. 1.57 and 1.96 kg/d, respectively). The AnimalNASEM model was the most accurate and precise for primiparous cows because it did not present significant slope and mean bias (−0.01 and 0.08 kg/d, respectively), also presented lower RMSE (0.60 kg/d or 2.53%) and the higher R^2 (0.95). Regarding multiparous cows, NRC2001 and AnimalNASEM had closer accuracies, with RMSE values of 1.49 and 1.64 kg/d, respectively, but NRC2001 model had a more significant mean bias (−1.22 vs. 0.87 kg/d). RationNASEM model was neither precise nor accurate on predicting DMI for pens of multiparous cows, with lower R^2 (0.63), higher RMSE (2.27 kg/d or 8.2%) and higher mean and slope bias (1.56 and 0.21 kg/d). In conclusion, primiparous DMI was more precisely predicted by the updated AnimalNASEM equation. On other hand, the updated model was not as effective for multiparous cows, and NRC2001 and AnimalNASEM equations had similar accuracy and precision on predicting DMI.

Key Words: feed intake, model evaluation

1490T Preference of corn and different corn milling co-products in lactating Jersey cows.

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Manipulations at the intersection of nutrition, behavior, and management may be an important location to apply novel feeding strategies that take advantage of affordable feeds and a cow's natural behavior to increase feed intake. The grain fuel sector continues to modify methods in how corn-ethanol is produced. This has resulted in different feed co-products that can be utilized by the dairy industry. The objective of this study was to determine how preference of various corn milling co-products differ and also compare them to corn grain. Eight lactating Jersey cows (169 ± 4 DIM, 29.7 ± 4.55 kg/d milk yield, 21.3 ± 2.78 kg/d DMI) were utilized. The treatments tested included dried corn distiller's grains and (DDGS) in meal form, high protein DDGS (HPDG) in meal form, pelleted DDGS (PEDG), and finely ground corn (CORN). During the experiment, cows consumed a corn-silage based TMR with 59.8% DM, 17.3% CP, 25.6% starch, and 26.0% NDF. Cows were offered 0.45 kg each treatment in a randomized arrangement within the feed bunk for 1 h or until the feed was fully consumed. Feeds were offered for 9 d with all treatments presented the first 4 d. The most preferred feed was then removed, and the remaining 3 feeds were offered for 3 d. The process was repeated for the last 2 d. Feed preference was ranked from 1 to 4 with 1 being the most preferred and 4 the least. The rankings of each treatment for each cow were summed and averaged. The PEDG was chosen as the most preferred feed (1.00 ± 0.25; mean ± SD) followed by CORN (2.14 ± 0.38), DDGS (2.50 ± 0.58), and HPDG (4.00 ± 0.00). The results were then examined using the Plackett-Luce model to determine the probability a treatment would be chosen first based on the current data set. The probability of PEDG being selected first was observed to be 98.8 ± 1.60% while the probability of CORN, DDGS, and HPDG being selected first were 0.93 ± 0.69, 0.21 ± 0.73, and 0.07 ± 0.86%, respectively ($P < 0.03$). Dairy cows readily consume corn and DDGS. Results of this study demonstrate preference is increased over corn when DDGS are pelleted.

Key Words: dried distillers grains and solubles, corn, feed preference

1491T Enhancing net food production by using "leftover" feeds for high-producing dairy cows. M. Mills*, S. Naughton, J. Liesman, and M. VandeHaar, *Michigan State University, East Lansing, MI.*

The environmental impact of dairy production is a concern for consumers. However, cows can convert human-inedible byproduct foods, or "ecological leftovers," such as wheat straw or bakery waste, into high quality foods for people. Our goal was to demonstrate that feeding carefully-formulated diets high in byproducts will increase net food production for humans with minimal effects on milk production. Multiparous Holstein cows (n = 30; 113 ± 28 DIM, 700 kg BW) were fed a control diet containing 20% byproducts (CON) or a diet containing 70% byproducts (BYP). BYP was 25% corn silage, 8% straw, 15% gluten feed, 15% bakery waste, 12% beet pulp, 8% soyhulls, and 17% supplements. CON was mostly corn silage, alfalfa, and corn grain. Cows were fed a 50:50 mix of both diets for 1 wk, then CON or BYP for 4 wk, mix for 1 wk, and then opposite diet for 4 wk. Data were analyzed using PROC MIXED of SAS 9.4 with treatment and period as fixed effects and cow as random. Cows fed CON consumed 30.8 kg DM, produced 53.9 kg milk with 3.47% fat, 2.94% protein and 4.98% lactose, and gained 0.1 kg BW per day. Cows fed BYP consumed 1.0 kg more feed ($P = 0.02$) and produced 0.8 kg less milk ($P = 0.2$) with 0.18% less fat ($P < 0.01$), 0.04% less protein ($P = 0.02$), and 0.05% less lactose ($P < 0.01$). BW and BCS gain were not altered. Cows fed CON converted 44% of feed energy (using Atwater energy values), 32% of feed protein, and 64% of feed lysine to milk energy, protein, and lysine. These values were similar for cows fed BYP. Corn grain, soybean meal, the grain portion of corn silage, and the leaves of alfalfa were considered human edible. When evaluating efficiency on a human-edible nutrient basis, cows fed BYP were much more efficient than those fed CON (215 vs. 66% for energy, 186 vs. 84% for protein, and 284 vs. 163% for lysine; all $P < 0.01$). Although the BYP diets resulted in a slight decrease in the yield of milk components, dairy cattle can increase the amount of food available for people when they are fed diets high in human-inedible byproducts. A food production system without dairy cattle might emit less methane but also require more land to grow food.

Key Words: byproduct, sustainability, feed efficiency

1492T Effect of a non-polar mycotoxin binder on milk mycotoxin concentration and milk production in commercial dairy farms, preliminary results. J. P. Russi*[1], O. H. Campanella[2], and A. E. Relling[2], *[1]One Idea LLC, Merced, CA, [2]The Ohio State University, Columbus, OH, [3]The Ohio State University, Wooster, OH.*

The objective of this study was to evaluate the effect of a mycotoxin binder made by creating a non-polar molecule using purified lecithin as described in patent # US20220331280A1 (Russi, 2022). Over 50 dairy farms were screened for mycotoxins in milk (aflatoxin M_1) and 6 were selected (190 ± 81 heads per farm) for the study. On those farms, a pre-supplementation bulk sampling was used on 2 consecutive days to measure milk yield, milk composition, and mycotoxin concentration (PRE). Cows were supplemented with 40 g/d of purified lecithin mycotoxin binder for 8 d, with a bulk sampling on d 7 and 8 of the same variables as previously (DURING). After d 8 the supplementation of the mycotoxin binder was stopped, and cows were fed with the same diet as before. Similar bulk samples were taken on 2 consecutive d 7 and 8 d after the treatment was stopped (POST). A total of 6 samples per farm were taken from the tank. Samples were averaged by sampling point, milk yield was averaged based on the bulk yield and number of milking cows. Data were analyzed with a mixed model using the fixed effect of sampling time and the random effect of farm. Repeated measurements were used to consider the lack of independence between the samples from each farm. The use of the mycotoxin binder decreased ($P < 0.01$) mycotoxin concentration in milk after 8 d of use, and the concentration of mycotoxins in milk increased after the product was stopped (Table 1). Milk yield increased ($P = 0.03$) with the use of mycotoxin binder compared with the pre-and post-use of the treatment. The mycotoxin binder did not affect ($P \geq 0.15$) the percentage of fat, protein, or lactose. Therefore, the use of this non-polar mycotoxin binder is an efficient way to decrease milk mycotoxin and increase milk production. More research needs to be conducted on the effect of different doses.

Key Words: binder, lecithin, mycotoxin

1493T Evaluation of a rumen-protected methionine product using appearance of methionine in plasma in response to feeding and abomasal infusion. C. J. R. Jenkins[1], J. D. Stypinski[1], G. M. Fincham*[1], J. Albrecht[2], O. R. Drehmel[2], M. F. Scott[2], C. Soderholm[2], and P. J. Kononoff[1], *[1]University of Nebraska–Lincoln, Lincoln, NE, [2]Milk Specialties Global, Eden Prairie, MN.*

Four lactating, ruminally cannulated Jersey cows, 232 ± 54.2 DIM and 488 ± 2.38 kg of BW, were arranged in a 4 × 4 Latin square design to measure the appearance of plasma methionine (Met) resulting from

Table 1 (Abstr. 1492T). Effect of purified lecithin as a mycotoxin binder on milk aflatoxin M_1 and milk yield and composition

Item	PRE	DURING	POST	SEM	*P*
Mycotoxins, ppb	43.7	21.8	43.1	9.69	<0.01
MY, L in 12 h	16.6	16.97	16.30	1.37	0.03
Fat, %	3.77	3.69	3.74	0.06	0.37
Protein, %	3.45	3.47	3.42	0.03	0.15
Lactose, %	4.83	4.86	4.86	0.03	0.18

feeding a rumen-protected Met (RPM; Milk Specialties Global, Eden Prairie, MN) product compared with abomasal infusion of DL-Met. Additionally, a comparison of RPM delivery method (top-dress vs. bolus dose via the rumen cannula) was made. In each of the 7 d periods, cows were assigned to one of 4 experimental treatments: (1) control; no supplemental Met (CON), (2) 24 g/d of Met delivered as RPM top-dress (RPM-TD), (3) 24 g/d of Met delivered as RPM via the rumen cannula (RPM-RUM), and (4) 24 g/d of Met delivered as DL-Met 99% (INF). Cows received the same basal diet throughout the experiment, which was formulated to be deficient in methionine (−5.0 g Met using the NASEM, 2021 model). Milk samples were collected twice daily on d 5–7 and were analyzed for fat, true protein, lactose, and SNF. Blood samples were collected via venipuncture of the coccygeal vein at 2, 4, 6, and 8 h after feeding on the last 3 d of each period and were analyzed for free AA. Milk protein % increased ($P < 0.01$) in response to RPM and INF treatments and averaged 3.67, 3.82, 3.86, and 3.79 ± 0.116% for cows receiving CON, RPM-TD, RPM-RUM, and INF treatments, respectively. Plasma Met also increased ($P < 0.01$) in response to RPM and INF treatments and averaged 12.7, 22.3, 26.6, and 38.1 ± 1.64 μ*M* for CON, RPM-TD, RPM-RUM, and INF treatments, respectively. The results suggest that the RPM product was effective at delivering Met post-ruminally and warrants further experimentation to determine relative bioavailability and metabolizable Met compared with other commercial RPM sources.

Key Words: rumen-protected methionine, amino acid, abomasal infusion

1494T Effects of level and duration of acidogenic supplementation in the prepartum diet on the productive performance and metabolism of dairy cows. J. T. R. Carvalho[1], J. H. Carneiro[1], J. C. S. Lourenço[1], L. S. Nogueira[1], R. Zimpel[2], V. B. Carvalho[3], A. Vieira-Neto[4], and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*DSM, São Paulo, SP, Brazil,* [3]*Phibro Animal Health Corporation, Campinas, SP, Brazil,* [4]*Merck Animal Health, Colorado Springs, CO.*

The objectives were to evaluate the effects of feeding diets with 2 levels of negative dietary cation-anion differences (DCAD) during the last 4 or 3 weeks of gestation on performance and metabolism in dairy cows. The experiment had a 2 × 2 factorial arrangement design, with 2 levels of negative DCAD: −50 or −150 mEq/kg/DM, and 2 durations of acidogenic supplementation during the prepartum period: 21 d (3W) or 28 d (4W). One hundred seventy-seven Holstein cows from a commercial herd were blocked by parity and expected calving date, and randomly allocated to 1 of 4 treatment: 3W-50 (n = 42), 3W-150 (n = 43), 4W-50 (n = 46), and 4W-150 (n = 46). Data were analyzed using the procedure MIXED of SAS, containing the fixed effects of level, duration, parity, time, and their interactions, and the random effects of block and cow. Urine pH was lower at −150 level (5.83 vs. 6.96 ± 0.07; $P < 0.01$) and, as expected, acidogenic supplementation duration was lower at 3W (25.45 vs. 31.48 ± 0.62 d; $P < 0.01$). Cows tended to lose more weight during the postpartum period when fed for 4W (47.1 vs. 32.7 ± 6.53 kg; $P = 0.07$), and milk fat content tended to be higher for the 4W duration (3.95 vs. 3.75 ± 0.08%; $P = 0.06$). The remaining variables BCS, colostrum yield and Brix, milk composition, and SCC did not differ among treatments. There was also no difference for milk yield for duration (46.76 vs. 46.90 ± 0.84 kg/d; $P = 0.91$) or level (47.18 vs. 46.47 ± 0.84 kg/d; $P = 0.55$). Regarding metabolic variables, a tendency for smaller postpartum glucose concentrations in blood at the 3W (2.57 vs. 2.67 ± 0.04 m*M*; $P = 0.09$) and at the −50 level (2.57 vs. 2.68 ± 0.04 m*M*; $P = 0.05$) was observed. No differences were found for BHB concentrations in blood. Regarding postpartum total calcium, there was also no difference for duration (1.90 vs. 1.92 ± 0.015 m*M*; $P = 0.44$) or level (1.90 vs. 1.92 ± 0.015 m*M*; $P = 0.30$). Productive performance and metabolism were not influenced by manipulation of DCAD levels and durations, at least within the recommended range of 4 to 3 weeks and −50 to −150 mEq/kg DM level of acidogenic supplementation in the prepartum diet.

Key Words: DCAD, transition, calcium

1495T Effect of feeding 25(OH)D_3 with a negative DCAD diet to non-pregnant Holstein dry cows for 84 days on calcium metabolism, vitamin D metabolites, and health. A. O. Oyebade*, E. D. Sharman, M. Garcia, J. D. Chapman, and B. D. Humphrey, *Phibro Animal Health, Teaneck, NJ.*

Feeding 25(OH)D_3 to prefresh cows has been shown to improve calcium (Ca) status and milk yield. The aim was to evaluate the effect of feeding 25(OH)D_3 in combination with a fully acidogenic, high Ca diet for 84 d on Ca metabolism, vitamin D metabolites, and health in Holstein dry cows. Twenty non-pregnant, multiparous dry cows (675 ± 87 kg) were fed a non-anionic prefresh diet for 14-d and blocked by body weight (BW) and serum 25(OH)D_3 to treatment groups: control (CON), placebo (200 g ground corn); treatment (TRT), 3 mg of 25(OH)D_3 (13.61 g of Phibro Hy·D 100 + 186 g ground corn). Treatments were fed 1x/d with 0.5 kg of corn silage before feeding the remainder of diet. Cows were fed treatments for 84 d as part of a negative dietary cation-anion difference (DCAD) diet using Animate nutritional specialty product. Cows were weighed weekly, while urine and blood samples were collected every 14-d. Urine samples were analyzed for pH, Ca, and creatinine and blood samples for 25(OH)D_3, 25(OH)D_2, 24,25(OH)D_3, Ca, phosphorus (P), magnesium (Mg), creatinine, glucose, and nonesterified fatty acids (NEFA). Data were analyzed with the GLIMMIX procedure of SAS, differences are declared at ($P < 0.05$). Rectal temperature, respiration rate, BW gain, and DMI were not different between treatments. Serum P was greater for TRT than CON (5.52 vs. 5.10 mg/dL); however, Ca, Mg, glucose, and NEFA did not differ. No differences were detected for urine Ca and creatinine. Serum 25(OH)D_3 was greater for TRT than CON (207.18 vs. 55.18 ng/mL), with differences being evident from d 14. Serum 25(OH)D_2 were higher in CON vs. TRT (3.86 vs. 2.99 ng/mL), with differences observed from d 56. Serum 24,25(OH)$_2D_3$ was higher in TRT vs. CON (26.75 vs. 0.74 ng/mL). Supplementing 3 mg of 25(OH)D_3 with a fully acidogenic, high Ca diet for 84 d increased serum 25(OH)D_3 above 200 ng/mL and increased serum P. Results demonstrate the combined strategy is safe and effective for improving vitamin D_3 status in dry cows.

Key Words: 25(OH)D_3, prefresh, negative DCAD

1496T Different anionic salt products result in similar feed intake and performance in individually fed pre-partum cows. O. P. Sbaralho, C. V. de Almeida, M. S. R. Serrasqueiro, N. T. S. Grigoletto, C. S. Takiya, G. Poletti, R. G. Chesini, D. J. C. Vieira, N. P. Martins, B. M. Ceron, and F. P. Rennó*, *University of São Paulo, São Paulo, SP, Brazil.*

The supply of acidified diets during the prepartum period can reduce the odds of metabolic syndromes in dairy cows. The objective of this study was to evaluate different dietary anions sources in transition cows on DMI, milk yield, and composition. Twenty-four Holstein cows (614 ± 21.3 kg BW and 2.0 ± 0.9 parity number) were blocked (n = 12) by parity and expected calving date (ECD), and randomly assigned to the following treatments: control (CON), anionic salts (Animate, Phibro Animal Health) fed at 0.35–0.40 kg/d; or Meganion (MEG), anionic salts (Meganion, Barentz) fed at 0.24–0.34 kg/d. Both treatments targeted −15 mEq/100 g diet DM and were provided from 30 d of the ECD. Cows were individually fed during the study. Diets had a forage to concentrate ratio of 75:25 and 48:52 during the pre- and postpartum periods, respectively. Feed offered and refusals were weighed daily to determine DMI. Samples of feed ingredients and refusals were analyzed for DM content from pooled samples (every wk) collected daily. Cows were milked 2 × daily and samples were collected for 3 consecutive d every wk for analyses of solids by mid-infrared method (Lactoscan). Performance data were evaluated until 42 DIM. Data were analyzed using the Mixed procedure of SAS modeling the fixed effects of treatment, time, and their interaction, and the random effect of block. DMI was similar between groups either during the prepartum (12.7 and 12.5 kg/d for CON and MEG, respectively) or during the postpartum period (20.3 and 19.9 kg/d for CON and MEG, respectively). FCM yield was not affected by treatments (33.1 and 33.3 kg/d for CON and MEG, respectively). A tendency for interaction effect ($P \leq 0.09$) between treatment and time was observed for milk fat yield and milk fat concentration but no differences between treatment groups within wk were detected ($P \geq 0.11$) after testing the effects of slice. Yields of protein and lactose were similar between groups. Feed efficiency (FCM ÷ DMI) was similar between groups (1.54 and 1.59 for CON and MEG, respectively). Different anionic salt products resulted in similar performance and DMI during the transition period.

Key Words: anion, DCAD, transition cow

1497T Assessment of the interaction among mycotoxins and organic molecules on the efficiency of mycotoxin binders to adsorb aflatoxin B_1 and deoxynivalenol in vitro.

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The objective of this study was to evaluate the potential interactions among 2 mycotoxins and 2 amino acids (AA) on the absorption efficiency of 3 mycotoxin binders (MTB). The experiment was carried out under in vitro conditions to simulate a post-ruminal digestion model in 2 phases: 1) a gastric digestion simulation with pepsin, malic acid, citric acid, acetic acid and lactic acid at pH 2 for 2 h; followed by 2) an intestinal digestion simulation with bile salts and pancreatin extract at pH 6.5 for 2 h. The experimental design was a factorial 3 × 2 × 2 in duplicate with 2 periods. The main factors were the MTB [bentonite, activated carbon (AC) and yeast cell walls (YCW)]; mycotoxins [aflatoxin (AF) and deoxynivalenol (DON)]; and AA (lysine and methionine). Mycotoxins were incubated with: a) each MTB separately (Study 1); b) each mycotoxin (AF or DON) together with the 2 AA (Study 2 and 3); and c) all substrates together (Study 4). Results were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The average adsorption of AF in Study 1 was high (70%), being highest for bentonite (99%) and lowest for AC (44%). The adsorption of DON (34%) was lower than AF (70%) and similar among MTB. In the presence of AA (Studies 2 and 3), the adsorption of AF (67%) was not different from Study 1, being highest for bentonite (98%) and lowest for AC (29%). The adsorption average of DON (42%) was not different from Study 1, being highest for YCW (67%) and lowest for bentonite and AC (average 30%). When all substrates were incubated together (Study 4), the adsorption average of AF (84%) was similar among MTB. However, the adsorption of AC (70%) increased in comparison to Studies 1 and 2. The adsorption of DON (57%) was lower than AF (84%), being highest for YCW (70%) and lowest for bentonite (61%) and AC (42%). Additionally, contrary to what was observed in Studies 1 and 2, the adsorption of DON by bentonite and AC in Study 4 increased. Results of the in vitro test suggest that there may be a synergetic effect among mycotoxins and AA that could enhance their adsorption.

Key Words: mycotoxin binder, mycotoxin, amino acid

1498T Dietary association of alpha-amylase and proteinate trace minerals on nutrient digestibility and performance of dairy cows.

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Inorganic trace minerals (INO) have been found to impair the activity of exogenous enzymes (ENZ) in monogastric animals. To that end, we wanted to evaluate the effects of α-amylase (AMY) and proteinate trace minerals (PM) on nutrient apparent digestibility and performance. Eight ruminally cannulated Holstein cows (29.5 ± 3.66 kg/d milk yield and 216 ± 30 DIM) were blocked according to parity and were enrolled into a 4 × 4 Latin square (LS) experiment with 21 d-periods. Cows within block were randomly assigned to the following treatments (TRT): CON, diet with 100% INO (Co, Cu, Fe, and Mn as sulfates, Zn oxide, and Na selenite), ENZ: CON treatment plus AMY (Amaize, Alltech) at 0.5 g/kg DM, PM, diet with 100% PM (Co, Cu, Mn, and Zn provided as PM, and Se as Se-yeast; Bioplex and Selplex, Alltech), or PMA: PM treatment plus AMY. Cows were housed in a tie-stall barn and were fed individually. TMR and orts were weighed daily to determine feed intake and samples were collected during the last 7 d of each period for chemical composition using wet chemistry analysis. Total feces collection was performed between d 17 and 20 of each period to determine fecal excretion and chemical composition. Cows were milked 2 × daily, and samples were collected during 3 consecutive d for solids analysis. Data were analyzed by the Mixed procedure of SAS modeling the fixed effects of LS, period, inclusion of PM or AMY, their interaction, and the random effect of cow within LS. The DM and NDF intakes were lower ($P = 0.03$) with PM inclusion. Digestibility of nutrients (DM, NDF, CP) was similar between TRT. No effects of AMY or interaction between AMY and mineral source were detected for nutrient intake or digestibility. FCM yield was similar among TRT (31.4, 32.0, 31.4, and 32.0 kg/d for CON, ENZ, PM, and PMA, respectively). No differences among TRT were observed for milk concentration and yield of fat, protein, or lactose. Feed efficiency (FCM/DMI) was greater ($P = 0.04$) with PM inclusion (1.30, 1.36, 1.38, and 1.40 for CON, ENZ, PM, and PMA, respectively). Feeding PM benefited feed efficiency without altering nutrient digestibility in dairy cows.

Key Words: enzyme, organic mineral

1499T Close-up diet calcium and dietary cation anion difference dynamics with biomarkers, health, milk yield, and reproduction.

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The objective was to identify associations of close-up diet Ca concentration and dietary cation anion difference (DCAD) at the pen-level with the prevalence of elevated biomarkers [nonesterified fatty acids (NEFA; prepartum: ≥ 0.17 mmol/L, postpartum: ≥ 0.59 mmol/L), BHB (≥1.2 mmol/L), and haptoglobin (≥0.45 g/L)], disorder incidence (DI; inci-

dence of 1 or more of displaced abomasum, clinical ketosis, metritis), milk yield at wk 4 of lactation (WK4MY), 305-d mature equivalent milk yield at ~120 DIM (ME305), and pregnancy risk to first service (PRFS). Multiparous (MP) and primiparous (PP) cows (n = 11–24) from 72 farms in the northeastern US were enrolled in a prospective cohort study. Total mixed ration samples were analyzed at a commercial lab (CVAS; Waynesboro, PA) by wet chemistry methods for minerals. All outcomes were calculated by parity and PP and MP cows were analyzed separately. A simple linear regression was conducted on all possible explanatory variables. Variables were included in the full multivariable general linear model if $P < 0.20$ and a manual backward stepwise elimination process ensued until all variables had a $P < 0.10$. For PP, we observed a quadratic association between Ca and WK4MY ($P = 0.07$), such that WK4MY was greatest when Ca = 1.2% of DM. No outcomes were associated with DCAD for PP. For MP, Ca was positively associated with prepartum NEFA ($P = 0.06$) and PRFS ($P = 0.09$), and we observed a quadratic association with BHB ($P = 0.07$; BHB lowest at 1.0% of DM), WK4MY ($P = 0.06$; WK4MY greatest at 1.4% of DM), and ME305 ($P = 0.04$; ME305 greatest at 1.5% of DM). We observed a quadratic association between DCAD and BHB ($P = 0.04$; lowest at 6.0 mEq/100 g of DM) and DI ($P = 0.04$; lowest at 8.1 mEq/100 g of DM). For PP, dietary Ca was only associated with WK4MY (optimum inclusion rate of 1.2% of DM) and DCAD was not associated with any outcomes. For MP, feeding a moderate (~1.4% of DM) Ca diet and neutral or slightly positive DCAD diet may optimize postpartum performance. Caution should be used when interpreting the DCAD results as urine pH was not measured for evaluating diet acidification.

Key Words: calcium, dietary cation anion difference, transition cow

1500T Relationships between particle size and in situ starch disappearance of corn grain.

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The objective of this study was to evaluate the relationships between physical characteristics of dry ground corn and starch disappearance. A data set of dry ground corn samples (n = 101) routinely analyzed for mean particle size (MPS; µm), surface area (cm^2/g), starch concentration (% DM), 0 and 7 h in situ rumen disappearance (starchD; % starch) and rate of disappearance (K_d, % starch) was used. Samples were not ground before in situ assays. Data were evaluated for normality and Cook's distance using PROC REG of SAS (9.4) and 3 samples were removed. Linear and quadratic relationships were determined with PROC GLM of SAS. The overall average for 7 h starchD was 69.6% starch. Starch K_d linearly increased ($P = 0.001$, $R^2 = 0.74$, RMSE = 4.36) 0.06 units for every unit increase in surface area. Likewise, 0 h starchD linearly increased ($P = 0.001$, $R^2 = 0.85$, RMSE = 6.37) 0.12 units for every unit increase in surface area. Quadratic relationships were observed between mean particle size and starch K_d ($P = 0.001$, $R^2 = 0.61$, RMSE = 5.37), 0 h starchD ($P = 0.001$, $R^2 = 0.69$, RMSE = 9.30), and 7 h starchD ($P = 0.001$, $R^2 = 0.71$, RMSE = 7.57). All 3 of these metrics decreased as particle increased, before reaching a plateau from between 500 and 1000 µm and up. Additional quadratic relationships were observed between surface area and 7 h starchD ($P = 0.001$, $R^2 = 0.72$, RMSE = 7.41), and 0 h starchD and 7 h starchD ($P = 0.001$, $R^2 = 0.76$, RMSE = 6.88). The 7 h starchD increased as surface area increased, before beginning to plateau from approximately 400 cm^2/g and up. Similarly, 7 h starchD increased as 0 h starchD increased, with no noticeable plateau reached despite a seemingly lower increase at the upper limit of 0 h starchD. No linear or quadratic relationships were observed ($P > 0.10$) between starch disappearance metrics and starch concentration. Mean particle size and surface area of dry ground corn are highly correlated with in situ rumen starch disappearance.

Key Words: starch digestibility, processing, starch K_d

1501T Live or autolyzed yeast supplementation: Effects on ruminal bacteria populations, fermentation, and urinary N excretion in dairy cows.

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This study evaluated yeast (*Saccharomyces cerevisiae*) on ruminal bacteria populations, fermentation, and N excretion. Eight ruminal cannulated Holstein cows (28.4 ± 3.96 kg/d milk yield and 216 ± 30 DIM), blocked according to DIM and milk yield, were enrolled into a 4 × 4 Latin square (LS) experiment with 21 d-periods. Cows were randomly assigned to treatments (TRT): control (CON); LY, 0.125 g/kg DM of Vistacell (AB Vista); autolyzed yeast (AY) 1 (AY1) 0.625 g/kg of Levabon Rumen E (DSM Nutritional Products); or AY at 0.834 g/kg (AY2). Ruminal digesta samples were collected on the last day of each period every 2 h until 16 h after feeding for analyses of pH, NH_3-N, and VFA. Samples collected 8 h after feeding were used to quantify bacteria populations (*Anaerovibrio lipolytica*, *Butyrivibrio* group, *Fibrobacter succinogenes*, *Butyrivibrio proteoclasticus*, and *Streptococcus bovis*) by DNA extraction, RT-PCR, and 2-ΔΔCT method. VFA (molar %) was determined by gas chromatography. Urine output was estimated based on creatinine excretion rate and uric acid was analyzed using a commercial kit. Data were analyzed using the proc MIXED of SAS and compared by orthogonal contrasts. Ruminal pH was similar among groups. Ruminal NH_3-N was lower ($P = 0.04$) in cows fed yeast products than CON (13.9, 12.3, 10.9, and 10.7 mg/dL for CON, LY, AY1, AY2, respectively). Molar percentages of acetate, propionate, butyrate, isobutyrate, or isovalerate were not affected by treatments. Valerate (molar %) tended to be lower ($P = 0.07$) in cows fed yeast products. No differences in ruminal fermentation were detected between treatments. Urinary N excretion (g/d) was greater ($P = 0.03$) in cows fed LY in comparison with AY (145 and 112 g/d, respectively). Uric acid excretion was greater ($P < 0.01$) in cows fed yeast products than CON (18.9, 23.9, 22.2, and 19.9 mmol/d for CON, LY, AY1, and AY2, respectively). Relative quantification of bacteria populations did not differ among treatments. Feeding yeast can decrease ruminal NH_3-N concentration and can increase uric acid excretion.

Key Words: additive, *Saccharomyces cerevisiae*

1502T Effects of indigestible neutral detergent fiber dietary content on performance and feed sorting of dairy cows.

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The objective of this study was to evaluate increasing dietary levels of indigestible neutral detergent fiber (iNDF) on dry matter intake (DMI), sorting index, milk yield (MY), and milk composition. Twenty-four Holstein cows (129 ± 52.5 d in milk and 37.6 ± 3.91 kg/d MY) were blocked according to parity, MY, and DIM and were used in a 4 × 4 Latin square (LS) design experiment with 21-d periods. Cows within block were assigned to different dietary levels of iNDF (IND1 = 10.24%; IND2 = 9.57%; IND3 = 8.91%; or IND4 = 8.25% of DM). The dietary iNDF level was manipulated with different inclusions of corn silage made from corn plants harvested at 2 heights from the ground level [40 cm (SIL40) or 65 cm (SIL65)]. Cows in IND1 received 100% of SIL40, IND2 received 66.6% of SIL40 and 33.3% of SIL65, IND3 received 33.3% of SIL40 and 66.6% of SIL65, and IND4 received 100% of SIL65. All diets had a forage to concentrate ratio of 48:52 of DM, whereas the corn silage was the sole forage source. The level of iNDF was measured by incubating feed samples in the rumen of 2 cows for 288 h. TMR and orts were recorded daily. Milk samples were collected for 3 consecutive d of each period and analyzed for solids using mid-infrared method. Samples of TMR and orts were collected during 2 d of each period and analyzed for particle size distribution. Data were analyzed using the MIXED procedure of SAS modeling the fixed effects of treatments, period, LS, and their interaction. The difference between treatments was evaluated by multiple polynomial regression. DMI (kg/d) linearly increased ($P = 0.02$) with lower levels of iNDF (27.03, 27.18, 27.63, and 27.64 for IND1, IND2, IND3, and IND4, respectively). The same occurred for DMI (% BW, $P < 0.01$). Sorting for feed particles between 19 and 8 mm quadratically increased ($P = 0.03$) and sorting for feed particles between 8 and 4 mm quadratically decreased ($P < 0.01$) according to iNDF levels. MY linearly increased ($P < 0.01$) with lower levels of iNDF (35.6, 36.7, 36.9, and 37.0 kg/d for IND1, IND2, IND3, and IND4, respectively). Protein and lactose yields increased linearly ($P = 0.02$ and 0.01, respectively) with lower iNDF in the diet. Milk composition did not differ between treatments ($P > 0.05$). In conclusion, lower levels of iNDF in the diet of dairy cows increased MY and DMI.

Key Words: *in situ* degradability, lignin, uNDF

1503T Supplementation of isoacids on feeding behaviors and enteric methane emissions of lactating cows fed diets at varying forage fiber levels.

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Our objective was to examine the effects of isoacids (ISO; IsoFerm, Zinpro Inc.) supplementation on feeding behaviors and enteric methane (CH_4) emissions of lactating cows under 2 forage neutral detergent fiber (NDF) levels (FL). In a randomized complete block design trial (10-wk), 64 Holsteins (662 ± 71 kg body weight, 119 ± 51d in milk) were blocked by parity, d in milk, and prior milk yield (MY) or genetic merit, and randomly assigned to 1 of 4 diets (n = 16) with a 2 × 2 factorial arrangement, 2 FL comprising 18 (LF) and 23% forage NDF (HF), without or with ISO supplementation (40 g/d per cow). All diets had 16.5% crude protein, 28% NDF, and 1.72 Mcal/kg dry matter (DM) net energy for lactation. Particle size, rumination time (RT), and CH_4 emissions were measured during the last 2-wk using Penn State Particle Separator, CowManager tag, and GreenFeed unit, respectively. Data were analyzed using a model including FL, ISO, and FL × ISO as fixed effects and block as a random effect (lme4 in R). Compared with LF, HF cows consumed more medium (19–8 mm) and short (1.18–7.9 mm) particles but less long (>19 mm) and fine (<1.18 mm) particles, resulting in greater overall consumption of physical effective particles (66 vs. 62%; $P < 0.001$). In contrast, ISO increased the consumption of long particles but decreased short and fine particles in HF cows ($P < 0.001$) but not in LF cows. Additionally, HF cows had greater RT ($P = 0.009$) and chewing ($P = 0.006$) with lower resting time ($P < 0.001$) than LF cows. Moreover, ISO decreased RT (371 vs. 525 min/d), intake adjusted RT (min/kg DM or NDF), and chewing time (526 vs. 644 min/d) under LF diet only ($P < 0.001$), whereas adjusted RT did not depend on FL. As expected, HF cows had greater CH_4 production (g/d), yield (g/kg DM or NDF intake), and intensity (g/kg MY or fat-protein corrected milk) than LF cows. In contrast, ISO decreased CH_4 intensity (10.7 vs. 9.7 g/kg MY; $P = 0.003$) and tended to reduce CH_4 production (332 vs. 361 g/d; $P = 0.074$) irrespective of FL. Overall, ISO altered feeding behaviors that depended on FL but reduced enteric CH_4 emissions regardless of FL.

Key Words: particle size, rumination

1504T Resveratrol as a nutritional intervention to ameliorate heat stress effects on lactating dairy cattle.

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Resveratrol, a natural plant-based phenol compound improves cellular functions by preventing oxidative stress and inflammation. A study was conducted at the University of Tennessee dairy farm from June to July of 2022, to establish whether resveratrol can reduce the effects of natural heat stress on production and increase welfare of lactating cows housed in a freestall barn. Forty-eight Holstein cows (14 primiparous and 34 multiparous) were stratified by days in milking (DIM, 126 ± 33 d), milk yield (49.50 ± 8.86 kg/d), parity (2.29 ± 1.0) and fed TMR ad libitum once daily. The TMR consisted of corn silage, grass hay, grounded fine corn, soybean hulls, whole cottonseed and a premix of minerals and vitamins. Cows were randomly allocated to control diet (CON, TMR with no resveratrol, n = 24) and resveratrol diet (RES, TMR top-dressed with resveratrol at 500 mg/cow/day (Thrive Animal Health, LLC), n = 24). Both groups were fed their respective diets for 14 d before the initiation of the study. Following dietary adjustments, the available sprinklers and fans in the barn were disabled during the 21-d treatment period to expose cows to the prevailing summer conditions of temperature and humidity. Milking occurred twice daily, and samples were collected to determine milk solids. Rectal temperature and respiration rates were recorded thrice daily. Data were analyzed using the mixed procedure with repeated measures in SAS and significance was set at $P \leq 0.05$. Fixed effects included treatment, day, treatment by day interaction and cow was considered random. Cows were exposed to an average THI index of 75.15 ± 4.16. Only main effects of treatment were detected. Milk yield and contents of lactose and protein increased in the RES group when compared with the CON ($P < 0.05$). However, fat content and somatic cell count were not affected by treatments. The morning and afternoon respiration rates of RES group were higher ($P < 0.05$) than the CON group. Additionally, the afternoon rectal temperature of RES group was lower ($P < 0.05$) than the CON group. In summary, resveratrol may ameliorate heat stress effects in lactating dairy cows.

Key Words: dairy cattle, heat stress, resveratrol

1505T Metabolomic study to understand changes in serum and milk metabolic in Jersey cows under heat stress.

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Heat stress (HS)-exposed dairy cows concern for the dairy industry economic losses and suffer frequently from metabolic diseases induced by abnormal levels of certain metabolites. Metabolomic studies using

proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy have been performed to investigate the inherent metabolic pattern under HS by analyzing various ruminant biofluids. However, since the population of Jersey cows is smaller than that for Holstein cows, research on metabolomic aspects for Jersey cows has not been actively conducted. The objective of this study was to understand the changes in serum and milk metabolic in Jersey cows under HS. Nine Jersey cows were used in this study. Serum and milk samples were collected under different temperature humidity index [optimum temperature period (OTP): 55.26 ± 4.28 and high temperature period (HTP): 75.64 ± 1.43]. The metabolites were determined by ^{1}H-NMR spectroscopy, identified and quantified using Chenomx NMR Suite 8.4 software, and the obtained data were statistically analyzed by MetaboAnalyst 5.0. In the serum; formate, glucose, creatine and lactose were significantly ($P < 0.05$) higher in the OTP than in the HTP. In contrast, threonine, acetone, methanol and leucine were significantly ($P < 0.05$) higher in the HTP than in the OTP. In addition, glycine, serine and threonine; and glyoxylate and dicarboxylate metabolism were significance ($P < 0.05$) differed and had an impact value higher than 0.1. In the milk; glycine, ethylene glycol, lactose and cis-aconitate were significantly ($P < 0.05$) higher in the OTP than in the HTP. In contrast, trehalose, glucuronate, alanine and acetone were significantly ($P < 0.05$) higher in the HTP than in the OTP. In addition, 11 metabolic pathways were identified significance ($P < 0.05$) differed and had an impact value higher than 0.1, including D-glutamine and D-glutamate; and glycine, serine and threonine metabolism. In the future, these results would help suggest new strategies to ameliorate the effects of HS including disease susceptibility and metabolic disorders, in Jersey cows.

Key Words: heat stress, Jersey cow, metabolomic

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion 2

1506T Effects of dietary supplementation of a probiotic-yeast compound blend on rumen fluid metabolome of lactating dairy cows. G. Taiwo[1], M. Idowu[1], S. Taylor*[1], D. P. Compart[2], M. Ballou[3], and I. Ogunade[1], *[1]West Virginia University, Morgantown, WV, [2]Texas Tech University, Lubbock, TX, [3]Papillon Agricultural Company, Easton, MD.*

We evaluated the effects of dietary supplementation of a probiotic-yeast compound blend (Papillon Agricultural Company, Easton, MD) on lactating dairy cows' ruminal metabolome. A total of 26 Holstein lactating dairy cows (60 ± 15 DIM) were blocked by DIM and previous lactation performance and then randomly assigned to 1 of 2 dietary treatments for 56 d. The 2 dietary treatments were 1. A corn silage-based TMR (CON), 2. A CON diet + a probiotic-yeast compound blend (PROB). The additive was top-dressed onto the TMR upon feed delivery. Rumen fluid samples were collected on d 28 and 56 using a commercially available oro-ruminal sampling device. The rumen fluid samples were composited for each cow and were processed for untargeted metabolome analysis. A total number of 905 metabolites were detected and identified. The partial least squares discriminant analysis plot showed a separation between the 2 groups of dairy cows using the first 2 principal components with 16.1 and 12.2% of explained variance, indicating that the supplemental additive altered the rumen metabolome of the dairy cows. A total of 84 differentially abundant (FDR $\leq$0.05) metabolites were detected between CON and PROB. Compared with CON, ruminal concentrations of 46 metabolites, including several amino acids and peptides such as alanine, valine, adenine, homoserine, valyl-hydroxyproline, and tyrosyl-serine were greater (FDR $\leq$0.05) in dairy cows fed supplemental PROB whereas 38 metabolites, including caproic acid, chorismic acid, and picolinic acid were lower (FDR $\leq$0.05). To determine the rumen metabolic pathways that were enriched by the additive supplementation, we performed a pathway enrichment analysis using KEGG. Results showed that dietary supplementation of the additive enriched ($P \leq 0.05$) pathways related to pantothenate and CoA biosynthesis, pyrimidine metabolism, propanoate metabolism, and β-alanine metabolism. The results of this study demonstrated that dietary supplementation of a blend of a probiotic-yeast compound altered the ruminal metabolome which may have a significant effect on the performance of dairy cows.

Key Words: dairy cow, feed additive, rumen fermentation

1507T Effects of dietary supplement consisting of ginger, lemon balm, cinnamaldehyde, inactivated yeasts and clays on microbial rumen function. M. Romero-Huelva[1], E. Ramos-Morales[1], P. Romero[1], H. Khelil-Arfa[2], R. Breitsma[2], A. Blanchard[2], D. Yanez-Ruiz[1], J. W. Hickman[3], and G. Acetoze*[3], *[1]CSIC, Granada, Spain, [2]ADM International Sàrl, Rolle, Switzerland, [3]ADM Animal Nutrition Technology Center, Decatur, IL.*

Current dairy production systems require a well-balanced rumen function to optimize the use of dietary nutrients and to avoid metabolic disturbances in the host. Plant and yeast based nutritional additives have been used as strategy to achieve this goal, however, scarce data are available on their effect on rumen microbial function. This study assessed the effect of a blend consisting of ginger, lemon balm, cinnamaldehyde, inactivated yeasts and clays (Anco FIT; AF) on rumen fermentation pattern and microbial ecosystem using an in vitro continuous culture system. Fermenters were fed a diet consisting of 60% forage (corn silage + grass silage) and 40% concentrate (barley, corn, sunflower, urea). Treatments were, the diet alone (control) or supplemented (0.125 g/d) with AF, AF + double the amount of cinnamaldehyde (AFC) or AF + triple the amount of ginger (AFG). The trial consisted of 2 incubation periods (2 replicates/period) of 18 d each, with the first 5 d for adaptation of the rumen microorganisms to the in vitro conditions before the administration of the treatments. On d 15 and 16 of the incubation, samples of the fermenters were collected for the determination of volatile fatty acids (VFA), ammonia, lactate, N fractions (NH_3, peptidic and aminoacidic), the activity of carboxymethylcelullase, xylanase and amylase and the abundance of bacteria, protozoa, archaea and fungi. The effect of applying the different treatment was analyzed using one-way ANOVA and Period as blocking factor. The supplementation with AF, AFC and AFG promoted a greater fermentation activity as seen in lower pH values (6.12 vs. 6.25 6 h after morning feeding, $P < 0.001$) and higher ($P < 0.001$) total VFA concentrations (+14%) than the control. The supplementation of AF, AFC and AFG, numerically enhanced enzymatic activities, which may be associated to the increased concentration of total bacteria ($P < 0.05$). Protozoa and archaea abundances increased with the supplementation with AF ($P < 0.05$). The concentration of NH_3-N was 33 and 31% greater with the AF and AFG treatments ($P < 0.05$), respectively. Overall, the dietary supplementation with AF, AFC and AFG improved rumen function.

Key Words: rumen function, phytogenics, inactivated yeast

1508T Effects of energy-nitrogen synchronization on *in vitro* rumen fermentation of a rapidly degradable substrate. J. M. Arroyo[1], J. González[1], F. Diaz*[2], and M. D. Carro[1], *[1]Departamento de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Madrid, España, [2]Dellait Research Center, Brookings, SD.*

Synchronizing the release of energy and N available for ruminal microorganisms can influence rumen fermentation and microbial protein synthesis, but results have been inconsistent. The objective of this study was to analyze the effects of energy-N synchronization on the in vitro fermentation of a rapidly degradable substrate. Samples (350 mg) of a substrate (61:14:25 wheat:corn:sugarbeet pulp; 9.51% CP; DM basis) were weighed in glass vials and mixed with 35 mL of buffered sheep rumen fluid, and incubated at 39°C for 12 h. Purified soybean protein (3.64 mg N/vial) was added either at the start of the incubation (nonsynchronized; NSYN) or distributed in 3 equal doses at 0, 4 and 8 h of incubation (synchronized; SYN). Samples were obtained at 4, 8 and 12 h, and analyzed for CH_4, volatile fatty acids (VFA) and NH_3-N. The amount of fermentable organic matter (FOM) was estimated according to Demeyer (1991). Data were analyzed as repeated measures ANOVA (PROC MIXED, SAS)with treatment, time and its interaction as fixed effects and inoculum as random effect in the model. No treatment × time interactions were detected ($P > 0.10$) for any parameter but sampling time affected ($P < 0.05$) all of them except butyrate proportion. SYN treatment resulted in greater ($P < 0.01$) gas (71.5 vs. 76.6 mL/g DM; averaged values at 4, 8 and 12 h of incubation) and total VFA (2.99 vs. 3.14 mmol/g DM) production and FOM (31.3 vs. 32.9%), but lower ($P < 0.05$) NH_3-N concentration (43.2 vs. 33.2 mg/L) and CH_4 proportion in the gas produced (17.5 vs. 11.4%), without changes in individual VFA proportions, acetate/propionate rate (2.18 vs. 2.21) or CH_4/VFA ratio (3.72 vs. 3.49 mL/mmol). The reduced NH_3-N concentration of SYN could indicate either lower protein degradation or greater NH_3-N uptake by rumen microorganisms. Favoring ruminal synchronization of energy and N produced beneficial effects on rumen fermentation.

Key Words: energy-nitrogen synchronization, in vitro, rumen fermentation

1509T Effects of replacing alfalfa haylage with high-protein duckweed on ruminal fermentation in vitro. N. Stepanchenko*, D. E. Wasson, S. Welchez, L. F. Martins, R. A. Brennan, and A. N. Hristov, *The Pennsylvania State University, State College, PA.*

Duckweed (DW) is an aqueous plant with nutrient absorption properties. When grown on agricultural wastewater, it has high protein content (35–43%), making it possible to be included as a protein source in dairy cow diets. The objective of the current study was to evaluate the effect of different replacement rates of alfalfa haylage with high-protein DW (grown in dairy wastewater with various concentrations of nitrogen: 20 [DW1], 30 [DW2], 40 [DW3], and 50 [DW4] mg N/L) on ruminal fermentation in vitro. Two incubations were carried out for 24 h at 39°C using an automatic gas production system. Two cannulated Holstein cows fed a typical mid-lactation diet were used as donors of rumen inoculum. Incubations included DW at 0 (alfalfa haylage included at 1%, wt/vol, in the incubation medium), 20, 40, 60, 80, and 100% (substrate dry matter basis) replacement rates of alfalfa haylage. Total gas production was recorded through the incubation, gas samples were analyzed for methane, and medium samples were analyzed for volatile fatty acids (VFA), and ammonia concentrations. Data were analyzed using PROC MIXED of SAS with replacement rate in the model. Polynomial contrasts were used to test linear, quadratic, and cubic effects of DW inclusion. Final pH and total gas production (mL/g substrate) were linearly decreased ($P < 0.001$) by DW. The concentration of total VFA, as well as that of acetate, propionate, and butyrate, were linearly decreased ($P < 0.001$) by DW, indicating decreasing fermentation vigor with increasing DW inclusion. Methane production (as % of total gas) was not affected ($P \geq 0.32$) by DW1 and DW2 but decreased ($P < 0.001$) linearly with increasing the inclusion rate of DW3 and DW4. Ammonia concentration was not affected ($P \geq 0.45$) by DW3 and DW4 but tended to linearly decrease ($P = 0.08$) for DW1. Overall, in the conditions of the current study, higher levels of DW inclusion decreased rumen fermentation vigor. Methane production was inhibited up to 32% by DW grown in wastewater with higher N concentration; the mechanism of this inhibition is unknown and warrants further investigation.

Key Words: duckweed, ruminal fermentation, methane

1510T Effects of a *Bacillus*-based direct-fed microbial on in vitro gas production and fiber digestibility of commercial dairy TMR. K. A. Bryan*[1], P. Fantinati[2], J. C. Bodin[2], J. N. Joergensen[2], G. Copani[2], D. Vyas[3], M. Malekkhahi[3], S. Farooq[3], L. O. Lima[3], C. Nino-de-Guzman[3], B. Po[3], K. Arriola[3], and B. I. Cappellozza[2], *[1]Chr. Hansen Inc., Milwaukee, WI, [2]Chr. Hansen A/S, Hørsholm, Denmark, [3]University of Florida, Gainesville, FL.*

Besides its effects on health, direct-fed microbials (DFM) may benefit nutrient digestibility. More specifically, *Bacillus* spp. are important enzyme-producing bacteria, regarding the amount and the range of enzymes of interest produced. Based on this rationale, we hypothesized that incubation of a *Bacillus*-based DFM would improve nutrient digestibility of commercial dairy total mixed rations (TMR). Therefore, our objective was to evaluate the effects of a *Bacillus*-based DFM on in vitro nutrient digestibility of commercial dairy TMR. Nine commercial dairy TMR were sampled from different locations in US and used in the present study. The TMRs were incubated or not (CON) with a *Bacillus*-based DFM (DFM; Bovacillus; 2.2×10^9 cfu/gram; Chr. Hansen Inc., Milwaukee, WI) over a 48-h in vitro period. The dose of DFM was adjusted to the volume of the flasks being used and to represent the commercial dose of 4 g/head per day (8.8×10^9 cfu/head). In vitro gas production, dry matter (DM), and neutral detergent fiber (NDF) digestibility were evaluated at 24 and 48 h, whereas starch digestibility was evaluated at 7 h post-incubation. Data were analyzed using the PROC MIXED of SAS (v. 9.4; SAS Institute Inc., Cary, NC). Treatment was included into fixed effects and flask as the random variable. Hour was not used as a repeated statement, as each hour time points were ran independently. Significance was set at $P \leq 0.05$. Mean in vitro gas production increased by 2.9% when DFM was incubated with TMR for 48-h ($P = 0.05$), whereas no differences were observed at 24 h gas production ($P = 0.53$). Dry matter and NDF digestibility were greater for DFM-treated TMR vs. CON, with improvements that were in the order of 1.8 and 2.7% for DM at 24 and 48 h, and 11.8 and 12.0% for NDF digestibility at 24 and 48 h, respectively ($P \leq 0.03$). Lastly, no treatment effects ($P = 0.31$) were observed on mean in vitro starch digestibility (62.1 vs. 62.9% for CON and DFM, respectively; SEM = 0.49). In conclusion, we observed greater in vitro fiber degradability and gas production of TMR collected from different locations of United States over a 48-h period when a *Bacillus*-based DFM was added at dose representative of 8.8×10^9 cfu per head. Greater improvements in fiber degradability are likely due to enzymes produced by *Bacillus*-based DFM.

Key Words: *Bacillus* spp., fiber digestibility, TMR

1511T In vitro fermentation and methane generation of a dairy diet including cardoon seeds (*Cynara cardunculus*). M. Riahi[1], J. González[1], M. D. Carro[1], F. Díaz*[2], and J. M. Arroyo[1], *[1]Departamento de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Madrid, España, [2]Dellait Research Center, Brookings, SD.*

Cardoon (*Cynara cardunculus*) exhibits excellent adaptation to the Mediterranean region with high seed production characterized by high concentrations of oil, protein and fiber. However, studies assessing cardoon seeds inclusion in ruminant diets are scarce. An in vitro trial was conducted to evaluate the inclusion of cardoon seeds in dairy ruminant diets. Two dairy diets including either cardoon seeds (CS) or soybeans (SS) were formulated. Both seeds were included to achieve 5.5% fat content in both diets (12.5 and 15.8% for cardoon seeds and soybeans, respectively). Diets were iso-proteic (17.1%; DM basis) and contained 36.1 and 32.6% NDF for CS and SS, respectively. Samples (350 mg) of each diet were weighed in glass vials, mixed with 35 mL of a 4:1 mixture of Goering and Van Soest's medium (1970) and sheep rumen fluid, and incubated at 39°C. After 17 h of incubation, gas production was measured and samples were obtained to analyze CH_4, volatile fatty acids (VFA) and NH_3-N concentrations. The amount of fermentable organic matter (FOM) was estimated from VFA production. Data were analyzed as a mixed model with diet as fixed effect and ruminal inoculum as random effect. The CS diet showed lower ($P < 0.05$) gas production (212 vs. 223 mL/g DM) and greater ($P < 0.01$) NH_3-N concentration (243 vs. 223 mg/L) compared with SS. The greater concentration of NH_3-N for CS would indicate a high rumen degradability of cardoon seeds protein. No differences were observed between diets in total VFA (5.99 vs. 6.20 mmol/g DM, for SS and SC, respectively) and CH_4 production (50.9 vs. 48.5 mL/g DM), CH_4/VFA ratio (8.16 vs. 8.21 mL/mmol), molar proportions of individual VFA, acetate/propionate ratio (4.48 vs. 4.55), and FOM (54.0 vs. 55.5%). The highly lignified fiber of cardoon seeds did not cause a reduction in the FOM of the CS diet. Results show the potential of cardoon seeds for its inclusion in dairy ruminant diets, however in vivo studies to confirm these results are necessary.

Key Words: in vitro, cardoon seed, rumen fermentation

1512T Effects of lactate-utilizing microorganisms on *in vitro* rumen fermentation characteristics, methane production, and microbial abundance.

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This study aimed to determine the effects of supplementing lactate-utilizing bacteria (LUB) which were previously isolated from the rumen fluid of Holstein dairy cows (*Ligilactobacillus agilis* RNAL840106, LA; *Corynebacterium vitaeruminis* SCR, CV; and *Cereibacter* sp. RNAL840107, CS), on in vitro rumen fermentation characteristics, methane (CH_4), carbon dioxide (CO_2) production, and rumen microbial community. The treatments used were CON: basal diet, LA: basal diet + 2% *L. agilis*, CV: basal diet + 2% *C. vitaeruminis*, and CS: basal diet + 2% *Cereibacter* sp. The data were analyzed by ANOVA using the general linear model (GLM) procedure of SAS and mean separation was done with Duncan's multiple range test. All bacterial strains were grown in MRS broth and were supplemented at 2% at an OD value of 2.0. Results showed that supplementation of lactate-utilizing bacterial strains significantly decreased the CH_4 yield ($P < 0.05$) after 6, 12 and 24 h of incubation, while total gas production was not significantly different. Total volatile fatty acid and molar proportion of propionate significantly increased ($P < 0.05$) with LA and CV after 12 h of incubation, whereas CS decreased. Metataxonomic analysis revealed that *Euryarchaeota* (62.5–68.3%) and *Candidatus Thermoplasmatota* (2.4–2.8%), and *Bacteroidetes* (23.8–24.6%) and *Firmicutes* (45.7–47.8%) were the most abundant archaeal and bacterial phyla in all treatment groups, respectively. At the archaeal genus level, *Methanobrevibacter* (86.8–87.6%) was the most dominant, followed by *Methanosphaera* (7.3–8.1%), *Methanomassiliicoccus* (3.6–4.0%) and *Methanobacterium* (1.0–1.2%). At the bacterial genus level *Prevotella* (11.1–12.8%) was the most dominant, followed by *Novisyntrophococcus* (5.7–7.7%), *Butyrivibrio* (4.1–4.6%), *Treponema* (3.5–4.7%), and *Intestinimonas* (3.7–4.0%). In addition, the recovered relative abundances of supplemented LUB strains, *L. agilis* and *C. vitaeruminis*, in the in vitro rumen fermentation system were 2.1% and 0.87%, respectively; however, *Cereibacter* sp. was not detected. Overall, the supplementation of lactate-utilizing bacterial strains can decrease CH_4 production and alter the rumen microbial community.

Key Words: methane production, ruminant, microbial abundance

1513T Effects of a *Bacillus*-based direct fed microbial on inflammation and gastrointestinal tract permeability during feed restriction in mid-lactation Holstein cows.

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Objectives were to evaluate the effects of a *Bacillus*-based direct-fed microbial (DFM; Strateris ECL; United Animal Health, Sheridan, IN) on gastrointestinal tract (GIT) permeability and inflammatory biomarkers during feed restriction (FR) in Holstein cows. Thirty-six cows were randomly assigned to 1 of 3 dietary treatments (TRT): 1) control (CON; 7.5 g/d rice hulls; n = 12), 2) DFM10 (10 g/d *Bacillus* [*B. subtilis* and *B. pumilus* blend] DFM, 4.9×10^9 cfu/d; n = 12) or 3) DFM15 (15 g/d *Bacillus* DFM, 7.4×10^9 cfu/d; n = 12). Before study initiation, cows were fed their respective TRT for 32 d. Cows continued to receive TRT during the trial consisting of 3 experimental periods (P): P1 (5 d) served as baseline for P2 (5 d), during which all cows were restricted to 40% of P1 feed intake, and P3 (5 d), a "recovery" where cows were fed ad libitum. In vivo GIT permeability was evaluated on d 4 of P1 and d2 and 5 of P2 using oral chromium (Cr)-EDTA. Plasma [Cr] was measured for 24 h following infusion and area under the curve (AUC) was calculated. Effects of TRT, time, TRT × time, replicate, and parity were assessed using PROC MIXED of SAS. Plasma Cr AUC increased in all cows on d 2 (9%) and 5 of P2 (6%) relative to P1 ($P = 0.03$). Circulating lipopolysaccharide binding protein (LBP), serum amyloid A (SAA), and haptoglobin (Hp) increased in all cows during P2 (38%, 1.5-, and 16.5-fold, respectively; $P < 0.01$). Further, P2 inflammatory markers either tended to be quadratically increased (LBP; $P \leq 0.07$) or were increased (SAA and Hp; 2.1- and 4.8-fold, respectively; $P \leq 0.05$) in DFM15 relative to CON and DFM10. During P3, LBP and Hp tended to be increased in DFM15 relative to DFM10 ($P = 0.07$). Regardless of treatment during P2, IFN-γ, IL-8, IL-10, IL-36, IFN-γ inducible protein-10, macrophage chemo-attractant protein, macrophage inflammatory protein-1β, and vascular endothelial growth factor increased from d 3 to 5 (2.3-fold, 76, 32, 19, 66, 44, 37, and 54%, respectively; $P \leq 0.01$). In summary, FR increased inflammatory biomarkers and GIT permeability and feeding DFM15 appeared to alter key variables of immune activation.

Key Words: probiotic, Cr-EDTA

1514T Effects of a probiotic supplement on gastrointestinal permeability and biomarkers of inflammation during feed restriction in a ruminant model.

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Objectives were to evaluate the effects of a probiotic on gastrointestinal tract (GIT) permeability and biomarkers of inflammation during feed restriction (FR) in a ruminant model. Thirty-two Holstein heifers (295 ± 25 kg body weight [BW]) were randomly assigned to 1 of 2 top-dressed dietary treatments: 1) control (CON; 10 g/d dried lactose; n = 16) or 2) probiotic (PRO; 10 g/d *L. animalis*, *P. freudenreichii*, *B. licheniformis*, and *B. subtilis* [11.8×10^9 cfu/head/day]; n = 16). The trial consisted of 2 experimental periods (P) during which heifers received their respective dietary treatments. Period 1 (14 d) served as baseline for P2 (5 d), during which all heifers were feed restricted to 40% of P1 feed intake. GIT permeability was evaluated on d 12 of P1 and d 2 and 5 of P2 using the paracellular permeability marker chromium (Cr)-EDTA. Effects of treatment, time, treatment × time, and replicate were assessed using PROC MIXED in SAS. By design, FR decreased DMI (60%) and BW (19 kg) in all heifers ($P < 0.01$). During P2 and relative to P1, all heifers had decreased glucose, BHB, insulin, and L-lactate (4, 14, 45, and 19%, respectively; $P < 0.01$), but increased NEFA, serum amyloid A, haptoglobin (Hp), IFN-γ inducible protein (IP)-10, and macrophage chemo-attractant protein (MCP-1; 3.0-, 1.7-, 5.0-fold, 24%, and 24%, respectively; $P \leq 0.02$). On d 5 of P2, PRO-fed heifers tended to have increased lipopolysaccharide binding protein and Hp relative to CON (60% and 7.5-fold, respectively; $P \leq 0.08$). During P2, IFN-γ was

unaltered in PRO-fed heifers but tended to increase with time in CON (2.2-fold; $P = 0.10$). Further, IL-36 concentrations tended to decrease in CON (15%) but increase in PRO heifers (18%) from d 3 to 5 of P2 ($P = 0.10$). Plasma Cr 24 h AUC increased in all heifers on d 2 and 5 of P2 relative to P1 (10 and 14%, respectively; $P < 0.01$), but this was unaltered by dietary treatment. In summary, FR compromised GIT barrier function and stimulated an inflammatory response, but this did not appear to be ameliorated by PRO within the constraints of the present study.

Key Words: direct fed microbial, gut health

1515T ***Bifidobacterium pseudocatenulatum* alleviates calf diarrhea.** M. Y. Hu, W. J. Du, W. J. Si, Y. Gao, Y. F. Du, J. X. Hou, L. H. Yang, X. H. Wang, L. Xu, and Q. B. Xu*, *Huazhong Agricultural University, Wuhan, China.*

The objective of this study was to find the key microbiota of calf diarrhea resistance and their mechanisms of action. Fifteen 4 d-old Holstein calves were randomly assigned to 2 groups. The fecal microbiota transplantation (FMT) group was transplanted with fecal flora from healthy calves at a concentration of 10^8 cfu/mL and the control group was fed PBS buffer. After the fecal microbiota of healthy calves was transplanted into the diarrhea calves, the fecal morphology, score and blood biochemical indexes of diarrhea calves were alleviated to a certain extent. At the same time, the fecal microbiota of diarrhea calves was changed significantly, and the Shannon ($P = 0.049$), Ace ($P = 0.016$) and Chao1 ($P = 0.016$) indexes were significantly increased, indicating that the species richness was significantly increased. There were significant differences in species groups at the level of phylum, genus and species, and the abundance of *Escherichia coli* was significantly decreased ($P = 0.015$). LDA effect size analysis showed that *Metlanobrevibacter wolinii*, *Clostridium* sp., *Bifidobacterium catenulatum*, *Bifidobacterium pseudocatenulatum*, *Veillonella magna*, *Lactobacillus salivarius*, *Bifidobacterium asteroides*, *Prevorella copri* and *Clostridium ventriculi* could be used as biomarkers after FMT. After correlation analysis, it was found that there was a significant negative correlation between *Bifidobacterium pseudocatenulatum* and diarrhea index ($P = 0.002$). At the same time, short chain fatty acids (acetic acid ($P = 0.009$), propionic acid ($P = 0.006$), butyric acid ($P = 0.019$) and valeric acid ($P = 0.009$)), and the metabolites of microorganisms were significantly increased. The concentration of acetic acid was significantly increased by analyzing the culture supernatant of *Bifidobacterium pseudocatenulatum*. Further research found that *Bifidobacterium pseudocatenulatum* and sodium acetate could significantly alleviate diarrhea and intestinal inflammation in mice. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software). Comparisons between the 2 groups were made using the Mann–Whitney U test (2-tailed). Correlations were assessed by Pearson correlation test using "cortest" in R. The above studies showed that *Bifidobacterium pseudocatenulatum* could alleviate calf diarrhea.

Key Words: calf diarrhea, *Bifidobacterium pseudocatenulatum*, fecal microbiota transplantation

1516T Effects of dietary energy level and ensiling on ruminal fermentation characteristics and greenhouse gas emissions of total mixed ratio. J. Y. Kim*[1], Y. H. Joo[1], S. M. Jeong[1], M. J. Seo[1], C. H. Baeg[1], B. G. Choi[2], S. S. Lee[3], P. N. Seong[3], and S. C. Kim[1], [1]*Division of Applied Life Science (BK21 Four, Institute of Agricultural and Life Science), Gyeongsang National University, Jinju, Gyoeongsangnam-do, South Korea,* [2]*Department of Animal Science, Gyeongsnag National University, Jinju, Gyoeongsangnam-do, South Korea,* [3]*Animal Nutrition and Physiology Division, National Institute of Animal Science, Wanju, Jeollabuk-do, South Korea.*

This study evaluated the dietary ensiling effects on rumen fermentation characteristics and greenhouse gas emissions of total mixed ratio (TMR) with different forage-to-concentrate ratio. The experiment conducted in a 2 × 2 factorial design consists of the dietary energy (Low vs. High) and ensiling process (TMR vs. fermented TMR [FTMR]) with 4 replications. Two different TMRs formulated to meet the requirements of low- or high-energy diets for Hanwoo cows. The half from 2 TMR diets ensiled for 21 d at room temperature (20°C). Rumen fluid was collected from 2 cannulated Hanwoo cows before morning feed and mixed with Van Soest medium at a 1:2 ratio. The diets (0.3 g) were placed into the incubation bottle with the rumen fluid mixture (30 mL) in quadruplicates and incubated for 72 h. After incubations, total gas emission was measured and subsampled for CO_2 and CH_4 emissions. The bottle contents were centrifuged to collect the supernatant for pH, ammonia-N, and volatile fatty acid (VFA), and the residue for in vitro digestibility of dry matter (IVDMD) and neutral detergent fiber (IVNDFD). All data were analyzed using PROC MIXED of SAS, and mean separation was performed using Tukey's test at $P < 0.05$. The FTMR had slightly higher rumen pH ($P = 0.002$; 6.76 vs. 6.80%), propionate ($P < 0.001$; 20.3 vs. 21.2%), and butyrate ($P < 0.001$; 10.3 vs. 11.3%) than the TMR diet, while had lower rumen acetate ($P < 0.001$; 64.4 vs. 64.0%). The ensiling process did not affect to IVDMD and IVNDFD of the TMR diets, but those in the High diet were higher ($P < 0.01$) than the Low diet. The FTMR diet had lower total gas ($P < 0.001$; 40.3 vs. 36.4 mL), CO_2 ($P = 0.021$; 3.58 vs. 1.59 mL/g IVDMD), and CH_4 ($P = 0.011$; 5.89 vs. 4.81 mL/g IVDMD) emissions than the TMR diet. Therefore, this study could conclude that the ensiling of TMR had beneficial effects on nutrient digestibility and greenhouse gas emissions in the rumen.

Key Words: fermentation, total mixed ration, greenhouse gas emission

1517T Relationship between ruminal microorganisms and its impact on forage fermentation parameters. D. C. Rosler*[1], G. V. Kozloski[2], M. P. Mezzomo[2], and C. A. Pozo[3], [1]*University of Florida, Gainesville, FL,* [2]*Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil,* [3]*National University of Formosa, Formosa, Formosa, Argentina.*

The aim of this study was to evaluate the interactive role of bacteria and fungi on forage degradation in vitro. Dried and ground (1 mm screen) samples of ryegrass (*Lolium multiflorum* Lam.) were weighed (1.5 g) in triplicate in 160 mL flasks and incubated in vitro during 48 h in medium (50 mL buffer + 50 mL rumen inoculum) containing or not antimicrobial substances. A mixture of penicillin, chloramphenicol, and streptomycin (500 mg/L of each) was used as antibiotic, and cycloheximide (50 mg/L) was used as antifungal. In vitro fermentations were conducted anaerobically in a water-bath slow-stir system at 39°C. Treatments were: antibiotic (Ab), antifungal (Af), negative control (Nc, without antimicrobials), or positive control (Pc, with both Ab and Af). Three replicate assays were conducted and, in each assay, gas volume was measured at 3, 6, 9, 12, 24, 36, and 48 h of incubation. Data of cumulative gas production in each flask in each assay was fitted to a one-pool logistic model which generated 2 kinetic parameters: total gas production (mL/g DM) and rate of gas production (%/h). Statistical analysis was carried out using a general linear model and treatment means were compared through the Student *t*-test. All variables were affected by treatments ($P < 0.05$). The total gas production and the rate of gas production in Nc treatment were 129 mL/g DM and 4.1%/h, respectively. Compared with the Nc treatment, Ab decreased by 47% the total gas production ($P < 0.05$) without affecting the rate of gas production. On the other hand, Af treatment presented similar values of total gas production and rate of gas production to Nc. When both Ab and Af were included in the incubation medium (Pc), total gas production was considerably lower ($P < 0.05$) than Ab treatment (25 vs. 129 mL/g DM) and the rate of gas production was lower ($P < 0.05$) than Nc and Af (3.4 vs. 4.2 and 4.3%/h, respectively). In conclusion, bacteria had the main role in the degradation of ryegrass forage, with low participation of the fungi population in this process.

Key Words: attachment, fiber, microbiota

1518T ***In vitro* ruminal fermentation of dairy diets including almond hulls.** A. Recalde[1], M. D. Carro[1], R. Jiménez[1], S. Calero[1], B. Barrero-Domínguez[2], A. García-Sanchez[3], and T. de Evan*[1], *[1]Departamento de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Madrid, Spain, [2]Departamento I+D+i Dcoop S.C.A., Málaga, Spain, [3]Grupo de Prado, Córdoba, Spain.*

Almond hulls (AH) are frequently used in dairy ruminant feeding, but information on the effects of this by-product on ruminal fermentation is scarce. The aim of this study was to assess the in vitro ruminal fermentation of diets for dairy ruminants including increasing amounts of AH. The AH sample contained (as feed basis) 7.70% ash, 7.37% crude protein (CP), 23.7% total sugars, and 14.3% crude fiber. The experimental treatments were 4 diets having similar CP and fiber content: a control diet without AH, and 3 diets including 8 (AH8), 16 (AH16) and 24% (AH24) of AH (fresh matter basis). All diets contained 40% of alfalfa hay, and the AH partially substituted corn, wheat bran and sugar beet pulp. Diets were fermented in vitro with buffered ruminal fluid from 4 sheep for 120 h to measure gas production kinetics, and for 24 h to measure fermentation parameters (pH, volatile fatty acids [VFA] and NH_3-N). Data were analyzed as a mixed model (Proc Mixed of SAS) in which diet was a fixed effect and ruminal inoculum was a random effect. Linear and quadratic effects of including increased amounts of AH were tested by polynomial contrasts. Increasing AH level in the diet decreased ($P \leq 0.01$; linear and quadratic) the time at which gas production started (lag time), with no effects on other gas production parameters. This indicates a more rapid start of fermentation of AH-diets, possibly due to sugars content in AH. There were no differences between control and AH8 diets in any fermentation parameter at 24 h incubation, but increasing the AH level reduced ($P \leq 0.05$; quadratic) total VFA production and NH_3-N concentrations and increased final pH ($P \leq 0.01$; linear and quadratic), all which suggest a reduction of diet fermentability. The use of AH did not affect VFA profile, excepting that butyrate proportions decreased ($P = 0.05$; quadratic) as the dietary level of AH augmented. These results indicate that AH could partially substitute conventional ingredients in dairy ruminant diets without negatively affecting their rumen fermentation. High inclusion levels of AH can reduce the ruminal degradability of the diet, but in the practice, this would depend on the nutritive quality of the AH used.

Key Words: almond hull, ruminal fermentation, *in vitro* gas production

1519T The effects of a fat-protein matrix supplement on ammonia concentration, gas production, pH, and dry matter degradation in ruminal batch and continuous cultures. J. Vinyard, M. Johnson*, A. C. Silva Vicente, M. Siregar, G. Salas Solis, E. Sarmikasoglou, C. Hammond, K. Alves, S. W. Ma, L. Katz, R. Lobo, S. Castillo, and A. Faciola, *University of Florida, Gainesville, FL.*

Omega-3 fatty acids (FA) have been demonstrated to be anticarcinogenic and prevent cardiovascular disease, increasing the interest in increasing these FA in dairy products by including them in the diets of cows. The objective of this study was to determine the impacts of feeding a rumen protected matrix of fat and protein from flaxseed and peas (FPM; approximately 21% fat, 24% CP, and 5.4% omega-3 FA; % of DM) in vitro. Treatments for experiment 1 (E1) and experiment 2 (E2) were: 0 (0P), 2.5 (2.5P), 5 (5P), and 7.5% of DM (7.5P) inclusion of FPM. Three runs with 4 replicates per treatment were used for E1. 0.5 g of each TMR (16% CP, 33% NDF, 28% starch, 4.2% EE; as % of DM) was incubated in buffered ruminal fluid for 24 and 48 h in glass serum bottles. Gas pressure was measured at 0, 3, 6, 9, 12, 24, and 48 h to estimate total gas volume and DM digestibility, pH, and ammonia-N (NH_3-N) concentration were measured at 24 and 48 h after bottles were opened. The same treatments were arranged in a replicated 4 × 4 Latin square using 8 dual-flow continuous culture fermenters for E2. Four 10-d experimental periods (7 d adaptation and 3 d sample collection) were used and 53 g of DM of TMR were fed twice daily. Samples were collected from fermenter effluents at 3, 6, 9, and 24 h after morning feeding and composited to determine NH_3-N concentration. Fermenter pH was measured at 0, 1, 2, 4, 6, and 8 h after morning feeding. Data for E1 and E2 was analyzed using PROC GLIMMIX of SAS and data using time points was analyzed using repeated measures. In E1, treatment was fixed and run was random. In E2, square, fermenter within square, period, and the fermenter by treatment interaction were random and day and hour were considered fixed. In E1, inclusion of FPM increased mean pH and total gas volume at 5P and 7.5P, but there was no impact of FPM inclusion on NH_3-N concentration or DM degradability. In E2, mean and maximum pH were greater for 0P compared with 2.5P but were similar for 5P and 7.5P. There was no impact of treatment on minimum pH nor a treatment by time interaction in E1 or E2. However, further analysis is needed to fully determine the impact of FPM inclusion.

Key Words: flaxseed, fermentation, omega-3

1520T Effects of black cumin oil and acetate on methane production in non-lactating Jersey cows. S. C. Sherwood*, A. L. Carroll, S. C. Fernando, and P. J. Kononoff, *University of Nebraska–Lincoln, Lincoln, NE.*

Methane production in ruminants can be reduced through dietary manipulation. The objective of this experiment was to determine the effects of feeding supplement containing black cumin (*Nigella sativa*) oil and acetate (BCA) on methane production in Jersey cows. It was hypothesized that the antimicrobial activity of the thymoquinone in black cumin oil would lead to changes in the microbial community and substrate availability for methanogenesis leading to decreased methane production. Acetate was used as a solute to increase solubility of essential oils in black seed oil to increase absorption. The study employed a 2-period crossover design and used 12 multiparous nonlactating Jersey cattle averaging 568 ± 20.5 kg and 4.6 ± 0.17 body condition score. Each 28 d period consisted of 24 d of adaptation followed by 4 d of collection. Data were analyzed with a GLIMMIX procedure in SAS (9.4) with fixed effect of treatment and random effect of cow and period. Diets for both treatment and control groups consisted of 53.0% forage, 45.2% concentrate, 1.9% of either corn oil or BCA with a diet DM of 53.6%. The BCA mixture was created with 4 parts black cumin oil and one part acetate. Acetate was mixed with the black cumin oil to increase the solubility of the oil. Compared with the control, cows consuming BCA consumed similar amounts of DM ($P = 0.22$) averaging 9.8 ± 1.03 kg/d. No difference ($P = 0.51$) was observed in methane production and averaged 287.3 ± 29.48 L/d across treatments. Similarly, methane production per kg of DM consumed did not differ ($P = 0.69$) averaging 29.3 ± 2.17 L/kg across treatments. Similarly, O_2 consumption did not differ ($P = 0.24$) between treatments 3,639 ± 253.1 L/d nor did CO_2 production ($P = 0.42$) 3,834 ± 294.4 L/d. No difference ($P = 0.64$) was observed in respiratory quotient (RQ) and averaged 1.05 ± 0.013 L/L. Results of this study suggests that feeding black cumin oil along with acetate does not decrease enteric methane production in cattle compared with those fed corn oil.

Key Words: methane, black cumin oil, acetate

1521T Effects of red seaweed (*Gracilaria* sp.) on *in vitro* rumen fermentation and methane production. B. Chae[1], S.-J. Kim[2], S. Cho[1], H.-R. Park[3], I. Cheon[1], and N.-J. Choi*[1], [1]*Department of Animal Science, Jeonbuk National University, Jeonju, Korea,* [2]*BOSA Co., Ltd., Seongnam, Korea,* [3]*Korea Agriculture Technology Promotion Agency, Iksan, Korea.*

The present study was conducted to evaluate the effects of additional levels of red seaweed (RS) *Gracilaria* sp. on rumen fermentation and methane production. The 5 treatment groups used in this in vitro experiment consisted of a basal diet (control) with the addition of different levels of RS (0.25, 0.50, 1.00, and 2.00% based on feed DM). The Basal diet was 35% non-structural carbohydrates with 16% crude protein, the ratio of forage and concentrate being 4:6. The rumen fluid was collected from a slaughterhouse. Then, the 5 diets were anaerobically incubated in vitro using strained rumen fluid and McDougall's artificial saliva buffer (1:4) for 6, 12, and 24 h. To assess the fermentation parameters, ruminal pH, total gas production, methane emission, ammonia nitrogen, and volatile fatty acid were determined. To test the differences in rumen fermentation parameters among additional levels of RS in diets, one-way ANOVA was applied using the statistical analysis system R software (R Core Team, 2022). The results suggested that methane emission was significantly decreased at all levels of RS at 6 and 12 h incubation ($P < 0.05$). At 24 h incubation, the basal diet plus 0.25 and 0.50% of RS decreased methane emission by 13.90 and 14.08%, respectively ($P < 0.05$). Also, the ruminal pH was slightly lower at all levels of RS, but all the values were in the normal range (5.8~7.2) for rumen function. Except for the treatment containing 2.00% RS at 24 h, ammonia nitrogen concentrations were significantly decreased with every RS inclusion level at all incubation times ($P < 0.05$). At 24 h incubation, although total volatile fatty acids were not significantly affected by RS addition, treatments with 0.25 and 0.50% of RS decreased the proportion of acetate and increased the proportion of propionate compared with the control ($P < 0.05$). These findings showed that an appropriate addition of RS (0.25 to 0.50%) to the basal diet could inhibit methane emission and protein degradation without negative effects on rumen fermentation, which may improve nutrient utilization and animal growth.

Key Words: seaweed, methane, rumen fermentation

Ruminant Nutrition: Protein and Amino Acids 2

1522T Lactation persistency and environmental advantage of a low-protein diet balanced for amino acids compared to a high-protein diet fed to dairy cows. J. Guyader*[1], I. Kok[2], M. Schilde[2], K. S. Edelmann[3], and C. Parys[1], [1]*Evonik Operations GmbH, Hanau, Germany,* [2]*Schothorst Feed Research, Lelystad, the Netherlands,* [3]*Evonik Operations GmbH, Isernhagen, Germany.*

This work aimed at evaluating the effect of reducing the crude protein (CP) level of a dairy ration while supplementing a rumen-protected methionine [Mepron, 85% DL-methionine (Met), Evonik Operations GmbH] on performance and nitrogen (N) excretion of lactating cows. This study was completed at Schothorst Feed Research, The Netherlands. Forty Holstein cows (58 ± 4 DIM; 39.6 kg ± 8.1 kg/d milk; mean ± SD) were used in a randomized block design experiment with a 1-wk covariate and 10-wk experimental period. Cows were fed either a high-CP diet (HP; 17.3% CP in DM, 1.94% of Met in metabolizable protein) or a low-CP diet supplemented with 0.05% Mepron to ensure that both diets were not limiting in Met supply (LP; 16.0% CP in DM, 2.25% of Met in metabolizable protein). Both diets were offered as a TMR and included the same proportion of corn (35.2%) and grass silage (23.8%) in diet DM. Lower dietary CP was achieved by reducing the proportion of both canola and soybean meals, which was compensated with additional cereal grains. Individual DM intake and milk yield were recorded daily. Milk composition was analyzed weekly. Nitrogen excretion was determined during wk 10 using acid-insoluble ash as an internal marker in the feed and feces. Creatinine was used to estimate total volume of the urine. Data were analyzed in R with a model containing the fixed effects of covariate (milk parameters only), treatment, week, and treatment by week interaction (milk parameters only), and the random effect of cow. On average, DM intake, milk yield and milk composition were similar between treatments, but a significant treatment by week interaction was found for milk and milk protein yield. Indeed, between wk 1 and wk 10, milk yield reduced by 4.8 kg with HP, but only by 1.5 kg with LP ($P = 0.021$). Similarly, milk protein yield decreased by 57 g with HP whereas it increased by 61 g with LP ($P = 0.035$). Total manure N decreased by 12% with LP ($P = 0.036$) and milk N efficiency tended to increase with LP (30.0 vs. 33.3%; $P = 0.108$). In summary, reducing dietary CP while supplementing Mepron improved the persistency of milk and milk protein production, and decreased N excretion into the environment.

Key Words: rumen-protected methionine, low-protein diet, nitrogen excretion

1523T Effects of level and oscillation of dietary crude protein on ruminal conditions. M. G. Erickson*[1], G. I. Zanton[2], and M. A. Wattiaux[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI.*

Our trial examined dietary crude protein (CP) feeding pattern (O = oscillating, S = static) at 2 levels of dietary CP (LP, 13.8%; HP, 15.5%) to test for hypothesized effects on ruminal conditions. Using a 2 × 2 factorial arrangement, we assigned 8 mid- to late-lactation Holsteins (M = 135, SD = 9 DIM) to treatment sequences in a Latin Rectangle with four 28-d periods. O alternated 2 diets (O-LP 12.2–15.5%, O-HP 13.8–17.3% CP) every 48 h so average dietary CP equaled that of the corresponding S treatments (S-LP, S-HP). Dietary differences were implemented by exchanging soybean hulls, ground corn, and expeller soybean meal with solvent soybean meal. On d 25–28 of each period, we collected fluid from the ventral rumen *per cannula* at −2, 0, 2, 4, and 11 h relative to 1x daily feeding. We measured pH with a portable meter and used colorimetric methods to quantify NH_3-N and total amino acids (TAA) on a flow injection analyzer. Data were analyzed using a linear mixed model with fixed effects for CP level, CP feeding pattern, their interaction, period, day, hour, and all treatment by time interactions, and a nested random effects structure. During d 16–27 of the fourth period, we monitored ruminal pH and redox potential (Eh) at 1-min. intervals with indwelling loggers and tested for differences in the daily minimum, mean, and maximum associated with diet changes in the oscillating condition (n = 4 cows). Ruminal pH (6.37 ± 0.03) and concentration of TAA (2.65 ± 0.11 m*M*) were unaffected by treatments, but NH_3-N was greater for HP than LP (2.32 vs. 1.84 ± 0.12 mg/dL NH_3-N, $P < 0.001$). For all variables, non-significant feeding pattern by day interactions indicated that rumen conditions were unaffected by dietary changes in the O condition ($P = 0.326$ to 0.726). This finding was consistent with indwelling logger measurements, which showed similar daily pH and redox potential across higher- and lower-CP phases within the oscillating CP feeding pattern ($P = 0.117$ to 0.833). In conclusion, large variation in dietary N concentration had little effect on ruminal pH and NH_3-N and TAA concentrations.

Key Words: rumen, oscillating, protein

1524T A ^{15}N isotope technique to estimate lysine bioavailability of rumen-protected lysine. K. L. Clark* and C. Lee, *Department of Animal Sciences, The Ohio State University, Wooster, OH.*

Rumen protected Lys (RP-Lys) containing ^{15}N-Lys was used to measure rumen bypass (RB), intestinal digestibility (ID), and bioavailability (BA) of RP-Lys prototypes. This method used ^{15}N-Lys in RP-Lys as a tracer to measure milk secretion and urine excretion of ^{15}N that is originally from RP-Lys to estimate BA. In a 4 × 4 Latin square design, 4 ruminally cannulated cows were randomly assigned to 4 treatments: negative control (NC), positive control (PC), RP-Lys prototype 1 (PT1), and RP-Lys prototype 2 (PT2). In 12-d periods, cows were fed a common diet with 7 d of washout and received one of the 4 treatments which was pulse-dosed into the rumen or abomasum on d 8. The PC and NC treatments were a pulse dose of L-Lys into the abomasum and rumen, respectively. Those treatments were necessary to determine RB and ID of RP-Lys because ^{15}N from RP-Lys can enter the blood circulation through both rumen and intestinal absorption. Total collection of feces, urine, and milk occurred from d 7 to d 12, and ^{15}N secretion or excretion were quantified. The MIXED procedure of SAS was used for all data analysis (random effect: cow; fixed effect: period and treatment). The PC treatment had the greatest ($P < 0.01$) ^{15}N secretion (% of ^{15}N dosed) in milk compared with other treatments. As a proportion of ^{15}N dosed, urinary excretion was greatest ($P < 0.01$) for NC (29%) followed by PT1 and PT2 (21%), and then PC (9%). ^{15}N secretion in milk or ^{15}N excretion in urine (as proportion of ^{15}N dosed) for PC and NC were used to develop a regression assuming PC is 100% rumen undigestible and NC is 100% rumen digestible. The regression was used to estimate RB and ID of PT1 and PT2. However, milk ^{15}N secretion was not appropriate for the regression because results were about 200% of rumen bypass for PT1 and PT2. When urinary ^{15}N excretion (% of ^{15}N dosed) was used, PT1 and PT2 resulted in 62.5 and 69.0% of RB (SEM = 2.0; $P = 0.02$), 69.3 and 71.1% of ID (SEM = 1.7; $P = 0.44$), and 43.4 and 49.4% of BA

(SEM = 2.5; $P = 0.09$), respectively. Therefore, urinary ^{15}N excretion was appropriate to identify Lys absorbed from the rumen or abomasum.

Key Words: intestinal digestibility, rumen bypass, stable isotope

1525T Utilization of nitrogen fractions derived from ^{15}N-labelled faba bean in *in vitro* batch-culture. Þ. H. Sigurðardóttir*[1], S. E. Räisänen[1,2], A. Halmemies-Beauchet-Filleau[1], O. Pitkänen[1], A. Honkanen[1], T. Kokkonen[1], F. L. Stoddard[1], A. Simojoki[1], E. Sahlstedt[3], K. Rinne-Garmston[3], and A. Vanhatalo[1], *[1]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland, [2]Department of Environmental Systems Science, Institute of Agricultural Sciences, Zurich, Switzerland, [3]Natural Resources Institute Finland (Luke), Helsinki, Finland.*

The aim of this study was to evaluate the utilization of N fractions derived from faba bean (FB) by rumen microbes in vitro. Faba bean was grown using ^{15}N-enriched N-fertilizer. The ^{15}N-labeled FB was fractionated into water-insoluble (IN), water-soluble (SN), non-protein (NPN) and NDF- (NDN) nitrogen fractions. Whole ^{15}N-labeled FB was used as a control treatment. The 4 fractions and FB were incubated for 10 h in an automatic Gas Endeavor in vitro system and the incubations were replicated 3 times. Purified mono-, di- and polysaccharides were used as an energy source. The ratio of buffer to rumen fluid, obtained from fistulated cows, was 2:1 and the concentration of ^{15}N was 5.6 mg/L for all fractions except NDN, being 2.8 mg/L. The inoculum was sampled at 10 h, and pH, VFA and NH_3-N concentrations were analyzed. Subsequently, the inoculum was separated into low- (Solid-N) and high-speed (Bact-N) centrifugate and NH_3-N pools, and ^{15}N enrichment of each N pool was measured. The recovery-% of each pool was calculated as the ratio of total ^{15}N recovered from each N pool. Statistical analysis was performed using PROC MIXED in SAS, with treatment as fixed and incubation as random effect. The average pH varied ($P < 0.01$) from 5.42 in NDN to 6.84 in NPN. The NH_3-N concentration was greatest ($P < 0.01$) for FB (14.7 m*M*) followed by SN (13.9), NPN (9.2), IN (3.6), and NDN (0.6). Total VFA production varied ($P < 0.01$) from 54.4 in NPN to 136.6 m*M* in NDN. Solid-N ^{15}N recovery was greatest ($P < 0.01$) for NDN (99.5%), followed by IN, NPN and SN (95, 94 and 93.3%, respectively), and was lowest FB (82.5%). Bact-N ^{15}N recovery was greatest ($P < 0.01$) for FB (16.3%), followed by SN, NPN and IN (6.2, 5.6 and 4.9%, respectively) and NDN had the lowest recovery (0.5%). ^{15}N recovery for NH_3-N pool was greatest ($P < 0.01$) for FB (1.2%), followed by NPN (0.4%), SN (0.2%), IN (0.06%), and was negligible for NDN. As expected, ruminal metabolism of N differed between the N-fractions of FB being greatest for the soluble true N and non-protein N and lowest for the fiber bound N. Further investigation of the rate of N utilization by rumen microbes is needed.

Key Words: N-fraction, ^{15}N-labelling, faba bean

1526T Effects of rumen-protected methionine on the plasma amino acid profile of F_1 Holstein × Gyr cows grazing intensively managed Mombaça grass. V. A. Oliveira*[1], J. P. A. Rezende[1], T. L. Pento[1], F. Lopes[2], S. I. Arriola-Apelo[3], and M. A. C. Danes[1], *[1]University of Lavras, Lavras, MG, Brazil, [2]Adisseo Latam, São Paulo, SP, Brazil, [3]University of Wisconsin, Madison, WI.*

The objective of this study was to evaluate the effect of rumen-protected Met (RPM; Smartamine M; Adisseo Inc., France) supplementation on plasma amino acid (AA) profile of lactating grazing cows. Twenty-four multiparous F_1 Holstein × Gyr cows (31.0 kg/d, 100 DIM) were used in a randomized block design for 9 weeks to evaluate the supplementation of a control diet with RPM to adjust Lys:Met ratio from 3.1 to 2.8 (CNCPS). The supplementation of RPM varied from 10 to 15 g/d, depending on the production of the cow. Cows grazed as a group and were allotted to a new paddock of Mombaça grass every day using sward height as the target entry criteria. Cows were milked twice daily, and individually fed a concentrate mixture 3 times a day, after each milking and at 11:00 a.m. Blood samples were collected from a coccygeal vessel into heparinized test tubes at 11:00 a.m., before concentrate was fed, on the last day of wk 3, 6 and 9. The samples centrifuged and plasma was stored at −20°C until analysis. Plasma AA concentration was analyzed by isotopic dilution, liquid chromatography, and electrospray ionization mass spectrometry after derivatization. The model included treatment, week and week x treatment interaction as fixed effects, in addition to block as random effect. Least squares means were compared using Tukey's test. The supplementation of RPM did not increase plasma Met concentration (35.7 vs. 31.4 μmol/L, $P = 0.22$). Plasma concentration of essential AA tended to increase for the RPM treatment (878.4 vs. 811.4 μmol/L, $P = 0.06$), while concentration of non-essential AA tended to decrease for RPM (1,229.0 vs. 1,307.2 μmol/L, $P = 0.08$). More specifically, RPM reduced plasma concentration of Glu (46.6 vs. 50.9 μmol/L, $P = 0.05$), Gly (308.3 vs. 350.0 μmol/L, $P = 0.03$) and tended to decrease plasma concentration of Gln (194.8 vs. 215.0 μmol/L, $P = 0.07$). Milk protein yield was higher (894 vs. 835 g/d, $P = 0.01$) for RPM. These results suggested that Met was a deficient AA in the control diet and that and increase in plasma Met is not necessary for a positive production effect of RPM supplementation.

Key Words: protein, tropical pasture

1527T Feeding rumen-protected methionine and calcium salts enriched in omega-3 fatty acids increase fatty acid and methionine intakes, increase plasma methionine concentrations, and alter milk fatty acid profiles in periparturient dairy cows. T. L. France*, K. Juarez-Leon, A. Javaid, N. D. Seneviratne, A. F. Ortega, and J. W. McFadden, *Cornell University, Ithaca, NY.*

The objective was to investigate the effects of feeding rumen-protected (RP)-Met and calcium salts (CS) of fatty acids (FA) enriched without or with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; i.e., n3FA) in periparturient cows. In a randomized complete block design study, 75 multiparous Holstein cows were assigned to 1 of 4 treatments (n = 18–19/diet): 1) Met deficient (−Met) with CS not enriched in n3FA (−n3FA; 0% n3FA; EnerGII; Virtus Nutrition, USA), 2) Met adequate (+Met; Smartamine M; Adisseo Inc., France) with −n3FA, 3) −Met with CS enriched in n3FA (+n3FA; 4% n3FA; EnerG-3; Virtus Nutrition), or 4) +Met with +n3FA from wk −3 to +4 wk relative to parturition. Cows were fed corn silage-based total mixed rations pre- and postpartum, formulated to provide Met at ≤0.96 or ≥1.13 g/Mcal ME for −Met and +Met, respectively. Calcium salts were fed at 1.5% ration dry matter pre- and postpartum. Milk was collected twice weekly. Blood was collected on wk 1 and 3 of lactation. Monthly feed was analyzed for AA concentrations. Fatty acid and AA intakes were calculated by multiplying DMI by FA or AA concentrations. Pre- and postpartum data were analyzed separately using PROC MIXED of SASv9.4. Preplanned contrasts included: 1) effect of Met (−Met vs. +Met), 2) effect of n3FA (−n3FA vs. +n3FA), and 3) effect of co-supplementation (+Met/+n3FA vs. +Met/−n3FA and −Met/+n3FA). Postpartum DMI was greater in +Met, relative to −Met ($P = 0.01$) and prepartum DMI was unaffected by treatment. Pre- and postpartum Met intakes were greater in +Met/+n3FA, relative to +Met/−n3FA and −Met/+n3FA (25.6 vs. 20.6 g/d pre-; 62.4 vs. 48.6 g/d postpartum; $P < 0.01$). Pre- and postpartum EPA and DHA intakes were greater in +Met/+n3FA, relative to +Met/−n3FA and −Met/−n3FA ($P <$

0.01). Milk EPA and DHA content was greater in +Met/+n3FA, relative to +Met/−n3FA and −Met/+n3FA ($P \le 0.01$). Milk EPA and DHA yields were greater in +Met/+n3FA, relative to +Met/−n3FA and −Met/+n3FA (1.45 vs. 1.17 g/d EPA; 0.67 vs. 0.32 g/d DHA; $P < 0.01$). Plasma Met was greater in +Met/+n3FA, relative to +Met/−n3FA and −Met/+n3FA (28.0 vs. 22.4 μM; $P < 0.01$). In conclusion, feeding RP-Met and CS enriched in n3FA increased Met and n3FA intakes, milk n3FA content and yield, and plasma Met levels.

Key Words: methionine, omega-3, transition period

1528T Using the plasma AA dose-response method to validate metabolizable methionine of a rumen-protected methionine product.

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Metabolizable Met is essential when optimizing dairy cow diets limited in Met. The plasma free-AA dose-response technique has been established to determine Met bioavailability within ± 2 to 4% points. Our objective was to measure the relative Met bioavailability and metabolizable Met of a RP Met product (RPM; Milk Specialties Global) (80% Met). Ten multiparous Holstein cows (190 ± 50 DIM) fitted with ruminal cannulas were used in a replicated 5 × 5 Latin square with 7-d periods. Treatments were (g/d): 1) 0 (CON), 2) 12 Met abomasally infused (12INF), 3) 24 Met abomasally infused (24INF) 4) 12 Met from RPM (12RPM), and 5) 24 Met from RPM (24RPM). The RPM prototype was fed in 3 daily aliquots at 0500, 1300, and 2100 h with the infusion being continuously into the abomasum via the ruminal cannulas. Intake, milk yield and components were collected the last 3 d of each period. Blood was collected from the tail vein the last 3 d of each period at 2, 4, 6 and 8 h after the morning feeding (0500 h). Data was analyzed using the PROC MIXED to generate means. For the REG procedure total sulfur AA (TSAA) % of (total AA-TSAA) was regressed against 0, 12, and 24 g of infused or fed Met to generate the linear regression variables. Significance was declared at $P \le 0.05$. Average milk yield was 41.2 (±0.67) kg/d and DMI was 27.9 (±0.91) kg/d. For all treatments milk protein, plasma Met, cystathionine, and TSAA concentrations were different from CON. The other sulfur AA varied in their responses (Table 1). The estimated bioavailability of the Met prototype was 73.8% CI of +2.0 (0.04557/0.06176). The metabolizable Met from the RPM was calculated to be 590 g/kg [(80 × 73.8)/10].

Key Words: bioavailability, methionine

1529T Effects of 2-hydroxy-4-(methylthio)butanoate supplementation on productive performance of periparturient dairy cows.

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The aim of this study was to evaluate the effects of 2-hydroxy-4-(methylthio)butanoate (HMTBa) supplementation during the transition period on the productive performance of dairy cows in a commercial herd in Paraná State, Southern Brazil. A total of 141 Holstein animals (59 heifers and 82 cows) were randomly distributed in a 2 × 2 factorial arrangement. The treatments were 0 (control) or 35 g of HMTBa supplementation (Novus International Inc.) in prepartum (21 d) or postpartum (21 d) periods. Cows were blocked considering the expected calving date, parity, and 305-d milk yield in the previous lactation. Treatments were provided individually and top-dressed once a day mixed with corn meal. An equivalent amount of corn meal was fed to the control animals. Milk samples were collected twice weekly during the first 30 d of lactation. The data were analyzed as a mixed model, considering the fixed effects of HMTBa, period of supplementation and their interaction, and the random effects of block and cow. In general, no interactions were detected ($P > 0.05$) between HMTBa supplementation in the pre and in the postpartum periods. Milk yield was not affected by treatments ($P = 0.35$). The cows that received HMTBa supplementation in postpartum increased milk fat content (4.01 vs. 3.89%, $P = 0.02$) and showed higher MUN (13.2 vs. 12.3 mg/dL; $P < 0.01$). The HMTBa supplementation in the prepartum increased lactose content (4.76 vs. 4.71%, $P = 0.01$), but when HMTBa was supplemented in the postpartum, lactose content decreased (4.70 vs. 4.77%, $P = 0.01$). Treatments did not affect milk protein content ($P = 0.83$). Similarly, HMTBa supplementation during prepartum or postpartum did not affect milk casein and somatic cell linear score. Our results suggest no benefits to the productive performance of dairy cows when HMTBa is supplemented during prepartum. On the other hand, HMTBa supplementation during postpartum increases milk fat content. No interactions were detected between HMTBa supplementation in the pre and in the postpartum periods.

Key Words: methionine analog, milk fat, transition period

1530T Effects of replacing canola meal with extruded soybean meals on lactational performance and enteric gas emissions in dairy cows.

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Mechanically extracting oil through an extrusion process increases rumen-undegraded protein content of oilseed meals. This study investigated the effects of an equal crude protein (CP)-base replacement of canola meal (CM) with extruded soybean meals (ESBM) processed at 2 extruder temperatures, 149°C (LSBM) and 171°C (HSBM), on lactational performance and enteric gas emissions of dairy cows. Fol-

Table 1 (Abstr. 1528T). Milk protein concentration and plasma sulfur AA concentrations (μM)

Item	CON	12INF	24INF	12RPM	24RPM	SEM	$P =$
Milk protein	2.99^{b}	3.06^{a}	3.08^{a}	3.06^{a}	3.07^{a}	0.029	0.003
Methionine	23.7^{c}	35.1^{b}	45.0^{a}	30.8^{b}	40.2^{a}	3.09	<0.0001
Cystine	21.6	22.8	22.5	22.1	22.5	0.60	0.27
Cystathionine	1.69^{c}	2.25^{ab}	2.47^{a}	2.05^{b}	2.32^{a}	0.13	<0.0001
Homocystine	3.15	2.85	2.64	3.24	2.72	0.22	0.20
Taurine	49.6^{b}	53.4^{ab}	57.9^{a}	50.7^{b}	54.8^{a}	2.50	0.003
TSAA	99.7^{c}	116.3^{b}	130.4^{a}	109.0^{bc}	122.0^{a}	4.85	<0.0001

$^{a–c}$Means within rows differ at $P < 0.05$.

lowing a 2-wk covariate period, 48 Holstein cows averaging (±SD): 117 ± 64 d-in-milk (DIM) and 45 ± 8 kg/d milk yield (MY) were assigned to 1 of 3 treatments in a 9-week randomized complete block design experiment. Cows were blocked based on lactation number, DIM and MY. Treatment diets contained (diet DM basis): CM, 13.7% CM (with 42.6% CP), LSBM, 12.1% LSBM (with 47.8% CP), and HSBM, 12.5% HSBM (with 46.1% CP) with similar inclusion of other feed ingredients. Enteric CH_4 emission was measured using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS and orthogonal contrasts were used to evaluate the effect of replacing CM with ESBM and extrusion temperature of ESBM (LSBM vs. HSBM). Block and block × treatment were random effects. Dry matter intake (DMI; 27.1 kg/d; SEM = 0.65), MY and energy-corrected MY (ECM; 41.6 kg/d; SEM = 1.24) and ECM feed efficiency (1.52 kg/kg; SEM = 0.04) did not differ among diets. Milk urea N (10.0 vs. 11.4 mg/dL; SEM = 0.34) was lower and fat concentration (4.07 vs. 3.76%; SEM = 0.14) was greater ($P \leq 0.04$) for CM vs. ESBM. Milk fat yield, milk true protein content (MTP), and body weight (BW) change tended to be greater ($P \leq 0.10$) for CM vs. ESBM. When compared with LSBM, MTP tended ($P = 0.10$) to be lower for HSBM. Daily CH_4 emission (389 g/d; SEM = 13) and emission yield (i.e., per kg of DMI) were not affected by diets. Replacement of CM with ESBM on an equal CP basis in the diet of dairy cows, resulted in similar daily DMI, CH_4 emission, and ECM yield, but decreased milk fat concentration, tended to decrease MTP and BW gain and increased MUN. Extrusion temperature of ESBM had no effect on lactational performance of the cows.

Key Words: extruded soybean meal, canola meal, dairy cow

1531T Supplementing low crude protein diets with rumen-bypass amino acids improved nitrogen efficiency in lactating dairy cows. M. S. Seleem[1], W. Zhaohai[1], X. Chengqian[1], Z. Ying[1], M. D. Hanigan[2], and D. Bu*[1,3], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [2]*School of Animal Sciences, Virginia Tech, Blacksburg, VA,* [3]*Joint Laboratory on Integrated Crop-Tree-Livestock Systems, Chinese Academy of Agricultural Sciences (CAAS), Ethiopian Institute of Agricultural Research (EIAR), and World Agroforestry Center (ICRAF), Beijing, China.*

The objective of this study was to investigate the impacts of supplementing rumen-bypass Lys (RPL) and rumen-bypass Met (RPM) to diets with 2 levels of crude protein (CP) on nutrient intake, milk production, milk composition, nitrogen efficiency, and amino acid (AA) plasma concentrations in lactating cows. Fifteen multiparous Holstein cows were used in a 3 × 3 Latin square design with 21-d periods. Treatments were [1] a high-protein (HP) basal diet containing 16.4% CP [MP balance −130 g/d], [2] a medium-protein diet containing 15% CP plus RPL (60 g/cow/d) and RPM (25 g/cow/d) (MPLM) [MP balance −314 g/d], [3] low-protein diet containing 13.6% CP plus RPL (60 g/cow/d) and RPM (25 g/cow/d) (LPLM) [MP balance −479 g/d] according to the NASEM (2021) model. Individual DMI and milk yield were recorded daily; milk samples were collected on d 19, 20, and 21 of each period. Tail vessel samples were collected on d 15, 18, and 21 of each period. The last 7-d data of each period were analyzed using the PROC MIXED of SAS (v9.4) with treatment, square, cow, period, and sampling time. Cow within the square was the random effect, while all others were fixed in the model. Compared with the HP diet, the intake of dry matter (23.6 kg/d vs. 21.7 and 21.6; $P = 0.002$), CP (3.8 vs. 3.2, and 2.9 kg/d; $P < 0.001$), NDF (6.8 vs. 6.2, and 6.2 kg/d; $P < 0.001$), ADF (4.8 vs. 4.3, and 4.3 kg/d; $P < 0.001$), and OM (22.1 vs. 20.4, and 20.3 kg/d; $P = 0.006$), but not starch intake (6.3 vs. 6.4, and 7.0 kg/d; $P < 0.001$), was lower for the supplemented diets. Milk production and composition were not affected by RP-AA supplementation relative to the HP diet ($P = 0.36$). MUN concentrations were decreased for the low CP diets with AA compared with the HP diet (9.02 vs. 7.19, and 5.94 mg/dL; $P = 0.001$), indicating improved N efficiency. Plasma amino acid concentrations were not affected by the treatment except for proline, which increased for the MPLM diet ($P = 0.04$). We conclude that milk production can be maintained when feeding low CP diets to lactating dairy cows if the absorbed AA balance is maintained through RPL and RPM feeding.

Key Words: rumen-bypass amino acid, low-protein diet, nitrogen efficiency

Animal Behavior and Well-Being 3

1600W Using machine learning techniques to classify respiratory disease persistency in dairy calves. E. Casella[1], M. C. Cantor*[2], M. M. Woodrum Setser[1], J. H. C. Costa[1], and S. Silvestri[1], [1]*University of Kentucky, Lexington, KY,* [2]*Penn State University, College Park, PA.*

Bovine respiratory disease (BRD) has major implications for economics and calf welfare. The objective of this study was to use a machine learning approach to diagnose BRD development (pre-sick −5 to d −1, BRD+ diagnosis d 0) and BRD persistency (relapse or chronic status from d 1 to d 10) in dairy calves with a cost-sensitive feature selection process. We included 159 group housed calves from one facility who were offered ≥10 L/d for the first 43 d. Calves had daily health exams (outward BRD signs, and biweekly lung scores). Two abnormal outward signs of disease, and lung consolidation ≥3 cm^2 was BRD+ with antimicrobial treatment (d 0). BRD+ on d 14 was classified as relapse status (23 calves) with gradient boost algorithms and BRD+ on d 21 was classified as chronic status (47 calves) with support vector machine learning. The cost-sensitive feature selection process (CFS) made prediction accuracies for pre-sick and BRD persistency given budget constraints (start-up costs, and maximum cost/calf/d), and day relative to d 0 (higher F1-score weight assigned to earlier predictions). Start-up costs and cost/calf/d were split into a budget scenario (Low $7,000, $1.5, respectively) or (High $15,000, $2.5, respectively). We considered prediction accuracy poor ≤65%, moderate ≥70% or high ≥80%. Our results show that for pre-sick calves, the CFS at −4 d was moderate for Low (76% accuracy vs. High 87% accuracy) with respect to optimal F-score. Features selected for BRD for Low were activity behavior, weight, age, and IgG and for High were activity behavior, health exam, age, and IgG. For relapse status, the CFS Low had a poor prediction accuracy 65% at d 4 post BRD+ diagnosis. However, for chronic status, the CFS Low had a high prediction accuracy of 81% at 2 d post BRD+ diagnosis. The CFS Low for BRD persistency only selected age and weight. Conversely for BRD persistency, the CFS High selected activity behavior, health exam, and age. We suggest that chronic BRD status may be detectable in dairy calves with high accuracy as early as 2 d after initial BRD diagnosis. Future research should evaluate the practicality of identifying chronic BRD using CFS approaches on farm.

Key Words: precision technology, health

1601W Measuring the impacts of estrus and parturition on rumen temperature and environment, milk productivity, behavior, and physiological attributes in Holstein dairy cows. J. S. Lee*[1], H. K. Ryu[1], Y. H. Jo[1], S. R. Lee[1], H. W. Jin[2], H. K. Ko[3], and H. G. Lee[1], [1]*Department of Animal Science and Technology, Sanghuh College of Life Sciences, Konkuk University, Seoul, Republic of Korea,* [2]*Dongbang S&D Co., Ltd., Seoul, Republic of Korea,* [3]*National Agricultural Cooperative Federation Agribusiness Group, NongHyup, Gyeonggi-do, Republic of Korea.*

We investigated rumen temperature and environment in estral and pregnant cows using a bolus sensor. Cows in estrus and non-estrus were also assessed milk yield, behavioral and physiological changes in study animals. To assess the rumen temperature and environment, 25 of bolus sensors were inserted into estral (n = 21) and pregnant (n = 4) Holstein cows. We then monitored temperature and activity within the rumen using the wireless bolus sensor, recorded drinking, feeding and mounting behavior, and measured milk yield, milk compositions, vaginal temperature and levels of intravaginal mucus resistance. Data were analyzed by means of repeated measures analysis using SAS version 9.4 (SAS Institute Inc., Cary, NC). We found that cows in estrus exhibited more acts of mounting (16.3 vs. zero times/day; $P < 0.001$), decreased feeding (9.1 vs. 10.5 times/day; $P = 0.016$), increased vaginal temperature (39.0°C vs. 38.5°C; $P = 0.013$), and decreased vaginal mucus resistance (200Ω vs. 250Ω; $P < 0.001$), compared with non-estral cows. Besides, rumen environment via bolus sensors demonstrated that increased levels of rumen activity were most significant in estrus cows at the highest activity levels ($P < 0.001$). The estrus group exhibited increased rumen temperature ($P = 0.012$). On the other hand, the parturition group showed decreased rumen temperature in a rate of over 39.5°C ($P < 0.001$) and average activity levels ($P = 0.01$) compared with non-parturition cows. There were no changes in the milk yield and milk compositions. In conclusion, the results of this study not only provide basic physiological data related to estrus and parturition in Holstein lactating cows, but also suggest that monitoring of rumen temperature and activity might be used as an effective smart device for estrus and parturition detections.

Key Words: bolus wireless sensor, estrus, rumen temperature

1602W Development of an animal-based global welfare score usable in dairy cattle breeding. P. Lemal*[1], M-N. Tran[2], M. Schroyen[1], and N. Gengler[1], [1]*University of Liège–GxABT, Gembloux, Belgium,* [2]*Association Wallonne des Eleveurs-ELEVEO, Ciney, Belgium.*

Evaluation of welfare in dairy cattle is a challenge especially at the animal-based level. The objectives of this study were thus to develop an animal-based welfare score but also to evaluate its possible use in selection for welfare. The Welfare Quality Assessment Protocol (WQP) for cattle is currently considered a reference for welfare but only provides a score for the farm. A total of 2702 dairy cows were assessed for at least one animal-based measure of the WQP in 28 Walloon farms with a mean of 2.54 audits by farm and a maximum of 5. To develop this animal-based welfare indicator, a maximum of individual measures was used and combined with farm level indicators. Indeed, some welfare criteria were the same for the whole herd and could not be individualized (e.g., availability of water). To optimize this indicator, animal-based measures were selected based on 4 criteria: (a) if available, at least one measure was kept by welfare criteria described in the WQP to stay in line with the welfare principles; (b) the more representative measures based on genetic correlations among them were prioritized to indirectly select for the non-chosen measures; (c) measures with relevant genetic correlation with production traits (milk production, fat percentage, protein percentage, somatic cell count) were preferred to allow the concomitant selection for this indicator and for high production traits and low somatic cell count; (d) measures with a high heritability allowed to maximize heritability of the final indicator and thus to allow selection for welfare. On this basis, the individual measures selected were body condition score, udder cleanliness, number of lesions, lameness score, respiratory score, diarrhea score and avoidance distance. The final score obtained by combining these individual measures with farm level measures presented a positive genetic correlation with protein percentage (0.18 ± 0.14), a negative genetic correlation with somatic cell count (−0.14 ± 0.23) and a heritability of 0.25 ± 0.08. In conclusion, this study allowed the development of a first promising animal-based welfare score and showed that selection for welfare seems possible.

Key Words: welfare, score, genetics

1603W Feeding behavior patterns and performance in dairy calves are associated with food neophobia.

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Food neophobia is the aversion of novel feeds, which is commonly expressed in ruminants. The aim of this study was to determine if food neophobia is associated with feeding behavior and performance in dairy calves. Holstein calves (n = 28) were group housed at 4 d of age with an automatic feeding system that measured milk replacer intake, starter intake, and rewarded and unrewarded visits to the feeder. Calves were allocated up to 15 L/d of milk until d 46, then milk was reduced until calves were completely weaned at d 66. Feeder use was recorded until d 81. Feeding behaviors were summed from d 4 to d 81. Average daily gain (ADG) was the performance measure. Calves were subjected to a food neophobia test (FNT) at d 83 ± 3 for 30 min. The FNT exposed calves to 3 identical buckets containing a familiar feed (alfalfa hay), an empty bucket, and a novel feed (corn silage based total mixed ration). Behaviors recorded included time spent manipulating each bucket, inactive, walking, grooming, attentive to each bucket, latency to approach each bucket, frequency of vocalizations, and total quadrants of test arena entered. Behaviors from the FNT were subjected to principal component analysis that revealed 3 factors ('feed neophilia,' 'active,' and 'feed apathetic') that explained 60.6% of behavioral variance. The factor 'feed neophilia' had high loadings for time interacting with both feed buckets. Regressions assessed associations between calves' factor scores and their feeding behavior and ADG. Factor 1 ('feed neophilia') had a negative association with rewarded visits to the milk feeder ($P = 0.03$). Factor 2 ('active') had a positive association with total starter consumed ($P = 0.01$) and a negative association with the age at which calves first consumed ≥1 kg of starter ($P = 0.01$). Factor 3 ('feed apathetic') had positive associations with ADG ($P = 0.01$) and the number of rewarded visits to the milk feeder ($P = 0.05$). These results indicate that food neophobia is not highly associated with feeding behavior in the home pen or performance of calves. However, other personality traits measured with the FNT are associated with feeding behavior and growth.

Key Words: precision technology, weaning, personality

1604W The effect of the social environment on labor duration and dystocia of dairy cows housed in group maternity pens.

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Management practices during calving can impact cows' calving experience. The objectives of this study were to evaluate the effect of opportunities to isolate ("blind") and stocking density in group maternity pens on the duration of labor (min; start of rhythmic abdominal contractions to calf delivery or calving assistance) and frequency of dystocia (stage II labor >70 min, including unassisted and assisted calvings). Holstein dairy cows (primiparous = 120; multiparous = 223) were enrolled 21 ± 3 d before calving. Cows were assigned randomly to 1 of 4 treatments of a 2 × 2 factorial arrangement including: 1) high vs. low stocking density (7.7 to 12.9 m^2 vs. 15.4 to 25.8 m^2 lying space/cow), and 2) presence of a blind (yes vs. no). Pens were replicated 4 times (experimental unit: stocking density × blind × replicate = 16). Video was continuously observed for 3 h before calving to assess the start of rhythmic abdominal contractions (stage II labor) and time of delivery (calf's hips fully expelled) or time of calving assistance. Frequency of dystocia was considered the percent of cows with dystocia per experimental unit (number of cows with dystocia ÷ total number of calvings). A cox proportional hazard and mixed linear model were used for analysis of duration of labor and frequency of dystocia, respectively. Cows had a lower hazard of calving (i.e., greater duration of labor) in pens without a blind than with a blind (HR = 0.72; 95% CI: 0.53–0.97), in high compared with low stocking density pens (HR = 0.75; 95% CI: 0.56–1.0), primiparous compared with multiparous cows (HR = 0.71; 95% CI: 0.55–0.9), and dystocia compared with normal calving (HR = 128.3; 95% CI: 54.2–303.6). Frequency of dystocia was not affected by stocking density (high = 62.3 vs. low = 63.7 ± 5.2%; $P = 0.85$) or a blind (yes = 58.9 vs. no = 67.2 ± 5.2%; $P = 0.27$). More primiparous cows had dystocia than multiparous cows (74.3 vs. 51.7 ± 5.2%, respectively; $P = 0.005$). These results suggest the presence of a blind, stocking density, parity, and dystocia may affect duration of labor. Dystocia is likely influenced by parity but not stocking density or a blind.

Key Words: welfare, dystocia, maternity pen

1605W Precision and accuracy of a behavior monitoring collar inter-device for rumination, feeding activity and idle time of lactating dairy cows.

J. V. R. Lovatti*[1], L. F. Garrido[2], J. F. Aires[3], K. A. Dijkinga[2], J. H. C. Costa[1], and R. R. Daros[2], [1]*Dairy Science Program, University of Kentucky, Lexington, KY,* [2]*Graduate Program in Animal Science, School of Life Science, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil,* [3]*Animal Science Department, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.*

Inter-device precision and accuracy are fundamental to use data from precision dairy technologies. This study aimed to validate the behavior monitoring collar (BMC; CowMed, Santa Maria, RS, Brazil), comparing first with visual observation, and second with inter-device precision and accuracy for rumination, feeding activity, and idle time of lactating dairy cows. Holstein cows (n = 23; parity: 1.3 ± 0.6; and milk yield: 34.88 ± 8.66 kg/d) were housed in a voluntary milk system free stall barn. Cows were fitted with 2 BMCs, positioned longitudinally in the middle of the collar's left side. Visual observations were made over 2 periods of one day (0700 to 1100 h; 1400 to 1700 h); these 7 h per cow were summarized for each behavior to assess the agreement of visually recorded behavior and BMC data. Also, 21 d of BMC data was summarized per cow for both BMCs to assess the inter-device agreement. Pearson correlation (r), Lin's concordance correlation coefficient (CCC), linear regression, and Bland-Altman plots (BAP) were calculated. For visual observations, we found high correlations for feeding activity ($r = 0.87$, $P < 0.01$; CCC = 0.86); very high for idle time (0.93, $P < 0.01$; CCC = 0.625), but moderate correlations for rumination (0.50, $P < 0.29$; CCC = 0.48). The BAP was deemed acceptable and without bias; BAP mean differences ± SD were 0.78 ± 7.85, −0.48 ± 8.14, and 7.16 ± 7.72 min/h for rumination, feeding activity, and idle time, respectively. The slope of the linear regression was different from 1 for all behaviors. For inter-device comparison, we found very high correlations for feeding activity (0.91, $P < 0.01$; CCC = 0.91) and idle time (0.92, $P < 0.01$; CCC = 0.91), and high for rumination ($r = 0.90$, $P < 0.01$; CCC = 0.90). The BAP was deemed acceptable and without bias; BAP mean differences were −8.18 ± 68.23, 10.83 ± 88.36, and −1.90 ± 78.60 min/d for rumination, feeding activity, and idle time, respectively. The slope of the linear regression was different from 1 for all behaviors. In summary, this study validates the precision of BMC inter-device for recording the behavior of lactating dairy cows.

Key Words: precision livestock farming, accelerometer, PDT

1606W Clinical metritis alters the behavior pattern in cows during the early postpartum. J. Cardoso[1], J. Halfen*[3], K. Cardoso[1], C. Brauner[1], R. Ferreira[2], and E. Schmitt[1], [1]*Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil,* [2]*Santa Catarina State University, Chapecó, Santa Catarina, Brazil,* [3]*Virginia Tech, Blacksburg, VA.*

Uterine disease is associated with poor reproductive efficiency, milk yield decreases and higher culling rate. The early diagnosis can anticipate the treatment and supportive therapies, moreover to minimize metritis associated loses. The aim of this study was to identify changes in the behavior of cows with metritis or metritis plus other diseases. Fifty-eight Holstein cows were monitored by behavior using neck accelerometer (C-Tech, Chip Inside) to determine the rumination, activity, and idleness time, from 2 to 8 d in milk. Cows were monitored daily by veterinarian and enrolled to the groups C (control; no clinical disease detected; n = 30), M (animals with clinical metritis; n = 10) or M+ (clinical metritis plus other diseases; n = 18). The data was analyzed as total time, in minutes, the animal expressed each behavior in a 24-h period. The effect of groups, time, and their interaction on behavior variables were assessed using mixed models for repeated measures. Different covariance structures were tested for each model, and it was accepted the one with smaller Akaike Information Criteria (AIC). Differences between groups at a specific time point were determined by Tukey HSD pairwise comparison. All the analyses were performed using the JMP (JMP Statistical Discovery LLC), and $P < 0.05$ was considered statistically significant. Cows with metritis or metritis plus other diseases had increased activity and decreased idleness. Also, cows with metritis plus other diseases had decreased rumination time when compared with the control group (Table 1). Cows with metritis or metritis plus other diseases changed the idleness in the 3rd day after calving when compared with healthy cows (540 ± 15 C; 662 ± 20 M+; $P = 0.0005$). In conclusion, the findings of the present study are promising for the development of alternatives for the early metritis diagnosis.

Key Words: activity, idleness, rumination

1607W Breed differences for rumination and grazing time in lactating organic cows. T. Muratori*[1], B. J. Heins[2], and C. D. Dechow[1], [1]*Penn State University, University Park, PA,* [2]*WCROC, Morris, MN.*

Understanding how cows behave when grazing can improve pasture management and maximize the productivity and health of the herd. We aimed to investigate differences among breeds with data collected through the RumiWatch System (RWS), focusing on the total time spent ruminating, grazing, and eating; and defining their patterns across the day. The RWS is a wearable sensor system that monitors dairy cows' feeding behavior. The sensors detect head movements and chewing activity, which can estimate the amount of feed consumed, the frequency and duration of feeding bouts, rumination, and other behaviors. Cows were either Holstein (n = 2), GrazeCross (GC; Jersey × Normande × Viking Red; n = 6), or ProCross (PC; Holstein × Montbeliarde × Viking Red; n = 5). Data were collected over 8 consecutive days for 13 animals and exported as hourly totals. Cows were kept on pasture for the duration of the study, and no concentrate was provided. Variables include rumination time and bites, grazing bout duration and bites, eating time, and chews. Hourly data were summed to create daily totals. All statistical analyses were conducted with the MIXED procedure of SAS; breed group, day, hour of the day, and the interactions of hour with breed and day were independent variables. A repeated effect of time in hours from the start of the trial with cow as the subject was included. Analyses for 24 h totals excluded the effects of hour and the repeated effect was day. The 24 h period of graze bouts was different ($P < 0.05$) between GC (least-squares-means = 574.14 min) and PC (501.51). The 24-h eating time was higher ($P < 0.05$) for GC (604.07 min) than PC (540.76 min). Total events tended to be different ($P < 0.10$), with Holsteins having the highest number of actions (rumination chews, eating chews, drinking gulps, and other movements) in a day (73,867) and PC the fewest (61,343). The 24-h rumination time ranged from 398.63 min (PC) to 465.8 (HO) and was not significantly different. All 3 groups had peak grazing activity during the 20th hour of the day. Understanding grazing behavior could improve grazing strategies to improve feed efficiency and enhance the performance of herds on pasture.

Key Words: cow, RumiWatch, behavior

Table 1 (Abstr. 1606W). Behavior of cows during the early postpartum

Mean (min/day ± SE)	C	M	M+
Rumination	463 ± 10^{a}	417 ± 18^{ab}	398 ± 13^{b}
Activity	260 ± 10^{a}	209 ± 17^{b}	189 ± 13^{b}
Idleness	537 ± 12^{a}	629 ± 21^{b}	649 ± 16^{b}

1608W Evaluation of time budgets and vaginal temperature of pastured Holstein cows offered shade or sprinklers. K. Braman*, J. Drewry, and A. Stone, *Mississippi State University, Starkville, MS.*

The study aim was to evaluate lactating cow behavior associated with pasture heat abatement strategies (HAS): 80% shade cloth (SH) and PVC sprinkler systems (SP). Lactating Holstein cows (n = 46) were assigned to SH or SP pens (n = 23 each) based on parity, production, days bred, and DIM. Cows were housed in freestalls before a 7-d pasture acclimation period with no access to HAS, 1 d of HAS acclimation, 3 d of observation, and then crossed over to repeat the 1 and 3 d acclimation and observation periods. Every 30 min a drone was used to evaluate heat abatement use (HAU). Vaginal temperature (VT) was collected every 5 min. Steps, lying bouts (LB), and time lying (TL) were recorded using a triaxial accelerometer. Temperature-humidity index (THI) data was collected. PROC GLIMMIX of SAS was used to evaluate the fixed effect of HAS and THI on HAU, VT, steps, LB, and TL. In the morning (1000 to 1200), cows in SH had greater HAU when VT increased, but cows in SP that had greater HAU when VT decreased ($P < 0.01$). In the afternoon (1230 to 1430), cows in SP with lower VT had greater HAU compared with SH and no HAU ($P < 0.01$). At greater THI, cows that walked more had increased VT in both HAS ($P < 0.01$ and $= 0.04$ for SH and SP, respectively). Cows using SP had decreased VT compared with cows using SH ($P < 0.01$). As THI and VT increased, LB decreased for SH ($P = 0.01$) but increased for SP ($P = 0.04$). For both SH and SP, TL increased as THI and VT increased ($P < 0.01$ and 0.04, respectively). In SP, TL increased as VT × LB increased ($P < 0.01$) and decreased as HAU increased ($P < 0.01$). The interaction of THI × VT affected steps with cows in SP increasing steps as THI × VT increased ($P < 0.01$) and cows in SP decreasing steps as THI and VT increased ($P < 0.01$). Cows in SP walked more at greater THI × VT ($P < 0.01$) whereas cows using SH walked less at greater THI × VT ($P < 0.01$). Cows in SH took fewer steps at greater HAU and THI ($P = 0.03$) compared with cows in SP or no HAU. Cows used both SH and SP on pasture. Providing SP may be useful at reducing VT and alleviating heat stress in pastured dairy cattle.

Key Words: heat stress, heat abatement, pasture

1609W Assessing the frequency of sole ulcers according to housing system type in Québec dairy cows. B. Fouquette*[1], J. C. Arango Sabogal[2], A. Desrochers[1], L. Des Côteaux[1], and M. Villettaz Robichaud[1], [1]*Faculté de médecine vétérinaire, Departement of Clinical Sciences, Université de Montréal, Saint-Hyacinthe, Québec, Canada,* [2]*Faculté de médecine vétérinaire, Departement of Pathology and Microbiology, Université de Montréal, Saint-Hyacinthe, Québec, Canada.*

Most studies and recommendations on lameness and hoof lesions focus on free stall barns. In Québec, most dairy farms are tie-stall systems. Our objective was to evaluate the frequency of sole ulcers (SU) in dairy cows according to their housing system. Data on hoof trimmings performed between 2015 and 2022 were obtained from the database of the Québec Hoof Trimmers Association. Cow and herd data information were obtained from the local DHI. In total, 39,658 dairy cows (95% Holstein) from 357 herds (78% tie-stalls) were included based on the following criteria: having at least one lactation and one trimming session and having available information on housing system. The productive life of each cow was calculated as the difference between the culling date and its first calving date. Median productive life was 34.5 mo (IQR: 34.6 mo). Overall, no hoof lesions of any type were observed in 22,037 cows (55.6%; 95% CI: 55.1–55.6%) throughout their productive life. SU was diagnosed at least once in the productive life of 6,004 cows (15.1%; 95% CI: 14.8–15.5) and was more frequently observed in cows housed in tie-stall (n = 4,273; 71.2%; 95% CI: 70.0–72.3) than free-stall farms (n = 1,731; 28.8%; 95% CI: 27.7–30.0). This study shows that almost half the cows have at least one hoof lesion during their productive life. Frequency of SU lesions differ according to the housing type. Following steps include exploring the association between SU lesions and length of productive life while accounting for housing type.

Key Words: sole ulcer, housing system, dairy cow

Animal Health 3

1610W Comparison of calf morbidity, mortality, and future performance across categories of passive immunity. P. Crannell and A. Abuelo*, *Michigan State University, East Lansing, MI.*

The objective of this retrospective cohort study was to compare dairy calf morbidity, mortality, growth until weaning, and reproductive efficiency until first calving among the categories of poor (<5.1 g/dL total protein), fair (5.1–5.7 g/dL), good (5.8–6.1 g/dL), and excellent (>6.2 g/dL) transfer of passive immunity (TPI). The records from 4,336 calves (2,272 female, 2,064 male) with 2–7-d-old total protein records in a commercial farm were analyzed. Data from both sexes were used to evaluate preweaning health and mortality, whereas only the female's data were used to investigate average daily gain (ADG), reproductive and production performance. Associations of TPI categories with disease events, reproduction indices, first-lactation milk yield and ADG at weaning were evaluated by survival analysis and mixed models. Compared with calves with excellent TPI, calves in the inferior TPI categories showed increased risk of diarrhea: poor [hazard ratio (HR) = 1.49; 95% CI: 1.22–1.82], fair (HR = 1.32 [1.16–1.51]), good (HR = 1.14 [1.02–1.29]). However, the risk of pneumonia differed only between the calves in the poor and excellent TPI groups (HR = 1.39 [1.05–1.84]). The preweaning mortality risk was also higher in calves with poor TPI (HR = 4.29 [1.98–9.27]) compared with excellent TPI. However, mortality risks were not statistically different between calves with fair or good TPI and those with excellent TPI. Similarly, calves with poor TPI had a 64, 55, and 24% lower risk of reaching first insemination, successful insemination, or first calving, respectively. However, there were no differences in ADG, number of inseminations, or first-lactation 305ME across TPI groups. Our results confirm the positive effects of optimal TPI in calf preweaning health and postweaning reproductive efficiency. The 4 proposed categories of TPI can assist in decreasing the incidence of diseases that occur in the first weeks of life (i.e., diarrhea), but their effect on other diseases or future performance might be more limited. Although conducted in one herd, this study can be used to illustrate the effect of TPI on future calf performance.

Key Words: calf health, colostrum, neonatal period

1611W Effects of milk-derived bioactive peptide Val-Pro-Pro on diarrhea of pre-weaning calves. X. Zong*, Y. Du, L. Yang, and Q. Xu, *Huazhong Agricultural University, Wuhan, Hubei, China.*

A well-known milk-derived bioactive tripeptide, VPP (Val-Pro-Pro) has good anti-inflammatory, anti-hypertension, and anti-hydrolysis properties. However, whether VPP can alleviate calf intestinal inflammation is unclear. In this experiment, the effects of VPP on growth, diarrhea incidence, serum biochemical indices, short-chain fatty acids, and fecal microorganisms were examined in pre-weaning Holstein calves. Eighteen one-month-old calves with similar birth date, body weight, and genetic background were randomly assigned equally to 2 groups (n = 9). The control group was given 50 mL of phosphate buffer saline by oral gavage before morning feeding, whereas the VPP group received 50 mL of VPP solution (100 mg/kg body weight/d) by the same way. The study lasted for 17 d, with the first 3 d used for adaptation. Initial and final body weights were determined, and daily dry matter intake and fecal score were recorded throughout the study. At 2 h after morning feeding on d 14, 10 mL of blood was collected by the jugular vein and serum hormone levels and antioxidant and immune indices were measured. Fecal microorganisms were collected on d 0, 7, and 14, and 16S rDNA sequencing was performed. The values for blood and growth parameters used for statistical analysis are means ± SD, n = 10, and they were analyzed by *t*-test using GraphPad Prism 9.0 (GraphPad Software Inc., La Jolla, CA). Oral administration of VPP did not significantly affect calf average daily feed intake and body weight, but the growth rate in body weight was significantly higher in the VPP group than in the control group on d 7 ($P < 0.05$). Compared with the control, VPP significantly decreased serum TNF-α and IL-6 contents ($P < 0.05$), and concentrations of nitric oxide and IL-1β also decreased but not significantly ($0.05 < P < 0.1$). After 7 d of VPP, relative abundances of g_Lachnoclostridium, uncultured_bacterium, and g_Streptococcus in fecal samples increased significantly ($P < 0.05$). Compared with the control, VPP significantly increased concentrations of the fecal short-chain fatty acids n-butyric acid and isovaleric acid ($P < 0.05$).

Key Words: calf, VPP, calf diarrhea

1612W A therapeutic diet for foot-and-mouth disease–infected Holstein Friesian crossbred calves improves immune response by suppressing inflammation and oxidative stress. A. Somagond*[1], B. H. Manjunatha Patel[1], A. K. Pattanaik[2], N. Krishnaswamy[1], G. B. Manjunatha Reddy[3], G. K. Gaur[2], and J. J. Loor[4], [1]*ICAR–Indian Veterinary Research Institute, Bengaluru, Karnataka, India,* [2]*ICAR–Indian Veterinary Research Institute, Izzatnagar, Bareilly, UP, India,* [3]*ICAR–National Institute for Veterinary Epidemiology and Disease Informatics, Bengaluru, Karnataka, India,* [4]*University of Illinois, Urbana, IL.*

Foot and mouth disease (FMD) affects dry matter intake (DMI) and performance due to mucosal erosions developed on the tongue and gums. A soothing therapeutic diet (TD) in a different physical form compatible with oral lesions could ensure optimal feed intake. A TD was offered in the mash or cooked form to 18 Holstein Friesian crossbred male calves (10–12 mo old). They were randomly distributed across 3 treatment groups, namely, TD-Mash (TDM), TD-Cooked (TDC), and TDC-Customized Nutrient Supplement (TDCNS), with n = 6/group. A group of 4 calves served as Control-Mash (CONM). All TD groups were infected with the FMD virus in a bio-containment facility (BSL-III). The diet consisted of ground finger millet straw, crushed maize and wheat, unrefined brown sugar, groundnut cake, vegetable oil and sodium bicarbonate. The TD was offered at 1.5% of BW. The blood collection was done at 0, 3, 5, 12, 19, 26, 33, and 42 d post-infection (DPI). The tongue and foot healing was scored on a 1–5 scale. The experiment continued for 42 d when the calves had recovered completely. Data were analyzed using the Linear Mixed Model in SPSS v.22. Among the hematological parameters only the platelet count and TLC of CONM and TDM differed significantly with TDC and TDCNS groups ($P < 0.05$). The biochemical variables remained unaffected. Cortisol and T_4 (Thyroxine) levels of treatment groups differed significantly with CONM ($P < 0.05$) on the 3rd DPI. The oxidative stress biomarkers catalase, Glutathione peroxide, and Superoxide dismutase increased significantly in treatment groups from 5th to 26th DPI ($P < 0.05$). The pro-inflammatory cytokines (IL-1β, IL- 6, TNF-α), anti-inflammatory (IL-4, IL-10) and acute phase proteins (haptoglobin, serum amyloid-A, ceruloplasmin) of treatment groups differed significantly with CONM ($P < 0.05$). The TD inhibited lipid peroxidation as indicated by malondialdehyde ($P < 0.05$). The tongue and foot healing score was highest on 12th and 24th DPI in the TDCNS group. In conclusion, feeding TD improved the immune response by reducing inflammation and oxidative stress indicating calves were immunocompetent. Changes in the physical form of TD helped enhance the healing of FMD lesions.

Key Words: cooked, foot and mouth disease, therapeutic diet

1613W Barriers to recording calf data on Ontario dairy farms.

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Establishing accurate illness and treatment rates in calves is crucial, yet calf health records are often incomplete. The aim of this study was to investigate the barriers to recording calf illnesses and treatments on Ontario dairy farms. An online survey was completed by a convenience sample of 88 Ontario dairy farms in 2022 and contained 34 questions regarding farm demographics, factors that would improve recording compliance, and current practices surrounding record keeping and analysis. Multivariable models were built through backward stepwise elimination to assess associations between explanatory variables and the following outcomes: likelihood of making changes based on records analysis, factors that would increase the use of electronic recording methods, and whether farms did or did not record 100% of calf illnesses and treatments. Pearson's chi-squared test was also used to investigate associations between explanatory variables and why a calf illness or treatment may not be recorded. Farms were more likely to record 100% of antimicrobial treatments if they recorded using a computer software system compared with those that did not (OR = 3.45; 95% CI: 1.18, 10.14; $P = 0.02$). For supportive therapies, non-family employees were more likely to record 100% of treatments compared with owners (OR = 6.08; 95% CI: 1.18, 31.22; $P = 0.03$). Farms that kept records in the calf barn were less likely to report that illnesses were not recorded due to time constraints ($P = 0.008$) or because calf records are not analyzed ($P = 0.04$). Also, farms that recorded calf treatments in a paper book were more likely to report that treatments were not recorded because calf records are not analyzed ($P = 0.04$). The most commonly indicated factors that would improve illness recording practices were recording with a mobile app (n = 19/71; 26.8%) and for the recording system to be easy to use (n = 22/71; 31.0%). Recording practices may be improved by ensuring records are kept near the calves and that recording methods allow for data analysis. An easy-to-use mobile app solution may also improve recording if it could be used in the calf barn, provide data analytics, and allow for time-efficient data entry.

Key Words: data analytics, dairy calf, record

1614W Neonatal calves infected with *Cryptosporidium parvum* have impaired transporter expressions in their jejunum epithelium.

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Infectious diarrhea is a major cause of weight loss and delayed growth as well as increased morbidity and mortality in neonatal calves. Intestinal transporters translocate a wide variety of substances across cellular membranes, although their expression, localization, and regulation have not been fully implicated in the pathogenesis of infectious diarrhea. The aim of this study was to get a deeper insight into the substrate transporters in neonatal calves' jejunal epithelial cells infected with *Cryptosporidium parvum* using next-generation sequencing. Ten neonatal calves (Holstein-Friesian) were randomly allocated into, 1 – control (CTRL, n = 5) and 2 - infected by oral application of 2×10^7 *C. parvum* oocysts (LE-01-Cp-15 strain, n = 5) on d 1 of life. On d 8, calves were slaughtered and total RNA was extracted from the jejunum mucosa. Stranded mRNA libraries were sequenced on the Illumina HiSeq 2500 (San Diego, CA). Processed reads were searched against the bovine reference genome (ARS-UCD 1.2). Differentially expressed genes (DEG) were defined as having log2 (fold change) > 1.5 and false discovery rate <0.05 using DESeq2. Gene ontology analysis was performed on DEG using DAVID (v2022q4) bioinformatics tools. Out of the total 12,908 genes, 193 and 87 genes were down and upregulated in the infected calves' mucosa, respectively. The DEG included ATP-binding cassette (ABC) and solute carrier (SLC) transporters comprising downregulation of ABCC2, ABCD1, SLC28A2, SLC38A3, and SLC5A4 in the infected group. In contrast, SLC10A2 and SLC4A4 were upregulated in the infected group. These transporters are localized at cellular membranes (brush border) and are associated with transmembrane transport of substrates including organic/inorganic anions/cations, bicarbonate, monocarboxylates, cholesterol and fatty acids, bile acids, vitamins, and amino acids/oligopeptides. The current results provide novel prospects for the pathogenesis of diarrhea in neonatal calves infected with *C. parvum*, which include the deregulation of specific transporters in mucosal epithelial cells that control acid–base balance as well as nutrient absorption.

Key Words: transcriptomics, solute carrier, absorption

1615W The respiratory infectome of dairy calves characterized by a total RNA sequencing approach.

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We collected samples from 2 dairy farms in New South Wales, Australia (F1 and F2) to identify the species that infect the upper respiratory tract of calves using untargeted RNA transcriptomics. We collected nasal swabs from n = 60 calves 5–54 d old. We sequenced 10 RNA libraries, from pooled and individual animals with and without clinical respiratory disease. Total RNA sequencing was done using Illumina Stranded Total RNA with Ribo-Zero library preparation and sequenced on an Illumina Novaseq. Of the total RNA, most corresponded to bovine transcripts. Of the total transcriptome bacterial RNA ranged from 3 to 0.05%, and viruses 0 to 1%. Few sequence reads were classified as eukaryotic non-bovine. *Mycoplasma* sp., namely *M. bovoculi*, *M. dispar*, and *M. bovirhinis*, were the most prevalent bacterial species identified consistently in F1 and F2; other bacterial species were less prevalent. In contrast, the virome of the animals from F1 and F2 differed; the abundance was quantified using Kallisto and differences between groups assessed using DESeq2 in R. In F1, we identified Astroviruses (4 different genotypes), Kobuvirus, Torovirus, and Bovine Respiratory Syncytial Virus (BRSV). In F2, the virome was predominantly Bovine Rhinitis A (BRAV) but also Influenza D, Bovine Rhinitis B, Bovine Coronavirus, and Influenza. Calves with respiratory disease in farm 2 had a relatively higher abundance of Bovine Rhinitis A. In all libraries *Babesia* sp. was detected and in 2 libraries, cryptosporidium. Two of the libraries in which cryptosporidium was detected, were from animals with respiratory disease, however, it is not possible to establish causality and there is potential contamination of field samples. While the bacteriome of the farms was similar, the infectome differed. *Mycoplasma* other than *M. bovis* may play an important role in respiratory disease. The identification of the viral species identified that may also play a role in respiratory disease should also be further explored.

Key Words: metagenomics, virome, bovine respiratory disease

1616W Untargeted metatranscriptomic methods to characterize the enteric infectome of calves with and without diarrhea.

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We conducted a metagenomics study to explore the range of microbial species infecting calves with and without diarrhea. Rectal swabs were taken from 60 calves 4 to 54 d old from 2 farms (F1 and F2) in New South Wales, Australia. The mean fecal score of 56 healthy calves at the time of sampling was 0 and from the 4 calves with diarrhea was 2 (scale 0 to 3). RNA was extracted from each sample and pooled (pools of similar age, same farm and disease status). A total of 11 libraries were sequenced and their content classified at a high hierarchy taxonomy level varied for viruses (92–0.04%), bacteria (99–7%), parasites (0–41%) and for the bovine host (6–0.5%). Bacterial genus diversity was similar in healthy calves with high abundance of *Prevotella*, *Bacteroides*, and one healthy group had a high abundance of *Phascolarctobacterium*. The 3 libraries for scouring calves had an altered abundance for *Campylobacter*, *Bacteroides*, *Parabacteroides*, *E. coli* compared with healthy calves. Alpha diversity was computed using Shannon and Simpson index, and Beta diversity was assessed using NMDS. Viral abundance among groups was assessed using DESeq2 in R. The transcriptome from a 43-d-old scouring calf had 43% RNA of *Bovine torovirus* which was present on F1, but not F2. On F2, one library from 2 young calves 7 to 8 d old consisted of 92% rotavirus A RNA, while the library from 2 scouring calves 24 to 25 d old had only 0.04% of sequencing reads as viruses. We identified several picornavirus species (unclassified bovine picornaviruses, *Hungarovirus*, *Parechovirus*, *Enterovirus F*, and *Kobuvirus*) and 4 genetic types of *Astroviruses* and *Caliciviruses* (bovine *Calicivirus* unclassified, *Norovirus GIII*, *Nebovirus* unclassified). No fungi were detected but *Esophagostomum*, *Cryptosporidium*, *Giardia*, and *Trichomonanididae* were found and F1 had *Eimeriidae* and *Entamoeba*. The *Trichomonanididae* (unclassified species) had the highest eukaryotic abundance in 6 libraries, 11–41% of the total RNA in healthy calves. This research will guide the next phase of investigation into associations with disease and to develop laboratory culture, experimental infection models and diagnostic assays.

Key Words: metagenomics, calf diarrhea, virome

1617W Expression of virulence factors and antimicrobial resistant genes in total RNA sequenced from rectal swabs from diarrheic calves.

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Our objective was to evaluate the fecal microbiome in non-diarrheic (n = 55) and diarrheic (n = 5) calves aged 4 to 54 d old from 2 farms using an untargeted RNA sequencing approach. We sequenced 11 total-RNA pooled libraries, of which 3 were from diarrheic calves. The sequences obtained were screened using virulence factor (VF) database and AMRFinder to identify antimicrobial resistance (AMR) genes. We used a threshold of >90% coverage and >90% identity to reference sequences as a threshold for detection and abundance estimation. We determined abundance of VFs and AMR genes by mapping short reads to the genes using Kallisto and determined differential expression statistics between libraries using DESeq2 in R. Two libraries from scouring animals had a distinctive increase in expression of virulence genes, with 10 with statistical significance ($P < 0.05$). One library had increased VFs from *Escherichia coli* and *Campylobacter jejuni* and had a co-prevalence with a rotavirus A (2 calves 6–7 d old) with 8 VFs differentially expressed ($P < 0.05$). The second scouring library had an increase of *C. jejuni* associated virulence genes but no association with viral pathogens (2 calves 24–25 d old) with 19 genes differentially expressed ($P < 0.05$). A third scouring library had no increased VFs compared with healthy calves, and scouring from this calf was associated with a Torovirus (calf 43 d old). Overall, antimicrobial resistance genes against tetracycline, aminoglycosides, cephamycin, sulfonamide, macrolide, lincosamides, streptothricin, one β-lactamase (bla_{ACI-1}), glycopeptides, and nitroimidazole were identified. Tet(Q), followed by sul2 and aph(3″)-Ib were highly expressed across all libraries. Only 2, (aph(3″)-Ib, aph(6)-Id, sul2), had significantly higher expression ($P < 0.05$) in diarrheic calves. Next steps include integrating RNA sequences with genomic DNA and phenotypic characterization of the isolates to understand the bacterial mechanisms for virulence, the genetic context of the genes and the mobile genetic elements that may facilitate their spread.

Key Words: calf diarrhea, virulence, antimicrobial resistance.

1618W Withdrawn.

1620W Arrival risk factors associated with morbidity and mortality in veal calves in Québec, Canada.

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Morbidity and mortality remain important concerns for veal production which traditionally involves very young calves coming from dairy farms. There is little information available about specific studies that examine the relationship between transfer of passive immunity (TPI) and health outcomes in veal calves in Québec. Therefore, the objective of this study was to investigate the risk factors, including TPI failure, associated with morbidity and mortality in veal calves. A prospective cohort study was conducted on 59 batches of milk- and grain-fed veal calves' facilities in Québec, Canada. Thirty calves per batch were randomly sampled for transfer of TPI estimation using serum Brix refractometry (<8.4%, inadequate). Recorded morbidity and mortality were modeled through generalized linear mixed models using a logit link, as function of a continuous variable (arrival weights) and of categorical variables (inadequate TPI, arrival season, and calves' suppliers), the included variables were based on causal diagrams informed process. Moreover, those models were executed after missing data imputation. Data was available for 1,729 calves. Our study reported that 1,084 calves had a serum Brix <8.4% (62.7%) compatible with inadequate TPI. The total proportion of morbidity was 25%, while the total mortality proportion was 3.9%. Calves with inadequate TPI had higher odds of morbidity (odds ratio (OR) = 1.5, 95% confidence interval (95% CI): 1.2–2.1) than those with adequate TPI. A similar, but non-significant association was found between inadequate TPI and odds of mortality (OR = 1.2, 95% CI 0.7–2.1). Finally, calves arriving during the fall season had increased odds of mortality compared with calves that arrived in winter (OR = 6.1, 95% CI 1.2–28.7). Inadequate TPI can affect the health of veal calves, as measured through its impact on morbidity. However, other factors, for instance the season of arrival, also play an important role.

Key Words: risk factor, transfer of passive immunity, Brix

1621W Effects of *Cryptosporidium parvum* infection on intestinal short-chain fatty acids and free fatty acid receptor expression in neonatal calves.

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Short-chain fatty acids (SCFA), produced by different classes of microbes, are known for health-promoting effects in the intestine and discussed as a link between the intestinal immune system and the microbiota. The present study aimed to investigate the impact of a *C. parvum* infection on SCFA and lactate concentrations in abomasum, jejunum and colon and expression of free fatty acid receptors (FFAR1, 2, 3, 4) and genes encoding intestinal immune responses in the mucosa of jejunum, ileum and colon of 8-d-old calves. Five male Holstein calves were infected on d 1 of life with 2×10^7 *C. parvum* oocysts and 5 calves were not. Calves were slaughtered on d 8. SCFA in intestinal content were determined by gas chromatography and lactate by a clinical chemistry analyzer. The expression of FFAR and genes related to local immune responses in the mucosa were analyzed by qRT-PCR and FFAR2 protein expression by Western blotting. For statistical analyses, the SAS MIXED procedure was used including the fixed effects group, gastrointestinal segment and their interactions. Acetate concentrations were lower and lactate concentrations tended to be lower in colon of infected than control calves. Pooled LSMeans of the *FFAR1* and *2* gene expression were lower in infected than control calves. Infected calves showed reduced hydroxycarboxylic acid receptor (*HCAR*) 1 and 2, superoxide dismutase 1, and tumor necrosis factor α gene expression and increased indoleamine 2,3-dioxygenase 1 (*IDO1*), interferon gamma (*INFG*), interleukin 1 β (*IL1B*) and nitric oxide synthase 2 (*NOS2*) expression compared with control calves. *HCAR1* and *2* gene expression was positively correlated to the *FFAR1* and *2* gene expression. In contrast, *IDO1* and *IL1B* gene expression was negatively correlated to *FFAR1* and *2* and *INFG*, and *NOS2* to *FFAR1*. FFAR2 protein expression tended to correlate positively with the *FFAR2* gene expression. The present study revealed significant changes in the intestinal SCFA concentrations and *FFAR1* and *2* gene expression of *C. parvum* infected calves which seemed to be related to the intestinal immune response.

Key Words: diarrhea, cattle, short-chain fatty acid

1622W Evaluation of technologies for early detection of dairy calf pneumonia.

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The incidence of pneumonia in dairy calves is significant and can lead to morbidity or mortality. Early detection could reduce its impact. The aim of this study was to evaluate automated milk feeder (AMF) and accelerometers for their potential to provide early detection of calves with pneumonia. Calves from a commercial farm of approximately 700 lactating Holstein cows were followed over 3 different phases in 2022: phase 1 (February to April), phase 2 (July to September) and phase 3 (September to December). The AMF parameters used in this study were consumption (mL/d), consumption speed (mL/min), and the number of rewarded and unrewarded visits. Accelerometers were used to evaluate the number of lying bouts, the duration of lying bouts (min) and the lying time (h/d). A calf was considered sick when a treatment for pneumonia (first treatment) was given by the producer. Calves were paired, one healthy (no treatment) and one with pneumonia, by phase, and by days at the AMF (less than 2 d difference). Calves had access to the AMF at ± 7 d of age and a total of 72 calves were treated for pneumonia. A linear mixed model was used with day, treatment, and phase, interaction between day and treatment, and phase and treatment as fixed effects. Calf was considered as random effect. We were unable to detect a significant difference for consumption, and the number of rewarded or unrewarded visits. Consumption speed was lower for calves with pneumonia 1 d before treatment (496 ± 21.6 vs. 559 ± 22.7 mL/d; $P = 0.04$). Number of lying bouts were lower (19.9 ± 0.86 vs. 22.5 ± 0.88; $P = 0.04$) and lying time duration was higher (61.4 ± 2.29 vs. 52.4 ± 2.36 min; $P < 0.01$) for calves with pneumonia 1 d before treatment. Lying time per day was higher for calves with pneumonia 1 d (18.6 ± 0.18 vs. 17.6 ± 0.18 h; $P < 0.001$), 2 d (18.3 ± 0.19 vs. 17.4 ± 0.20 h; $P < 0.01$), 3 d (18.0 ± 0.19 vs. 17.5 ± 0.20 h; $P = 0.04$), and 4 d (18.3 ± 0.21 vs. 17.6 ± 0.22 h; $P = 0.04$) before treatment. This study, conducted under commercial conditions, shows that the automated milk feeder and accelerometers have the potential to provide early detection of pneumonia.

Key Words: calf, pneumonia, technology

1623W Distribution of navel size of newborn calves from 23 source dairies in California.

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According to NAHMS, the incidence of calf navel disease is very low (<2%). However, research studies in the Midwest and Northeast of the US observed a prevalence of navel disease that ranged from 14 to 30%. The main objective of this study was to describe the navel size of newborn calves in California dairies, as a proxy for navel disease prevalence. A secondary objective was to identify factors associated with navel size. A convenience sample of 23 source dairies were enrolled after visiting 11 calf raising facilities receiving calves from one (n = 8), 2 (n = 1), 3 (n = 1) and 10 (n = 1) sources. The study included HO (n = 502), JE (n = 230), and Crossbred (n = 173) calves [female (n = 749); male (n = 156)] ranging from 3 to 10 d of age. Measurements of navel diameter were performed by placing the caliper on the cranial side of the navel, proximal to the abdominal wall, on <20 (n = 3), 20 to <40 (n = 9), and ≥40 (n = 14) calves per source dairy. Navel size was classified as adequate (≤13 mm), uncertain (>13 to 20 mm), and inadequate (>20 mm). For our first objective, descriptive statistics were used to summarize navel size classification per farm using quartile distribution (Q1: first percentile; Q3: third percentile). For our second

objective, univariable association of navel size with breed, sex and age was conducted with linear regression models (continuous variable), or generalized logit regression (dichotomized: ≤20 mm, >20 mm) with source farm in the random effect. The proportion of calves classified with adequate navel size per farm was 37.7% (Q_1) and 51.9% (Q_3), whereas with inadequate navel size was 7.6% (Q_1) and 15.8% (Q_3). A significant association of navel size as continuous variable was identified with breed (HO: 15.4 mm, JE: 13.0 mm, Cross Beef: 15.4 mm), sex (female: 14.4 mm, male: 15.9 mm) and age (≤6 d: 13.9 mm, >6 d; 15.3 mm). No significant association of dichotomized navel size was identified with breed or age; however, a trend for a difference with sex was detected (male: 15% vs. female 9%). This study identified an important variation in the distribution of navel size classification between source dairies that warrants future research.

Key Words: navel disease, navel diameter, newborn calf

1624W Preweaning health of dairy heifers is associated with prepartum metabolism of dams. C. Van Dorp, B. Van Winters*, L. Ogilvie, B. Mion, and E. S. Ribeiro, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objective was to evaluate the associations of preweaning health of dairy heifers with prepartum metabolism of their dams. Pregnant cows (n = 273) housed in freestall pens equipped with automatic feeding bins were enrolled in the study 45 d before expected calving and followed until parturition. Body weight (BW), body condition score (BCS), feed intake, and energy balance (EBAL) were summarized weekly. Blood samples were collected on d −21 ± 3, −10 ± 1, −3 ± 1, and 0 relative to calving for analyses of target metabolites. After calving, singleton heifer calves were followed from birth to weaning by 63 d of age. All heifers were managed under the same conditions and data related to colostrum management and health problems (digestive and respiratory) were recorded. Dams were retrospectively categorized into 2 groups: dams of heifers diagnosed with a preweaning health problem (HP; n = 32) and dams of heifers not diagnosed with a preweaning health problem (NoHP; n = 93). Statistical analyses were performed by ANOVA using GLIMMIX of SAS, considering effects of group, parity, time, their interactions, season, and the random effect of cow. No differences in gestation length, birth BW, volume, type, and quality of colostrum fed, or total IgG fed in colostrum were observed between the 2 groups. However, a greater proportion of HP heifers had failure of transfer of passive immunity (19.5 ± 7.2 vs. 6.3 ± 2.6%; $P = 0.04$). Prepartum BW, BCS, feed intake, and EBAL of dams did not differ between groups. Nonetheless, dams of HP heifers had greater concentrations of haptoglobin (0.29 ± 0.04 vs. 0.18 ± 0.02 g/L; $P = 0.02$), glucose (4.4 ± 0.10 vs. 4.1 ± 0.06 mmol/L; $P = 0.07$), ferric reducing antioxidant power (45 ± 1.5 vs. 48 ± 0.9 µmol/L $FeSO_4$; $P = 0.10$), and glutathione peroxidase activity (200 ± 8.2 vs. 182 ± 4.9 nmol/min/mL; $P = 0.07$), and lower concentrations of urea (3.5 ± 0.12 vs. 3.9 ± 0.07 mmol/L; $P = 0.02$) and selenium (68.2 ± 1.0 vs. 70.9 ± 0.6 ng/mL; $P = 0.03$) in serum during the prepartum period. No differences in prepartum concentrations of NEFA, BHB, albumin, and Ca in serum were observed. In conclusion, prepartum metabolism of the dam is associated with the health status of their heifer calf during the preweaning period.

Key Words: DOHaD, disease

1625W Evaluating the effectiveness of a nonsteroidal anti-inflammatory drug as an early intervention strategy for neonatal calf diarrhea. A. Welk*[1], M. C. Cantor[1,2], H. W. Neave[3], and D. L. Renaud[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Science, Penn State University, State College, PA,* [3]*Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark.*

The objective of this study was to evaluate the effects of a nonsteroidal anti-inflammatory drug (NSAID) administered at the time of a milk feeding behavior alert on diarrhea severity and performance in dairy calves. For this double-blind controlled trial, 70 calves were fed up to 15 L/d of milk replacer with an automated milk feeder from 2 to 35 d of age. A milk feeding behavior alert (≤60% relative change in milk intake and/or drinking speed) was used to detect calves at-risk for diarrhea. At their first alert, calves were randomly allocated to receive a single dose of meloxicam (NSAID) or saline (CON). Fecal consistency was scored daily, and calves were diagnosed with diarrhea when they had loose feces for ≥2 d or watery feces for ≥1 d. Calves with low milk consumption (<4 L/d) received 2 L of electrolyte until diarrhea resolution (normal feces for ≥2 d). Body weight was collected at birth and weekly. A cox proportional hazard model evaluated the hazard of treatment on the number of days to diarrhea diagnosis relative to the alert. Mixed models evaluated the association of (1) treatment and duration of the diarrhea bout and (2) treatment and average daily gain (ADG) over the 35 d experimental period. A logistic regression tested the association of treatment with the odds of receiving electrolytes. On average calves triggered an alert at (mean ± SD) 9 ± 2 d of age and were diagnosed with diarrhea at 0.7 ± 2 d relative to the alert. NSAID and CON calves were diagnosed with diarrhea at a similar age relative to the alert (HR = 0.61, $P = 0.07$, 95% CI: 0.35 to 1.04) and had similar diarrhea durations (NSAID: 3.3 ± 0.3 vs. CON: 3.2 ± 0.3 d, $P > 0.10$). NSAID and CON calves also had similar ADG (NSAID: 0.97 ± 0.03 vs. CON: 0.93 ± 0.03 kg/d, $P > 0.10$). However, NSAID calves had reduced odds of being treated with electrolytes compared with CON calves (OR = 0.30, $P = 0.03$, 95% CI: 0.01 to 0.89). Overall, providing an NSAID at the time of a milk feeding behavior alert reduced the odds of a calf receiving electrolytes, but did not affect calf performance.

Key Words: automated feeding, sickness behavior, precision

1626W Inter-rater reliability of scoring systems for abomasal lesions in Quebec veal calves. L. Van Driessche*[1], G. Fecteau[1], J. Arsenault[2], L. Miana[3], Y. Chorfi[4], M. Villettaz-Robichaud[1], P. Hélie[5], and S. Buczinski[1], [1]*Department of Clinical Science, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, QC, Canada,* [2]*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Université de Montréal, St-Hyacinthe, QC, Canada,* [3]École Nationale Vétérinaire de Toulouse (ENVT), Occitanie, Toulouse, France, [4]*Department of Veterinary Biomedicine, Faculty of Veterinary Medicine, Université de Montréal, St-Hyacinthe, QC, Canada,* [5]*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universié de Montréal, St-Hyacinthe, QC, Canada.*

The objective of this study was (1) to determine inter-rater reliability of scoring systems for abomasal lesions in veal calves and (2) compare macroscopical lesions with histology. Abomasal lesions are common in veal calves and usually assessed by visual inspection at slaughter. A total of 76 abomasa of veal calves from 7 different slaughter days were retrieved from a veal slaughterhouse and scored by 4 independent raters after a short training session. The localization of the lesions was separated into pyloric area, fundic area and torus pyloricus. Three different types of lesions were used: erosions, ulcers and scars. To determine inter-rater reliability, Fleiss κ and Gwet's agreement coefficients type 1 (AC1) were used for the presence or absence of a specific lesion in one abomasal area, and intra-class correlation coefficient (ICC) was used for the number of lesions. Overall, a poor to very good inter-rater agreement was seen for the pyloric area and the torus pyloricus regarding the presence or absence of a lesion (Fleiss κ: 0.00–0.34; Gwet's AC1: 0.12–0.83), although a higher agreement was observed when combining lesions in the pyloric area (Fleiss κ: 0.09–0.12; Gwet's AC1: 0.43–0.93). For the fundic area, a poor to very good agreement was observed (Fleiss κ: 0.17–0.70; Gwet's AC1: 0.90–0.97). Regarding the inter-rater agreement for the number of lesions, a poor to moderate agreement was found (ICC: 0.11–0.73). When using the scoring system proposed by Brscic et al. (2011), a poor single random rater agreement (ICC: 0.42; 95% CI: 0.31–0.56) but acceptable average random rater agreement (ICC: 0.75; 95% CI: 0.64–0.83) was determined, indicating that a different score is seen between raters but the order of gravity of lesions is similar. Erosions and ulcers classified by histology were often considered erosions macroscopically and scars classified by histology were often mistaken as ulcers macroscopically. These results show that scoring abomasal lesions is challenging and suggest implementation of a new reliable scoring system. This can help in truly determining possible risk factors to prevent these lesions and thus improve the health of veal calves.

Key Words: ulcer, veal calf

1627W Ruminal pH and subacute ruminal acidosis prediction using artificial intelligence and individual Fourier transform infrared spectroscopy milk analysis. T. Touil*[1], F. Huot[1], S. Claveau[2], A. Bunel[2], D. Warner[3], D. Santschi[3], R. Gervais[1], and É. Paquet[1], *[1]Université Laval, Québec, Québec, Canada, [2]Agrinova, Alma, Québec, Canada, [3]Lactanet, Sainte-Anne-de-Bellevue, Québec, Canada.*

Low ruminal pH for a long period of time could lead to subacute ruminal acidosis (SARA). Subacute ruminal acidosis negatively impacts the health of cows and is associated with monetary losses for the dairy industry. The objective of our study was to predict ruminal pH (rpH) and SARA using artificial intelligence (AI) applied to Fourier transform infrared spectroscopy (FTIR) spectra obtained from routine DHI milk analysis. A total of 107 multiparous Holstein cows were selected from 12 commercial farms in Quebec, Canada, and the ruminal pH was monitored continuously for 90 d using smaXtec wireless boluses. In parallel, a balanced set of 2,040 individual milk samples were collected during the morning (AM, n = 1,020) and the afternoon (PM, n = 1,020) milking. Milk samples were analyzed to obtain FTIR spectra by Lactanet. FTIR and pH data were combined to generate 3 equally balanced data sets to develop AI models: one for the AM samples, another with the PM samples, and a data set composed of AM/PM samples with a total of n = 1,020 samples in each data set. Pre-processing methods including spectra filtering (SF) and taking the first derivative (FD) were tested, and different AI algorithms including partial least squares (PLS) and random forest (RF) were used to predict rpH and SARA. AI models were validated using 15-fold cross-validations. For rpH, the best prediction models presented a root mean square error (±interquartile range) of 0.27 ± 0.02 (SF-FD-PLS), 0.28 ± 0.02 (SF-FD-PLS), and 0.28 ± 0.01 (SF-FD-RF), for AM, PM, and AM/PM data sets, respectively. For SARA, the best models had an accuracy (±interquartile range) of 70 ± 3% (FD-PLS), 72 ± 6% (SF-FD-PLS), and 70 ± 6% (SF-FD-PLS), for AM, PM, and AM/PM data sets, respectively. Those results show that rpH and SARA could be detected from routine DHI milk samples with various levels of performances depending on the pre-processing steps, AI algorithm, and milk sampling time.

Key Words: subacute ruminal acidosis, FTIR, artificial intelligence

1628W Provision of active ventilation to outdoor hutches during summer improves immune function of dairy calves. E. M. Tabor*, G. A. Larsen, S. L. Field, A. R. Guadagnin, and J. Laporta, *University of Wisconsin, Madison, WI.*

The pre-weaning phase is critical for immune system development. Heat stress during this life stage can delay immune system development. We investigated the effect of heat abatement for outdoor hutch-housed calves in summer on immune function. We evaluated markers of innate and adaptive immune function of outdoor hutch-housed dairy calves with access to 2 types of ventilation during summer. Heifer calves were housed in outdoor hutches with passive (PASS, 0.05 m/s air speed, n = 16) or active ventilation (ACT, 1.1 m/s air speed via solar-powered fans, n = 16) from birth to 28 d of life. At d 28, fans were turned off, and heifers were monitored until weaning (d 56). Hemogram and leukogram were evaluated on d 1, 7, 14, 21, 28, 42, and 56 using a VetScan hematology analyzer. Circulating IgG concentration was measured on d 1, 14, and 28 via radial-immunodiffusion assay. On d 28, neutrophil phagocytosis and oxidative burst were assessed by flow cytometry, and the expression of immune-related genes in peripheral blood leukocytes was evaluated by qPCR (e.g., heat shock proteins *HSP72*, *HSP90*; heat shock factor 1 *HSF1*; toll-like receptors 2 and 4 *TLR-2*, *TLR-4*; interleukins *IL-2*, *IL10*, *IL1-β*; transforming growth factor β *TGF-β1*; tumor necrosis factor *TNF-α*; and co-stimulatory molecule *CD28*). Data were analyzed using PROC MIXED in SAS. Circulating IgG was not different between treatments ($P = 0.54$). Relative to heifers exposed to PASS, ACT heifers tended to have reduced white blood cells, neutrophil, and lymphocyte counts ($P < 0.08$), had a greater percentage of neutrophils undergoing phagocytosis ($P < 0.01$) and tended to have greater amounts of reactive oxygen metabolites produced by neutrophils ($P = 0.09$). The mRNA expression of *IL1-β* and *HSP90* tended to be upregulated ($P < 0.10$), whereas *TGF-β1* and *CD28* were downregulated ($P < 0.04$) in peripheral blood leukocytes of PASS relative to ACT. Active ventilation during summer increased heifer's neutrophil phagocytosis capacity, decreased neutrophil and lymphocyte counts, and tended to lower leukocyte mRNA expression of *IL1-β*.

Key Words: neutrophil, heat stress, pre-weaning

1629W Evaluating machine learning algorithms to predict locomotion scoring in dairy cattle. R. Neupane[1], S. Paudyal*[2], A. Aryal[3], and P. Pinedo[4], *[1]Christian-Albrechts-Universität zu Kiel, Kiel, Germany, [2]Department of Animal Science, Texas A&M University, College Station, TX, [3]Department of Construction Science, Texas A&M University, College Station, TX, [4]Department of Animal Science, Colorado State University, Fort Collins, CO.*

On farm locomotion scoring has been popularly used to evaluate dairy cow lameness. However, due to its subjective nature and individual evaluator bias, alternatives to manual locomotion scores (LS) are sought by utilizing automated approaches. In this study, accelerometer data were subject to machine learning algorithms (random forest [RF], naive Bayes [NB], logistic regression [LR]) to predict dairy cow LS. The hypothesis was that accelerometer-derived data, and the extracted features would predict LS that were comparable to visually observed LS by an individual. A total of 310 dairy cows were enrolled in the study and fitted with a leg-based accelerometer on the hind leg. A trained individual performed the LS (scores 1–5) in those animals biweekly. The scores were unevenly distributed into the 5 gait classes, therefore, were classified again into binary classes; moderate (scores 2 and 3), and severe lameness (scores 4 and 5). A training data set was obtained using 80% of accelerometer-derived data for model training, with the 20% of data set aside for model testing. Data augmentation using SMOT (Synthetic Minority Over-sampling Technique) was used to generate additional samples for model training. Performance metrics of accuracy, precision, recall, and F1-score were used for model evaluation. Three versions of each model type were tested: first with simple feature, slope feature, and third model with all features included. The RF had best results (0.89 ± 0.01 accuracy, 0.64 ± 0.04 recall, 0.93 ± 0.01 specificity, 0.64 ± 0.17 precision and 0.64 ± 0.06 F1 score, 0.79 ± 0.04 AUC-ROC) using all features. The model with NB yielded 0.63 ± 0.01 accuracy, 0.21 ± 0.17 precision, 0.45 ± 0.03 recall, 0.28 ± 0.04 F1 Score, 0.55 ± 0.09 AUC-ROC, and 0.66 ± 0.01 specificity utilizing simple features. Model using LR performed low with scores of 0.65 ± 0.01 accuracy, 0.24 ± 0.05 precision, 0.53 ± 0.03 recall, 0.33 ± 0.04 F1 score, 0.60 ± 0.03 AUC-ROC, and 0.67 ± 0.01 specificity utilizing all features. The model did not perform better than previous studies in predicting LS. The application of ML algorithms in accelerometer data should be further explored with larger data sets to develop an ability to predict LS with better accuracy.

Key Words: locomotion score, hoof trimming, machine learning

1631W Evaluating the use of electrolytes or milk replacer to improve surplus dairy calf health and growth outcomes.

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The objective of this study was to assess the impact of feeding milk replacer or 2 different oral rehydration solutions (ORS) on calf health and growth performance during a mid-transportation rest period (RP) after arrival to a calf-raising facility. Surplus dairy calves (n = 128) were transported in 4 cohorts from February to July 2022 for 12 h to a holding facility, rested for an 8 h RP, and transported for an additional 6 h to a calf-raising facility. During the RP, calves were randomly assigned to 1 of 3 treatments: 2 L of milk replacer (MR; n = 43), a high sodium ORS developed for diarrhea (ORS-D; n = 43), or a high potassium ORS developed for transported calves (ORS-T; n = 42). Calves were fed 2 L of their treatment twice, upon arrival and before leaving the holding facility. After unloading at the holding facility, calves were weighed, and health scored. After arrival, calves were weighed, health scored, and housed individually in hutches. After arrival, calves were health scored daily and weighed twice weekly for 14 d and again at 8 wk. The proportion of days with diarrhea (fecal consistency score >2 d/total d) and respiratory disease (>5 respiratory score d/total d), and short-term average daily gain (ADG) were assessed from unloading at the RP to 14 d after arrival to the calf-raising facility long-term ADG was assessed from unloading at the RP to 8 wk after arrival to the calf-raising facility. Linear mixed models were used to assess the impact of treatment on proportion of days with diarrhea, proportion of days with respiratory disease, short-term ADG, and long-term ADG. There was a tendency for calves in ORS-T to have a higher proportion of diarrhea (+16%; $P = 0.08$) and respiratory disease (ORS-T: +23%; $P = 0.06$) compared with MR. There was no treatment effect on short-term ADG (ORS-D vs. MR: $P = 0.13$; ORS-T vs. MR: $P = 0.51$) or long-term ADG (ORS-D vs. MR: $P = 0.24$; ORS-T vs. MR: $P = 0.62$). The results of this study suggest that feeding MR rather than an ORS during a mid-transportation RP could minimize negative health outcomes but does not affect growth outcomes after arrival to calf-raisers.

Key Words: calf, transportation, electrolyte

1632W Associations of maternal gestation length with colostrum quality, and calves' health and performance.

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Our objective was to investigate the associations of maternal gestation length (GL) with colostrum quality and health and performance of their calves from birth to end of first lactation. In Study 1, colostrum volume, IgG concentration, and total IgG secretion (volume × IgG concentration) were measured in 273 cows, which were categorized according to their GL in days (mean = 277; SD = 5) as short (SGL; >1 SD below the mean), average (AGL; within ± 1 SD from the mean), or long (LGL; > 1 SD above the mean). In Study 2, information of birth body weight (BW), health, culling, reproductive performance, and milk production from birth through the end of first lactation were collected from 3,381 heifers, and their dams were categorized based on GL (mean = 276; SD = 6) as described in study 1. Statistical analyses were performed using GLIMMIX of SAS and considered GL category, parity of dam, calf sex (study 1 only), calf breeding type (AI or ET; study 2 only), their interactions, and season. Colostrum of cows with SGL had higher concentration of IgG compared with colostrum of cows with AGL and LGL (back-transformed LSM: 94.2 vs. 74.2 vs. 74.1 mg/mL; $P = 0.04$). Volume (5.3 ± 0.2 L) and total secretion of IgG (432 ± 17 g) did not differ between groups. Birth BW differed between all groups and was positively associated with GL (SGL: 35.0 ± 0.3 vs. AGL: 38.7 ± 0.1 vs. LGL: 41.3 ± 0.3 kg; $P < 0.01$). Morbidity (79.3 ± 2.9 vs. 65.9 ± 1.1 vs. 64.4 ± 3.1%; $P < 0.01$), digestive (37.5 ± 3.4 vs. 28.2 ± 1.1 vs. 23.6 ± 2.7%; $P < 0.01$) and respiratory problems (57.0 ± 3.4 vs. 47.1 ± 1.1 vs. 47.5 ± 3.2%; $P = 0.03$), and multiple health problems (28.0 ± 3.1 vs. 20.4 ± 1.0 vs. 18.7 ± 2.4%; $P = 0.02$) from birth to first calving were higher in SGL calves than in AGL and LGL calves. Short GL calves had higher mortality than AGL calves, but both groups did not differ from LGL calves (16.2 ± 2.4 vs. 11.2 ± 0.7 vs. 14.5 ± 2.2%; $P = 0.03$). No differences in reproductive outcomes of heifers and first lactation cows, or milk production, health, and culling during first lactation were observed. Despite higher concentration of IgG in colostrum, SGL was associated with poorer health and survival of heifer calves from birth to first calving.

Key Words: DOHaD, production

1633W Exploring the impact of *Salmonella* Dublin in cross-bred dairy calves.

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Using data from an existing cohort study, the objective of this analysis was to explore the impact that *Salmonella* Dublin (*S.* Dublin) infection had on health and growth in young cross-bred dairy calves. A total of 160 cross-bred male and female dairy calves, between 3 and 10 d of age, arrived at a grain-fed veal facility. Calves were housed individually for the first 56 d after arrival where they were fed milk replacer twice daily and had free choice water and grain. At d 56, calves were weaned and moved into groups of 5. At arrival to the facility and departure, blood samples were collected, and *S.* Dublin was tested using an ELISA, with positivity declared at a percent positivity of >35%. One wk, 2 wk, and at departure to the facility, fecal samples were also collected and had a bacterial culture to determine *S.* Dublin presence. All calves that died had a post-mortem completed by a veterinarian with samples sent for bacterial culture. Calves had fecal consistency and respiratory scoring completed daily over the trial, with all treatments recorded. Logistic and generalized linear models were built using Stata to assess the impact of *S.* Dublin positivity. At arrival, 1 wk, 2 wk, and departure, 5.6%, 11.4%, 1.3%, and 28.5% were positive for *S.* Dublin, respectively. Overall, including post-mortems, 45.6% of calves were positive at least once during the trial. Calves that were positive for *S.* Dublin had a greater proportion of days with an abnormal fecal consistency score (>2) (24.5% *S.* Dublin vs. 15.9% negative; $P < 0.01$) and received a higher number of treatments for diarrhea (74.0% *S.* Dublin vs. 55.2% negative; $P = 0.02$). In addition, calves that tested positive for *S.* Dublin had a higher odds (odds ratio [OR]: 4.1; 95% CI: 1.5 to 11.0; $P < 0.01$) of mortality compared with calves that were negative for *S.* Dublin. There was also a tendency for calves that tested positive for *S.* Dublin to have a higher number of days with respiratory disease (respiratory score >5) ($P = 0.09$) and treatments for respiratory disease ($P = 0.05$). There was no impact on growth ($P = 0.49$). *Salmonella* Dublin can have a substantial impact and more research is needed to understand disease spread.

Key Words: infectious disease, antimicrobial resistant

1634W Inter- and intra-observer reliability of various indicators used for performing a welfare and health assessment of preweaned dairy calves.

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The objective of this study was to quantify the inter- and intra-observer reliability of various indicators included in a welfare and health assessment of preweaned dairy calves. After a literature review and a consensus building process involving many calf health and welfare experts, a total of 19 possible indicators were retained. Two methodologies (direct observation at the farms and using pictures and videos) were used to quantify the reliability of all indicators by 4 different observers. Animal-based indicators were observed on 3 farms (n = 40 calves), whereas for resource-based indicators pictures and videos from 20 different farms were obtained. Each observer scored the same calves 3 times in a random order over a 2-d period whereas it was performed in the same day for the pictures/videos. Intraclass correlation coefficients (ICC) were computed as reliability measures. Moderate to excellent reliability was considered when the coefficient was greater than 0.5. The majority of indicators had ICC >0.5 (Table 1). A poor reliability (ICC <0.5) was observed for cough, nasal secretion, and ear carriage. Inter- and intra-observer reliability were quantified for various indicators in a welfare and health assessment of preweaned dairy calves. That is an essential step to understand the importance of these indicators and implement them in multiple farms.

Key Words: calf, well-being, score

Table 1 (Abstr. 1634W). Inter- and intra-observer ICC of welfare and health indicators in preweaned dairy calves

Animal-based[1]			Resource-based[2]		
Indicators	Inter	Intra	Indicators	Inter	Intra
Diarrhea	0.960	0.976	Hygiene (calf bedding)	0.727	0.962
Hygiene (side)	0.787	0.850	Humidity (calf bedding)	0.783	0.952
Hygiene (rear)	0.873	0.845	Hygiene (walls)	0.923	0.970
Hygiene (carpus)	0.530	0.827	Hygiene (water source)	0.953	0.982
Weight	0.947	0.928	Hygiene (milk bucket)	0.970	0.998
Length	0.867	0.863			
Hip height	0.870	0.845			
Cough	0.364	0.318			
Nasal secretion	0.274	0.175			
Ocular discharge	0.673	0.823			
Ear carriage	0.357	0.487			
Sunken eyes	0.843	0.943			
Skin tent test	0.640	0.768			
Swollen navel	0.610	0.750			

[1]Indicators classified at the farm.

[2]Indicators classified using pictures and videos.

1635W Effects of kefir supplementation during the first 21 days of life on growth, diarrhea incidence, and antibiotic use in Holstein calves.

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Kefir is a probiotic souce that could be beneficial to calves in the preweaning period. Holstein calves (n = 60 Farm A, n = 40 Farm B, n = 40 Farm C) were randomized to receive a control (CON; 60 mL whole milk Farm A, no supplementation Farms B or C) or 60 mL of kefir (KEF) 1x/d in whole milk (WM) or milk replacer (MR) for the first 21 d of life. Farm A fed MR at 13.6% solids. Farms B and C fed salable or pasteurized WM, respectively. Kefir was prepared on each farm by fermenting WM with kefir grains for 24 h at 20–25°C. Initial body weight (BW) and stature were measured between 1 and 7 d of age. BW and stature were measured weekly at Farm A, and wk 4 and 8 at Farms B and C. Average daily gain (ADG) was calculated for wk 0 to 4 and wk 4 to 8. Feed intake was recorded on Farm A and efficiency was calculated. Health scores were recorded daily on Farm A and weekly at Farm B and C. Feed intake, efficiency, and growth were analyzed using the MIXED procedure of SAS v 9.4, with fixed effects of treatment, time, and their interaction by farm. Block was a random effect. Occurrence of health events and meeting target weight at weaning were evaluated using the GLIMMIX procedure. Farm A MR dry matter intake (DMI) was not different between treatments ($P = 0.54$). Calves receiving KEF had greater starter DMI at wk 8 ($P < 0.01$) compared with CON (1.28 and 1.14 ± 0.02 kg/d, respectively). Feed efficiency was not improved by KEF ($P = 0.48$). Body weight and ADG did not differ between treatments on all farms. The KEF calves on Farm B tended to have greater hip height ($P = 0.06$) and were more likely to reach target weight at weaning (OR: 15.5; 95% CI: 1.6–148.9; $P = 0.02$) compared with CON. Cumulative

d with diarrhea on Farm A was not different between treatments (7.8 ± 0.8; $P = 0.77$). Likelihood of medical intervention for scours was not different on all farms. Kefir supplementation during the first 21 d of life did not improve growth, diarrhea incidence, or need for antibiotic use. Early supplementation with KEF resulted in higher starter DMI before weaning, and further study could examine potential metabolic or intestinal development.

Key Words: calf, probiotic, antimicrobial alternative

1636W The effect of a rest period on health and growth of surplus dairy calves following long-distance transportation. H. M. Goetz* and D. L. Renaud, *Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada.*

The objective of this randomized controlled trial was to determine if providing a rest period to surplus calves transported 16 h mitigates the impact of long-distance transportation on health and growth after arrival to a calf-raising facility. Between September to November 2022, surplus calves ≥7 d old (n = 110) were enrolled 1 d before transportation and randomly assigned to one of 2 treatment groups: 1) continuous transportation by road for 16 h (n = 55) or 2) 8 h of transport, 8 h of rest, then a further 8 h of transport to a single calf-raising facility in Ontario, Canada (n = 55). Calves that received a rest stop were fed 2 L of milk replacer at the time of unloading for the rest period and again before reloading for the second leg of transportation. Calves were weighed before and after transportation and once weekly until they left the facility 11 wk after arrival. Additionally, fecal consistency and respiratory scores were evaluated before loading, after unloading, and twice daily for the 11 wk at the facility. Mixed effects Poisson regression models were used to evaluate the effect of treatment group on the incidence of abnormal respiratory and fecal consistency scores, cox proportional hazards models were used to evaluate associations between treatment group and treatment for diarrhea and respiratory disease, and a mixed linear regression model was used to evaluate the association between treatment group and growth. There was no difference in number of days with a fecal score ≥2 ($P = 0.65$) or respiratory score ≥5 ($P = 0.99$) between treatment groups. A total of 48 (43.6%) calves were treated for diarrhea ($P = 0.44$) and 48 (43.6%) calves were treated for respiratory disease ($P = 0.70$) at least one time after arrival which did not differ between treatment groups. Over the 77 d following transport, calves that received a rest stop had higher average daily gain (+ 94.9 g/d, $P = 0.02$, 95% CI: 17.5 to 172.2) than continuously transported calves. The results from this study suggest that rest periods may not mitigate the impact of long-distance transportation on health, but they may improve subsequent growth at the calf-raising facility.

Key Words: calf transport, male dairy calf, veal industry

1637W Hygiene management practices and ATP luminometry of feeding equipment in preweaned calves on dairy farms in Quebec, Canada. L. Van Driessche*[1], D. E. Santschi[2], É. Paquet[3], D. Renaud[4], É. Charbonneau[3], M. Steele[4], M.-L. Gauthier[5], A. Chancy[1], N. Barbeau-Grégoire[1], and S. Buczinski[1], *[1]Department of Clinical Science, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, QC, Canada, [2]Lactanet, Sainte-Anne-de-Bellevue, QC, Canada, [3]Department of Animal Science, University of Laval, Quebec City, QC, Canada, [4]Department of Population Medicine, University of Guelph, Ontario, Canada, [5]Laboratory of Animal Health, Ministry of Agriculture, Fisheries and Food, St-Hyacinthe, QC, Canada.*

The objective of this study was (1) to measure and compare the contamination of feeding equipment for preweaning calves on dairy farms in Quebec using ATP luminometry, visual assessment and bacteriological analysis and (2) obtain ATP luminometry cut-off values to determine contamination. On 50 commercial farms, a visual score was given and ATP luminometry measurements (expressed as relative light units [RLU]) were obtained using the Hygiena UltraSnap and a liquid rinsing technique for buckets, nipples, bottles, esophageal tube feeders (ET), the tube of automatic milk feeders (AMF), water samples and milk replacer (MR). Additionally, the total bacterial count (TBC) was determined on a subset of these samples using a dilution of 1:1,000 of the liquid on a 3M Petrifilm. After an incubation period at 35°C for 48 h, the TBC could be determined by counting the red-colored colonies using a detection threshold of <1,000 and >250,000 cfu/mL. Comparison between the different methods was done by the Spearman correlation, and a ROC curve was used to obtain optimal RLU cut-off values to minimize misclassification, with a threshold of TBC <100,000 cfu/mL. Characteristics of luminometry measurements are presented in Table 1. Overall, a higher correlation with bacteriological analysis was noticed for ATP luminometry compared with the visual score. This study gave new insights in the measurement of contamination of feeding equipment for preweaned calves on dairy farms in Quebec. By providing ATP luminometry benchmarks, an on-farm tool could become available to objectively determine contamination which could help to evaluate cleaning practices and improve the overall hygiene management and thus health of preweaned calves on dairy farms.

Key Words: calf, feeding equipment, ATP bioluminescence

Table 1 (Abstr. 1637W). Characteristics of RLU measurements taken from the feeding equipment, water and milk replacer available at 50 dairy farms in Quebec, Canada

Item	Bucket	AMF	Water	Nipple	MR	Bottle	ET
Median	621	301	190	132	94	43	15
Interquartile range	171–1,598	137–1,323	71–358	12–376	38–218	4–974	4–121
(number of samples)	(44)	(15)	(45)	(39)	(44)	(41)	(39)
Cut-off values	798	282	1,432	388	93	469	
(number of samples)	(40)	(14)	(43)	(5)	(40)	(36)	/
Sensitivity (%)	78.6	66.7	97.5	100	54.5	85.2	/
Specificity (%)	91.7	100	100	100	61.1	88.9	/

1638W Antimicrobial resistance profiles of *Escherichia coli* from dairy farms participating in an antimicrobial stewardship educational program for farm employees. A. Garzon[1], R. Portillo[2], G. Habing[2], N. Silva-del-Rio*[3], B. Karle[4], and R. Pereira[1], *[1]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, [2]Department of Veterinary Preventive Medicine, Ohio State University, Columbus, OH, [3]Veterinary Medicine Teaching and Research Center, School*

of Veterinary Medicine, University of California, Davis, Tulare, CA, [4]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Davis, Orland, CA.

The increase in antimicrobial-resistant (AMR) bacteria is one of the biggest public health threats of our time. Although current AMR is monitored through reports such as the US NARMS, there is a knowledge gap for on-farm AMR monitoring data. Our goal was to evaluate the AMR of *Escherichia coli* (EC) from pooled fecal samples before and after the implementation of an on-farm animal health and diagnostic training program for farm workers in antimicrobial stewardship in adult dairy cattle. Pooled fecal pat samples were collected from the hospital pen (cows treated with antimicrobials with a milk withhold period), the fresh pen (1 to 5 DIM), and the mid-lactation pens (90 to 150 DIM) in conventional dairies in CA (n = 9) and OH (n = 9). Fecal samples were collected as part of a larger study with a quasi-experimental design that assigned farms to the training intervention group (TG; 9 per state) or control group (CG; 3 per state). For the TG, farm worker(s) identified as having the task of diagnosing and treating adult cows on the farm participated in a training program on antimicrobial stewardship practices. Samples were collected at enrollment and 3 mo after completing the intervention. For each sample, EC was isolated. Standard culture, antimicrobial sensitivity testing using the broth microdilution approach, and categorization of isolates as susceptible, intermediate, or resistant were used. Logistic regression models were used to evaluate the association between EC AMR profiles and farm-level factors. A total of 504 EC isolates were tested for antimicrobial susceptibility. The prevalence of resistant isolates remained unchanged between the control and intervention farms after the training was delivered. Isolates from the hospital pens were 5.04 (95% CI: 1.11–22.97, $P = 0.032$) and 10.54 (95% CI: 2.17–51.18, $P = 0.001$) times more likely to be resistant to amoxicillin/clavulanic acid and chloramphenicol, respectively, than isolates from the mid-lactation pens. Our findings indicate a pen effect on the prevalence of AMR EC within the farm.

Key Words: dairy cattle, antibiotic resistance, antimicrobial stewardship

1639W Survey of *Salmonella* populations in cattle feed across the United States.

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Salmonella are non-spore-forming, gram-negative bacteria belonging to the family *Enterobacteriaceae*. The introduction of *Salmonella* into cattle is commonly transmitted through fecal-oral contamination of feedstuffs and water by animals shedding *Salmonella* on farm, including rodents, birds, and other livestock. Ingestion of *Salmonella* by cattle can be a cause of enteric, reproductive, and sometimes systemic infection. This survey was conducted to give further insight into the presence and diversity of *Salmonella* in feed and water in the cattle industry across the United States. From June 2016 to December 2022, 2,017 feed and water samples from 216 farms across the US were collected and tested for *Salmonella*. Of the samples collected, 720 were total mixed ration (TMR), 494 fermented feeds, 146 water, and 657 other feed stuffs. Fresh feed and water samples were collected, held on ice, and tested for *Salmonella* within 24 h. Black colonies on XLT-4 agar were considered presumptive *Salmonella*. DNA was extracted from each isolate and the CRISPR2 array region was sequenced. Of the 2,017 feed and water samples collected, 128 were *Salmonella* positive (6.3%). The highest prevalence of *Salmonella* was detected in TMR samples (11.3%) and water samples (8.9%). Corn silage and haylage samples had low prevalence of *Salmonella*, at 1.4% and 2.9%, respectively. A total of 184 *Salmonella* isolates were collected and identified. The most prevalent serovars were *S.* Cerro (23.4%), *S.* Kentucky (19.6%), and *S.* Montevideo (16.8%). A previous study also identified *S.* Cerro and *S.* Montevideo as 2 of the most abundant serovars in cow and calf fecal samples, suggesting feed and water as the transmission route for these pathogens in dairy cows. In TMR samples, the most abundant serovars were *S.* Bredeney (23.9%), *S.* Cerro (22.4%), and *S.* Kentucky (14.9%). For water samples, the most abundant serovars were *S.* Montevideo (46.5%), *S.* Cerro (20.9%), and *S.* Bredeney (16.3%). These data provide a better understanding of *Salmonella* populations in feed and water samples from across the US which can be used to guide future research into improved methods of *Salmonella* control.

Key Words: *Salmonella*, serotyping, feedstuff

1640W A comparison of quantitative PCR designs of the IS900 target for detecting *Mycobacterium avium* ssp. *paratuberculosis* in the herd environment and in infected dairy cows.

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Mycobacterium avium ssp. *paratuberculosis* (MAP) is the bacterium responsible for Johne's disease (JD). Infected dairy cows spend much of its life in a subclinical stage, while intermittently shedding MAP into the environment through its feces. Animal diagnosis is a very expensive national strategy. Screening the environment for the presence of MAP using quantitative polymerase chain reaction (qPCR) is a strategy with great advantage. Several commercial diagnostic tests used for the purpose employ licensed gene targets, having some advantage/limitation. IS900 is a multi-copy genetic element that combines high sensitivity and low-cost assay for detecting MAP. Over 10 qPCR strategies have been offered over the past 3 decades. In this study, we conducted an exhaustive analysis of the different molecular strategies. We assessed the sensitivity of the most popular IS900 qPCR designs. The limit of detection (LOD) is defined as the lowest level at which 95% of the positive samples are detected. The LOD of IS900 based strategies were analyzed using 7 replicates and stochastically evaluated by Logit regression to analyze binomial response variables (positive or negative) at 2 genome copies of MAP. The higher performance of IS900 was confirmed for the identification of JD herds using environmental samples (n = 100) and for detecting MAP infected cows of varying MAP shedding levels (n = 100). Dixon-type test was performed to eliminate extreme values. A Student's *t*-test was applied to the Ct values and the fluorescence signal differences (ΔRn) obtained by qPCR. The analytical sensitivity, the ΔRn, and PCR efficiency were all superior for IS900 to commercial kits. IS900 designs were found to be more sensitive for environmental and fecal samples than commercial diagnostic tests ($P < 0.05$). Our analysis confirmed that IS900 based strategies can serve as a more sensitive alternative to commercial kits for application in a national program for monitoring environmental samples to screening for JD positive herds.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, IS900 molecular target, fecal PCR

Breeding and Genetics 3: Omics, AI, and Emerging Technologies

1641W Impact of genomic prediction of daughter pregnancy rate in two reproductive programs combining estrus detection and timed AI in dairy cows. D. Melo*[1], R. Bruno[2], R. Bisinotto[3], and F. Lima[1], [1]*Department of Population Health and Reproduction, University of California, Davis, CA,* [2]*Zoetis Inc., Parsippany, NJ,* [3]*Department of Large Animal Clinical Science, University of Florida, Gainesville, FL.*

Genomic prediction of daughter pregnancy rate (GDPR) is a trait associated with improved pregnancy rates and estrus characteristics in dairy cows that allegedly reduce the need for timed AI. The objectives were to assess the impact of GDPR on time and pregnancy per AI (PAI) for the 1st service and breeding type in 2 reproductive programs combining estrus detection (ED) and timed AI (TAI). Holstein dairy cows were randomly allocated to reproductive program 1 (R1, n = 982) or 2 (R2, n = 942). In R1, cows were enrolled in a Presynch-OvSynch ($PGF_{2\alpha}$ at 36 ± 3 and 50 ± 3 DIM followed by ED and AI or for cows not showing ED, GnRH at 62 ± 3, $PGF_{2\alpha}$ at 69 ± 3, GnRH at 71 ± 3 and TAI at 73 ± 3 DIM). In R2, cows received a $PGF_{2\alpha}$ at 50 ± 3 followed by AI at ED until 82 ± 3 when cows not detected in estrus were enrolled in the OvSynch program (GnRH at 82 ± 3, $PGF_{2\alpha}$ at 89 ± 3, GnRH at 91 ± 3 and TAI at 93 ± 3 DIM). Data for GDPR were categorized in quartiles (Q1 = lowest and Q4 = highest). Data were analyzed using JMP from SAS. In R1, GDPR reduced ($P = 0.03$) days to 1st service (Q4 = 62.8 vs. Q1 = 65.6). In R2, GDPR reduced ($P < 0.001$) days to 1st service (Q4 = 68.5 vs. Q1 = 75.1). The percentage of cows bred after ED was greater for higher GDPR in R1 (Q4 = 53.4% vs. Q1 = 39.1%, $P < 0.001$) and R2 (Q4 = 79.0% vs. Q1 = 62.0%, $P < 0.001$). The percentage of cows pregnant for the 1st service was greater for higher GDPR in R1 (Q4 = 37.6% vs. Q1 = 28.3%, $P = 0.01$) but not in R2 (Q4 = 35.2% vs. Q1 = 27.7%, $P = 0.23$). Breeding type results indicated an impact of GDPR in R1 for cows inseminated after ED (Q4 = 36.1% vs. Q1 = 22.9%, $P < 0.01$), but not for cows inseminated after TAI (Q4 = 38.7% vs. Q1 = 32.3%, $P = 0.19$). In R2, no effects of GDPR were present for ED cows (Q4 = 33.3% vs. Q1 = 22.7%, $P = 0.19$) or TAI (Q4 = 42.5% vs. Q1 = 35.9%, $P = 0.64$). High GDPR (Q4) reduced the interval to 1st service and increased the proportion of cows detected in estrus independent of the reproductive program. However, the benefits of pregnancy were restricted to the R1, where shorter ED intervals had a significant impact on pregnancy outcomes of cows inseminated after ED.

Key Words: conception rate, ovulatory response, dairy cow

1642W Impact of heat stress on automated sensor estrous indicator traits in dairy cattle. G. R. Dodd*[1], C. M. Rochus[1], P. L. Rockett[1], F. Malchiodi[1,2], F. Miglior[1,3], F. S. Schenkel[1], R. L. A. Cerri[4], and C. F. Baes[1,5], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*The Semex Alliance, Guelph, ON, Canada,* [3]*Lactanet Canada, Guelph, ON, Canada,* [4]*Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada,* [5]*Institute of Genetics, Department of Clinical Research and Veterinary Public Health, University of Bern, Bern, Switzerland.*

Heat stress is known to disrupt biological functions in dairy cattle and subsequently limit reproductive ability. In particular, heat stress has been shown to influence the intensity and duration of estrous, which are strong indicators of reproductive outcome. Visual detection of estrous has proven to be challenging and labor intensive. Automatic activity monitors (AAM) are an effective alternative capable of accurately identifying estrous through behavioral changes in restlessness and movement. Several sensor-derived estrous indicator traits (SEI) have been associated with reproductive success including estrous duration (ED), maximum estrous strength (ES_{max}) and mean estrous strength (ES_{mean}). There is currently a lack of information on how environmental conditions affect SEI traits. Understanding this relationship will allow for more effective use of AAM data in breeding and artificial insemination programs. The objectives of this study are to estimate the temperature and humidity index (THI) threshold at which changes in SEI traits are observed, and perform a genetic evaluation of SEI traits modeling heat stress levels based on the temperature-humidity index (THI). This study will utilize 14,794 AAM records on 2,697 lactating Holstein cows collected between 2019 and 2021 from 3 US commercial herds fitted with collar-mounted accelerometer systems. Along with sensor records, the data includes parity, breeding and calving date information for individuals. Weather station data was obtained through National Center for Environmental Information, a publicly available resource provided by the US government. The THI value will be calculated for each record and a threshold will be established through segmented polynomial regression of the 3 traits on the THI values. Once a threshold has been established, a genetic evaluation of these 3 traits will be conducted using a reaction norm model to explore behavior of these SEI traits under heat stress environmental conditions. Results of this study will contribute to growing knowledge of SEI traits and provide context to possible genotype by environment interaction.

Key Words: heat stress, automated sensor trait, estrous detection

1643W Exosome mediated microRNA expression pattern in bovine oviductal fluid derived from different cycle stages. B. Shimelash Abebe*[1,2], D. Tesfaye[1], S. Gebremedhn[1], K. Schellander[1], and T. Wondie Alemu[1,3], [1]*University of Bonn, Institute of Animal Science (ITW), Bonn, Germany,* [2]*Gondar Agricultural Research Center, Gondar, Ethiopia,* [3]*McGill University, Department of Animal Science, Sainte-Anne-de-Bellevue, QC, Canada.*

The oviduct is not simply a passive organ required for transporting embryo to the uterus but also provides suitable microenvironment for the early embryo/gametes. Exosomes have been suggested as nano-sized molecular cargos that can mediate gamete/embryo-oviduct interactions by transferring selected RNAs and proteins. We hypothesize that exosomal messages in oviductal fluid is different in d 1 and 3, and between ipsilateral and contralateral side of the oviduct during estrus. These study aimed to investigates the expression pattern of exosomal miRNA in oviductal fluid from both sides of the ovulation and to determine the temporal expression pattern of exosomal miRNAs. Ten Simmental heifers were estrous synchronized before oviductal fluid flushing. Exosomes were isolated by ultracentrifugation and characterized by nanoparticle tracking analysis, and immunoblotting using exosome-specific protein markers. Exosomal miRNAs derived from the ipsilateral and contralateral side of the same animals were compared both at d 1 and 3 of the estrus cycle. Microarray Affymetrix GeneChip was used to identify differentially expressed miRNAs. Various bioinformatic tools were used to perform target gene prediction and pathway analysis for differentially expressed miRNAs. Results showed there was no differentially expressed miRNAs between both sides at both time points. However, 20 miRNAs were differentially expressed between ipsilateral side at d 1 vs. 3. Similarly, 21 miRNAs were found to be differentially expressed between the contralateral side at d 1 vs. 3. Interestingly, 12 exosomal miRNAs were found commonly expressed and all were upregulated in d 1 compared with d 3. This study identified a series of exosomal miRNAs expressed in bovine oviductal fluid. Target gene prediction and pathway analysis revealed that those miRNAs are involved in ECM signaling, TGF-β signaling and MAPK signaling. Future studies need to be performed to investigate potential horizontal transfer of exosomes to the oviductal cells or early stage embryos to elucidate the role of those miRNAs in early embryo development.

Key Words: exosome, bovine oviduct, microRNA

1644W Identification of novel mRNA isoforms and long non-coding RNA associated with pregnancy status in Holstein dairy cows. H. Sweett*[1,2], E. S. Ribeiro[3], S. J. LeBlanc[4], L. A. Favetta[5], S. Lam[2], F. Miglior[1,2], and A. Cánovas[2], [1]*Lactanet Canada, Guelph, Ontario, Canada,* [2]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* [3]*Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* [4]*Department of Population Medicine, Ontario Veterinary College, Guelph, Ontario, Canada,* [5]*Reproductive Health and Biotechnology Lab, Department of Biomedical Science, Ontario Veterinary College, Guelph, Ontario, Canada.*

Early pregnancy loss is a major challenge and economic loss in the dairy industry and can be affected by a non-receptive uterine environment. Our objective was to determine differences in endometrial expression of novel mRNA isoforms and long non-coding RNA (lncRNA) between non-pregnant and pregnant cows, to better understand the endometrium transcriptome, regulatory elements, and functional markers associated with pregnancy. This study used 82 Holstein dairy cows enrolled in a timed artificial insemination (AI) program at 35 ± 3 d in milk and AI at 62 ± 3 DIM. Endometrial tissue was biopsied from non-pregnant (NP; n = 18) and pregnant (P; n = 18) cows 15 d after AI. Pregnancy was classified based on recovery of an embryo or detection of IFN-tau in uterine luminal fluid. RNA-Sequencing analysis was conducted using CLC Genomics Workbench, where reads were annotated to the ARS-UCD1.2 assembly and differential expression of mRNA isoforms and lncRNA was measured ($FDR < 0.05$; $FC \geq |2|$). A total of 312 mRNA isoforms and 77 lncRNA were differentially expressed (DE) between NP and P cows. Among the DE mRNA isoforms, 64 were previously annotated to the bovine reference genome, 235 were novel lengths of known associated genes, and 13 were novel lengths of unknown genes. Gene prioritization analysis of the 179 known associated genes revealed 69 had a previous association with fertility-related traits and functional analyses revealed several immune-related terms ($FDR < 0.05$). Among the 77 DE lncRNA, we observed a significant association with intracellular signaling cascades involved in fertility including the MAPK, mTOR, PI3K-Akt, and Wnt signaling pathways. QTL annotation using the coordinates of the DE mRNA isoforms and DE lncRNA revealed that 12% and 35% were reproduction QTL, respectively. The identification of novel functional RNA variants may offer candidate markers in the bovine endometrium that can be targeted to determine and study early pregnancy establishment in Holstein dairy cattle.

Key Words: RNA-Sequencing, endometrium, bovine

1645W Genome-wide mapping and characterization of simple sequence repeats in the genome sequence of the world's highest-producing dairy breed—Holstein-Friesian. N. Yang*[1,2], M. Wang[1,3], A. T. Vincent[1], and E. M. Ibeagha-Awemu[3], [1]*Université Laval, Québec, Québec, Canada,* [2]*Yangzhou University, Yangzhou, Jiangsu, China,* [3]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada.*

Simple sequence repeats (SSR) are ubiquitously distributed in most genomes, among which microsatellites is a classical marker. The whole genome sequence of the Holstein-Friesian (HF) released in 2022 lacks SSR annotation, which is crucial to support the effective exploitation of SSRs for increased productivity of this important breed. Therefore, we performed genome wide-identification of SSR and compared the SSRs (1–6 bp motif), compound SSRs (CSSRs) and variable number tandem repeats (VNTRs, 7–30 bp) in HF genome (ARS-LIC_NZ_Holstein-Friesian_1) with the bovine reference genome (Hereford, ARS-UCD1.3). We identified totally 772,767 SSRs, which is less than in Hereford (776,712). We found more CSSRs (49,231) and VNTRs (247,810) in HF than in Hereford (48,763 CSSRs and 241,031 VNTRs). The SSRs accounted for ~0.52% of valid sequences with a density of 5,172.65 bp/Mb in HF. Mono-nucleotide SSRs were the most frequent accounting for 44.3% of total SSRs count and 37.1% of SSRs length, and with highest abundance (128.46 loci/Mb) and density (1,921 bp/Mb) followed by di- and tri-nucleotide SSRs. Most mono-, di-, tri-, tetra, penta, and hexa-nucleotide SSRs had repeat lengths of 12 bp, 14 bp, 15 bp, 16 bp, 20 bp, and 24 bp, respectively. The most abundant standard motifs of SSRs with greater than 10,000 counts were A, AC, AT, AGC, AACTG, ACG, AG, AAAT, ATCAG, and C. The CSSRs consisted of 23,341 SSRs (accounted for 6.38% of total SSRs). The majority of CSSRs consisted of 2 different motifs, while the length and gap length of most CSSRs were 30 bp and 0 bp, respectively. The abundance and density of VNTRs in HF genome were 92.98 loci/Mb and 1,892.07 bp/Mb, respectively. The most abundant motifs in VNTRs was 7 bp long, while motifs longer than 10 bp were rare. Our results unveiled the global patterns of distribution, abundance, and density of SSRs, CSSRs, and VNTRs in the HF genome, providing baseline reference for the further investigation of their possible roles and application in Holstein dairy breed productivity

Key Words: simple sequence repeat (SSR), compound SSR, variable number tandem repeat

1646W Colostrum microbiome as a predictor of future milk solids production. S. Jewell*[1], S. Krishnamoorthy[1], A. Miles[2], and H. Huson[1], [1]*Cornell University, Ithaca, NY,* [2]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.*

The objective of this study was to assess if colostrum microbiome populations differ among cows that go on to produce high and low concentrations of milk solids in lactation with the hope this could be used as a predictor of future production. Colostrum and 5 additional milk samples throughout lactation were collected from 275 Holstein cows from 2 commercial NY herds. The average fat, protein, and total solids percent composition across these samples (excluding colostrum) was calculated for each animal. Animals with averages in the top and bottom quartile of milk fat (HFAT and LFAT), protein (HPROT and LPROT), and total solids (HSOL and LSOL) were grouped and pairwise comparison of colostrum microbial composition were conducted between the high and low group for each solids component. The average milk fat composition across all cows was 4.8% with HFAT and LFAT averaging 9.3% and 1.7%, respectively. The average milk protein composition across all cows was 3.2% with HPROT and LPROT averaging 3.5% and 2.9%, respectively. The average total solids composition across all cows was 13.8% with HSOL and LSOL averaging 18.1% and 10.8%, respectively. For the microbiome analysis, metagenomic DNA was extracted from each colostrum sample and the V4 region of the 16s rRNA gene was amplified and sequenced according to Earth Microbiome Project protocols. Differences in amplicon sequence variants (ASVs) between groups were identified using DADA2, and taxonomic classification were performed by QIIME 2 using SILVA v.138 database at 99% similarity. Regardless of group, the 4 dominant phyla were *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Alpha and β diversity were calculated based on multiple methods. Significantly higher ($P < 0.05$) α diversity was observed in LFAT and LSOL relative to HFAT and HSOL, respectively. Pairwise PERMANOVA testing revealed a statistically significant difference ($P < 0.05$) in β diversity between LFAT and HFAT and LSOL and HSOL groups. In conclusion, we identified differences in colostrum microbial composition between cows that went on to produce high and low milk fat or solids.

Key Words: microbiome, colostrum, milk solids

1647W Genetic parameters and genome-wide association studies for mozzarella and milk production traits, lactation length, and lactation persistency in Murrah buffaloes.

S. Lazaro*[1,2], H. Tonhati[2], H. Oliveira[1,3], A. Silva[2], D. Scalez[3], A. Nascimento[2], D. Santos[4], G. Stefani[2], I. Carvalho[2], A. Sandoval[2], and L. Brito[1], *[1]Purdue University, West Lafayette, IN, [2]College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, SP, Brazil, [3]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [4]University of New England, Armidale, New South Wales, Australia.*

Genetic and genomic analyses of longitudinal traits related to milk production efficiency are paramount for optimizing water buffaloes breeding schemes. Therefore, this study aimed to: 1) compare single-trait random regression models under a single-step genomic best linear unbiased prediction (ssGBLUP) setting based on alternative covariance functions (i.e., Wood, WD; Wilmink, WL; and Ali-and-Schaeffer, AS) to describe milk (MY), fat (FY), protein (PY), and mozzarella (MZY) yields, fat-to-protein ratio (FPR), somatic cell score (SCS), lactation length (LL), and lactation persistency (LP) in Murrah dairy buffaloes (*Bubalus bubalis*); 2) combine the best functions for each trait under a multiple-trait framework; 3) estimate time-dependent SNP effects for all the studied longitudinal traits; and 4) identify the most likely candidate genes associated with the traits. A total of 323,140 test-day records were initially made available. The WD model had the best goodness of fit based on the deviance information criterion and posterior model probabilities for all traits. Moderate heritabilities were estimated over time for most traits (0.30 ± 0.02 for MY; 0.26 ± 0.03 for FY; 0.45 ± 0.04 for PY; 0.28 ± 0.05 for MZY; 0.13 ± 0.02 for FPR; and 0.15 ± 0.03 for SCS). The heritability estimates for LP ranged from 0.38 ± 0.02 to 0.65 ± 0.03 depending on the trait definition used. Similarly, heritabilities estimated for LL ranged from 0.10 ± 0.01 to 0.14 ± 0.03. The genetic correlation estimates across DIM for all traits ranged from −0.06 (186 to 215 DIM for MY-SCS) to 0.78 (66 to 95 DIM for PY-MZY). Numerous relevant genomic regions and candidate genes were identified for all traits, confirming their polygenic nature. The candidate genes identified contribute to a better understanding of the genetic background of milk-related traits in Murrah buffaloes and reinforce the value of incorporating genomic information in their breeding programs.

Key Words: lactation length, mathematical model, milk trait

1648W A comparison of various machine learning techniques and cross-validation schemes to predict dry matter intake using milk spectral data.

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Genetic selection for feed efficiency requires accurate yet cost-effective determination of dry matter intakes (DMI). Our goals were 1) to compare different machine learning methods for the prediction of DMI using milk spectral data and different cross-validation (CV) schemes, and 2) to determine whether key mid-infrared spectral regions are associated with DMI. We compared 5 methods: support vector machines (SVM), elastic net, gradient boosting machines (GBM), partial least squares regression and Bayes B. In addition to milk spectral data, the reference model included various cow factors, including days in milk, parity, body weight, change in body weight and milk component yields. The data set was derived from 4 research stations contributing to the US Holstein database on DMI. Data were based on 28-d averages, leading to 2,989 records on 1,291 cows from 32 different experiments or cohorts. The CV schemes were based on 10-fold cow-independent and experiment-independent CV, both done within each station separately as well as a joint CV study on all data. Herd-independent partitions of training and test sets were also conducted as a final CV study. The smallest root mean square error of prediction (RMSEP) were observed for cow-independent CV schemes with average RMSEP values across stations and the 5 methods ranging from 1.52 to 1.76 kg/d whereas corresponding experiment-independent CV schemes ranged from 1.62 to 2.54 kg/d. In both scenarios, Bayes B was generally among the smallest and GBM and SVM among the largest RMSEP within each station and in the joint CV analyses. No significant differences were observed between the methods for RMSEP in herd-independent CV with average RMSEP ranging from 3.0 to 3.2 kg/d. Hence predicting DMI across management systems may have limited utility; nevertheless, some standardized coefficients for various wavelengths, especially within the Fat A region (1,786 to 1,725 cm^{-1}), were often estimated to be even more important than energy sinks. This suggests that certain spectral regions may have merit for predicting DMI across herds.

Key Words: feed efficiency, milk spectral data, machine learning

1649W Estimation of genetic parameters for milk mid-infrared predicted methane production in dairy cattle.

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Methane emissions from livestock represent 18% of global greenhouse gas emissions and result in a 3–10% loss in energy intake. Therefore, integrating methane production into breeding goals is important to increase the economic and environmental efficiency of dairy cattle. As measuring CH_4 production is expensive and labor-intensive, predicted CH_4 (PCH_4) production based on milk mid-infrared spectra (MIR) could be an alternative. This study aimed to estimate the genetic parameters of weekly average milk MIR predicted CH_4 production and its genetic correlation with MY, FY, and PY. A total of 458 weekly average CH_4 production records (g/d) from 426 first lactation Canadian Holstein cows were used to build a prediction model using a linear Bayesian artificial neural network. Test-day milk MIR, season of calving, and age at calving were used for predicting PCH_4. Then a total of 25,448 weekly average PCH_4 production records and the corresponding test-day records for MY, FY, and PY from 5,041 cows distributed over 200 herds were analyzed. One single trait and 3 separate 2-trait random regression models with Legendre polynomial functions on weeks of lactations of increasing degree (1 to 4) were evaluated. The random regression models included herd-test date and polynomial regression for age-season of calving as fixed effects, and random polynomial regressions for herd-year of calving, animal additive genetic, and permanent environment effects. Legendre polynomials of the fourth degree had the best fit for the fixed and random effects. The estimated average heritability for the weekly average PCH_4 was 0.34, and its average genetic correlations with test-day MY, FY, and PY were −0.12, 0.42, and 0.03, respectively. This study found that weekly average PCH_4 production is moderately heritable, suggesting that it can be used for genetic selection to mitigate CH_4 production. However, the moderate genetic correlation with FY should be considered when selecting animals for lower PCH_4 production to avoid decrease in FY.

Key Words: CH_4 production, milk MIR data, random regression model

1650W Milk somatic cell transcriptome characterization of high and low residual feed intake Holstein dairy cows.

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Feed accounts for the largest expense on dairy farms, leading to the demand for improvement of breeding strategies for more feed-efficient animals. The most common trait measured to represent feed efficiency in cattle is residual feed intake (RFI), which accounts for the animals' actual feed intake and its expected feed requirements for maintenance and growth. Transcriptomics technologies, such as RNA-Sequencing (RNA-Seq), can identify changes in the expression of candidate genes, such as those related to feed efficiency. These candidate genes can be further investigated and ultimately enhance genetic selection decisions. Previous research identified a significant correlation between milk somatic cells and the mammary gland tissue transcriptome, therefore milk somatic cells were analyzed in this study. The objective of this study was to complete RNA-Seq analysis using CLC genomics workbench to identify differentially expressed (DE; FDR <0.05, |FC| > 2) genes in milk somatic cells of Low RFI (n = 22) and High RFI (n = 17) group of animals from a population of 200 milking Holstein cows. In total, 36 genes were DE between Low RFI and High RFI animals. Additionally, these DE genes were significantly enriched in 28 metabolic pathways (FDR <0.01), the majority of which are associated with host immune response including the RIG-I-like receptor signaling pathway. QTL annotation and enrichment analysis were performed using the genomic coordinates of the 36 DE genes. In total, there were 646 previously annotated QTL within the genomic regions of the positional candidate genes and 26.7% were QTL associated with milk production. There were also QTL annotated for feed conversion ratio and residual feed intake. QTL enrichment was also performed to account for the overrepresentation of milk traits in the QTL database. In total, 387 significantly enriched QTL were identified within our gene coordinates. In summary, this study identified potential candidate genes and genomic regions that should be further studied to determine their potential impact on Holstein dairy cows' feed efficiency. This knowledge may enhance genetic selection decisions to improve feed efficiency of Holstein cows.

Key Words: residual feed intake, RNA-Sequencing, dairy cow

1651W Diet digestibility in Holsteins estimated from fecal potentially degradable neutral detergent fiber was heritable and varied across herds and parity.

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Our objective was to analyze the digestive efficiency of Holstein cows using fecal potentially degradable neutral detergent fiber (pdNDF) to determine the association of digestive efficiency with parity, month of lactation (MOL), herd-year, and to estimate heritability. Fecal samples were collected in the morning and afternoon from 893 lactating cows from 3 Pennsylvania, 1 New York, 1 Ohio, and 1 Florida herd. Fecal samples were dried in a forced-air oven for 48 h at 55°C and composite fecal samples were made from morning and afternoon samples on an equal dry-weight basis. The composite samples were sent to a commercial laboratory to evaluate the NDF and undigestible NDF (uNDF) following a 240-h in vitro digestion with pdNDF defined as NDF − uNDF. Fecal pdNDF was analyzed with mixed models implemented in the Echidna program that included fixed effects of parity (1, 2, ≥ 3), herd-year, MOL, and the interaction of parity and MOL; the random effect was animal and there was no variation associated with permanent environment. Least-squares-means (LSM) of pdNDF for parity 1 (24.27 ± 0.34) was lower ($P < 0.001$) than from parity 2 (25.26 ± 0.40) and ≥ 3 (25.72 ± 0.39). The parity by MOL effect ($P < 0.001$) indicated that mo 1 of parity 1 had the lowest pdNDF with an increase as lactation progressed (LSM = 24.82 ± 0.42 for MOL 10), whereas pdNDF was more constant for multiparous cows (range 24.31 ± 0.72 to 25.30 ± 0.66). Fecal pdNDF among herd-years ranged from 18.41 ± 0.71 to 29.80 ± 0.81 ($P < 0.001$). The heritability estimate for pdNDF was 0.13 ± 0.07. Lower fecal pdNDF indicates the animal is more efficient in fiber digestibility, suggesting that early lactation, first parity cows digest fiber more efficiently than multiparous cows. There is significant variation in digestive efficiency across herds and fiber digestion was heritable.

Key Words: pdNDF, digestive efficiency, heritability

Extension Education 1

1652W Consumer confidence in the dairy industry is increased by knowledge of partnerships between producers, processors, and academia. S. Reichler[1], A. Stelick[1], S. Nasberg-Abrams[2], and N. Martin*[1], [1]*Cornell University, Ithaca, NY,* [2]*Columbia High School, Maplewood, NJ.*

Consumer confidence and trust are vital to the long-term success of the dairy industry. In New York (NY), the strong partnerships between producers, processors, and academia are key to providing consumers with safe, high-quality products. Our goal was to determine if exposing consumers to knowledge of these partnerships would improve their confidence in the NY dairy industry. To this end, we produced a 5 min NY dairy industry partnerships video. A survey was developed to evaluate consumer perceptions of the dairy industry, including questions about the consumers' attitudes toward i) dairy products, ii) farmers, processors, and academic institutions, and iii) intent to purchase dairy products. A Solomon 4-group-like design was used to test our hypothesis that consumers informed of the NY dairy industry partnerships video would positively perceive the industry's commitment to providing safe, high-quality dairy products. In total 348 consumers (ages 18–79 yr; 73% female, 25% male, and 2% other) were recruited through existing listservs and social media and completed the online survey. Results were analyzed using a previously described stepwise series of statistical tests that checks for pretest sensitization before testing main effects between treatment and control groups. Our results indicate that consumers who viewed our video showed increases ($P < 0.05$) in positive responses to the following questions; i) overall attitude toward dairy products and dairy product quality; ii) dairy farmers care about the quality of dairy products; iii) dairy processors ensure that consumers have high-quality products through partnerships with universities; iv) partnerships between dairy farmers, dairy processors and universities help provide consumers like me with safe, high-quality products, and; v) I trust that the dairy farmers and processors in my community are working hard to provide high-quality products to me and other consumers. No increase ($P > 0.05$) in positive consumer attitude was observed for intent to purchase questions. Our study highlights the importance of strong partnerships throughout the dairy industry in improving consumer confidence.

Key Words: partnership

1653W Survey of management practices on reproductive performance on South Dakota dairy farms. P. Villamediana* and M. Rovai, *Department of Dairy and Food Science, South Dakota State University, Brookings, SD.*

A survey was conducted from July 2022 to January 2023 regarding current South Dakota dairy cattle management practices that influence the reproductive performance on commercial farms. The bilingual survey (English and Spanish) was developed and administered to the farm's reproductive management assistant (producer/employee). The survey aimed to collect general farm reproductive management data along with the respondent's perception toward reproductive performance. A total of 20 questionnaires were completed, representing approximately 15.0% of all dairy farms in South Dakota. The median number of lactating cows on farms among respondents was 1,783 (range: 20–5,700), and Holstein was the most common breed (47.4% of herds), followed by Jerseys (36.7%), and just 15.9% represented by Brown Swiss and Holstein and Jerseys' crossbreds cows. Mean milk yield was 31.7 kg per day (35.2 and 26.6 kg of milk for Holstein and Jersey breeds, respectively) with a somatic cell count (SCC) lower than 200,000 cells/mL in 36.8% of the farms (23.8% of the cows). Culling rate was 28.2%; reproductive issues and productivity level were the most frequent primary reasons for removal. The mean voluntary waiting period cited by respondents was 61 (range = 28 to 75 d) and did not differ by breed. Automated activity monitoring system is used by 52.6% of the farms, and hormonal synchronization or timed artificial insemination programs were used in 68.4% of the herd. In vitro embryo transfer is used by 31.6%, whereas 10.5% use bulls with the whole herd. Pregnancy rate ranged between 30.0 and 40.0% for 36.8% of the respondents and over 40% for 10.5% of them. Lameness and mastitis were ranked as the most important cause of animal health concerns (40.0 and 45.0% of the respondents, respectively), whereas the greatest reproductive challenge raised by the respondents were conception rate and estrous detection (35.0 and 30.0%, respectively). Strategic workshops designed to better explain reproductive goals was perceived as a need by both dairy producer and employees. Several challenges and opportunities were identified to improve reproductive management practices (e.g., age to first calving, use of beef and sexed semen, and relationship between SCC and pregnancy rate). Results of this study can provide a useful benchmark regarding the commonly used reproductive outcomes on South Dakota dairy farms.

Key Words: dairy farm, survey, reproductive management

1654W A summary of breeding practices on Illinois dairy farms. B. R. Lenkaitis*[1], D. T. Nolan[2], F. C. Cardoso[2], C. A. Hayes[3], and J. P. Hutchins[1], [1]*Department of Agricultural and Consumer Economics, University of Illinois, Urbana, IL,* [2]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [3]*Department of Veterinarian Medicine, University of Illinois, Urbana, IL.*

Survey questionnaires requesting breeding information were mailed to 437 Illinois dairy herds. A total of 61 surveys were returned (14%). The objective of the survey was to determine the breeding decisions and costs of Illinois dairy farmers. Surveys were mailed out on April 18th, 2022, and collected on September 25th, 2022. Microsoft Excel (Microsoft, Redmond, WA) was used for the descriptive statistics of individual questions. Dairy farmers were asked to indicate their herd size and total bulk tank production. Respondents with herd sizes under 100 comprised 51% of responses, with the average herd size being 156 lactating cows. The average bulk tank production for all survey responses was 6,771.68 kg/farm per day. Survey participants were asked to select and rank what goals (production, health, and type) are most important in their breeding program. "Production (44%) and health (36%) were ranked first by respondents," indicating that most Illinois dairy farmers can easily trade off production and health. When dairy farmers were asked to rank areas of health, mastitis and infertility were both tied for first rank 34% of the time. Dairy farmers were asked whether they selected for Net Merit Dollars, Total Performance Index, Grazing Merit Dollars, Udder Composite, and Feet and Legs Composite when making breeding decisions. They were then asked to rank their selected indices in order of perceived importance. Net Merit was selected by 57% of farms. However, it was the index that was most commonly ranked first. Calving ease and milk, fat, and protein yields were selected the majority out of the 11 Predicted Transmitting Abilities that were listed. Out of the Predicted Transmitting Abilities that were chosen most the time, calving ease was the only one that did not rank in the top 5 for the entire pool of survey respondents. Survey respondents were asked about semen costs. The average reported costs for non-sexed, sexed, and beef semen were $25, $40, and $12/straw, respectively. In conclusion, most Illinois dairy farmers are production and fertility oriented with Net Merit Dollars, Udder Composite, and Feet and Leg Composite being the highest-ranked indices selected.

Key Words: breeding cost, breeding goal, breeding information

1655W Case Study: On-farm refractometer assessment of colostrum quality and passive immune transfer in dairy calves.

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On-farm assessment of colostrum quality is important to monitor the passive transfer of immunity (PTI) to calves. Colostrum quality and PTI were evaluated in a commercial dairy farm (South Dakota, USA). Calves were separated from their dams upon delivery and weighted. Colostrum was harvested, classified to first (≥50 IgG mg/mL) and second (≥20 to 50 IgG mg/mL) feedings using a colostrometer (BIOGENICS, Florence, OR), pasteurized at 60°C for 60 min, and frozen at −20°C. Before feeding, colostrum was thawed, and the Brix values (BV) were measured with a digital refractometer (MISCO, Solon, OH). Although colostrometer and Brix values are not the gold-standard methods, they are useful as calf-side decision tools. Holstein-Jersey heifer calves ($n = 73$; birth BW = 31.7 ± 4.4 kg) received 3.8 L colostrum within 30 min after birth and a second feeding 5.5 h later. Blood serum protein values were measured within 3 d after birth as a proxy of PTI using a digital refractometer (MISCO). Although the best quality colostrums were frozen, the BV before feeding revealed that only 53 and 33% of first and second colostrums, respectively were classified as good (BV ≥ 22.0%). Nevertheless, blood serum protein values averaged 6.58 ± 0.81 g/dL, surpassing the threshold of successful PTI (≥6.20 g/dL). Accordingly, all calves were weaned without major health problems. In conclusion, the typical colostrum cutoff of BV ≥ 22.0% was not related to successful PTI. Reasons could be: 1) BV might underestimate the colostrum quality, and 2) colostrum was fed within 30 min of life, resulting in successful PTI regardless of colostrum quality.

Key Words: dairy calf, colostrum quality, refractometer

1656W Evaluation of Healthy Farms Healthy Agriculture (HFHA) learning modules: Knowledge and intention.

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Animal health management resources on the Healthy Farms Healthy Agriculture (HFHA) website include youth-oriented online learning modules, which introduce concepts of disease transfer and risk management as well as techniques for talking about these concepts. Web accessibility was improved in modules published in early 2022. Four core modules cover (1) What is Biosecurity? (2) Routes of Infection and Means of Transmission, (3) Finding Sources of Disease Transmission Risk, and (4) Biosecurity Strategies. Two additional modules, Public Speaking for Biosecurity Advocates I and II, present a primer on public speaking using an example of an illustrated talk on livestock biosecurity. Learners are invited to respond to a post-completion survey in Qualtrics. As of January 24, 2023, 89 post-completion survey responses were received, and 58 usable responses have been summarized. Respondents were from Montana (20), Wisconsin (10), Pennsylvania (9), and at least 5 other states and were in grades 6–8 (12), 9–12 (38), or above (2). Modules were accessed through Wisc-Online (43), the HFHA website (5), and both sites (10). Most (43) felt confident about helping others practice good biosecurity, but only 2 had plans to make a formal presentation. Eighteen rated themselves as knowledgeable or very knowledgeable before and after completing the modules; all but 2 increased their knowledge after indicating they were initially slightly knowledgeable (28) or not at all knowledgeable (11). The top 4 behaviors learners planned to implement in the next 6 mo were (a) manage manure and dirty bedding effectively (36), (b) protect feed and water from contamination (34), (c) isolate and treat sick animals (33), and (d) vaccinate against diseases of concern effectively (33). The reported knowledge gain and intention to implement biosecurity practices suggest the modules are effective learning tools. Follow up surveys are designed to allow us to track the implementation of these intentions. Funding for this effort was partially supported by the National Institute of Food and Agriculture, US Department of Agriculture, under award number 2015–69004–23273, and Hatch project VT-H02907.

Key Words: biosecurity, online learning module

1657W Extension programming demonstrates that participants believe biochar would benefit agriculture and environmental sustainability.

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Dairy manure management is a critical aspect of every dairy operation. Often dairy manure is used as a fertilizer for crop production. Although dairy manure is very nutrient-rich, high concentrations of nutrients such as P and N can affect soil, water, and air. Texas A&M AgriLife is investigating the use of biochar as a soil amendment to manure-applied fields on water quality, soil health, and crop production. Since 2019, 3 Extension programs, one face-to-face research showcase (RS; $n = 19$), and 2 webinars (WEB; $n = 61$), were held for researchers, industry, and producers. The objectives were to increase the knowledge of what biochar is, its value as a soil amendment, and its perceived economic value. After each program, a 15-question survey was used to assess the perception of knowledge gain, overall value, and economic impact of biochar. Statistical analyses were only done for RS using SAS 9.2 (SAS Inst. Inc., Cary, NC) as sample size and completion of the WEB surveys prevented assessment of any valuable trends. With a small sample size, a matched pairs analysis was used to assess the change in understanding after RS on 2 topics: biochar and its application for crop production, and the use of biochar as a soil amendment and manure management. Significance declared at $P < 0.05$. The RS surveys ($n = 15$) represented 12,876 cows and 8,503 acres. There was a significant increase in the understanding of biochar ($P < 0.01$) and its perceived use to help improve water quality, soil health, and crop production ($P < 0.01$). Of the 15 RS surveys, 6 were producers and perceived the estimated economic impact of biochar to be near $12.08 to $17.72/cow/year and $8.84 to $13.96/acre/year with a 95% CI. Exploratory results from WEB surveys ($n = 21$) represented 16,745 cows and 4,550 acres and 100% increased their knowledge of biochar and believed it would benefit agriculture. The RS and WEB demonstrated that attendees believed their knowledge of biochar increased, perceived it as a beneficial soil amendment, and believed biochar would be economically valuable. Funding was provided by USDA-CIG, Project #: NR213A750013G032; NRCS, Grant #: NR213A750023C001; CBG-NLGCA, Grant #: 2020–70001–31552.

Key Words: biochar, extension programming, knowledge gain

Forages and Pastures 2

1658W Effect of growth stage/cutting time and intercropping of whole plant oat with whole plant faba bean forage hay on ruminal degradation and intestinal digestion in dairy cows. C. Nagy, D. A. Christensen, H. (B.) Lardner, V. H. Guevara Oquendo, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada.*

The objectives of this study were to determine the effect of growth stage/cutting time and intercropping of whole plant oat with whole plant faba bean forage hay on ruminal degradation kinetics and intestinal digestion in dairy cows. The intercropping oat and faba bean were grown in 3 fields and were cut at 3 growth stages: cutting stage 1 with whole oat plant at the inflorescence stage and whole faba bean plant at the flat pod stage; cutting stage 2 with whole oat plant at the milk development stage and whole faba bean plant at the milk pod stage; cutting stage 3 with whole oat plant at the soft dough stage and whole faba bean plant at the late pod stage. The rumen degradation was carried out using in situ technique with rumen cannulated lactating cows. The intestinal digestion was estimated using the modified 3-step in vitro method. The experimental design was a RCBD with the cutting stage as a fixed effect and growth fields and animals as random block effects. The data were analyzed using the Mixed model procedure in SAS 9.4. The results showed that with increasing cutting stage, the rumen potential degradable fraction (D) was decreased from 43 to 35% of DM and undegradable fraction (U) was increased from 28 to 34% of DM ($P < 0.05$) without affecting the degradation rate (K_d), the soluble fraction (S) and the effective degradable DM content (EDDM) with average of 5.95%/h, 29% of DM, and 508 g/kg, respectively. The cutting stage significantly impacted ($P < 0.05$) the effective degradable protein (EDCP) and NDF content (EDNDF). With increasing cutting stage, the EDCP was decreased from 158 to 126 g/kg DM and EDNDF from 190 to 135 g/kg DM. However, the intestinal digestibility of rumen undegradable protein (% dRUP) was not significantly affected by the cutting time with an average of 42% of RUP. In conclusion, the growth stage/cutting time affected rumen degradation kinetics, however, did not affect RUP intestinal digestibility of intercropped whole plant oat with whole plant faba bean forage.

Key Words: intercropping oat and faba bean forage, rumen degradation kinetics, intestinal digestion

1659W Raising heifers on pasture reduced heifer feed cost and improved income over feed cost in the first lactation of dairy cows. C. H. P. Camisa Nova*[1], K. F. Kalscheur[2], D. Jaramillo[3], and G. E. Brink[2], [1]*Animal and Dairy Science Department, University of Wisconsin–Madison, Madison, WI,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI,* [3]*US Dairy Forage Research Center, USDA-ARS, Marshfield, WI.*

Raising heifers on pasture rather than in confinement has been proposed to reduce heifer cost. Previous study has shown that heifers reared on pasture improved first lactation milk yield (MY) compared with heifers reared in confinement. Thus, it impacts economically dairy farms. The objective of this study was to evaluate the economics of heifers raised either on pasture or in confinement. Thirty-six Holstein heifers were divided into 2 regimens: 1) 18 heifers were rotationally stocked on grass pastures for 5 mo in the first year, then 6 mo in the second-year grazing season (3 groups of 6), and 2) 18 heifers were simultaneously reared in a free-stall barn (3 groups of 6) and fed a TMR. After first calving, all animals were managed under a conventional regimen for lactating dairy cows fed a TMR having corn and alfalfa silage and concentrate feeds. At 50, 100, 150, and 200 DIM, MY, DMI, and feed prices for both regimens were recorded for posterior evaluation. The IOFC in the first lactation was simulated using the milk price in Nov. 2022 (class III milk price) and the adjusted feed cost for lactating cows based on inflation from 2013 to the feed price in Nov. 2022. In the first grazing season with greater ADG for housed heifers (0.93 vs. 0.82 kg/d; $P = 0.025$), raising heifers on pasture decreased feed costs 26.7% compared with heifers raised in confinement (US$142.47 vs. US$194.48/heifer). During the second grazing season, with similar ADG comparing heifers in barns and on pasture (0.75 vs. 0.66 kg/d; $P = 0.260$), heifers on pasture decreased feed costs 58.4% compared with heifers raised in confinement (US$97.43 vs. US$234.18). After calving, MY was greater (+3.54, +4.05, +4.31, +6.40 kg; $P = 0.007$) as well as DMI (+1.80, +1.04, +0.64, +1.80 kg; $P = 0.0143$) for cows raised on pasture when heifers. Simulating the IOFC, it was greater for cows raised on pasture when heifers (US$+1.13, +1.59, +1.82, and +2.53/cow/d, respectively). In conclusion, raising heifers on pasture reduced feed cost as well as increased the IOFC through the first lactation compared with heifers raised in confinement.

Key Words: feed cost, heifer cost, pasture

1660W Effects triticale silage harvested at boot or soft-dough stages on performance of Holstein milking cows. M. Schultz*[1], G. Ferreira[1], J. Steger[1], K. Payne[2], W. Thomason[1], and S. Stewart[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA.*

This study evaluated the effects of harvesting time of triticale (*Triticosecale*) and dietary forage inclusion on milk yield and milk composition of lactating dairy cows. A field of triticale was harvested on April 28 (boot stage; BS) and May 25 (soft-dough stage; SDS) and ensiled in concrete-walled bunker silos for at least 60 d. The BS silage contained 9.5% CP and 61% aNDFom, whereas the SDS silage contained 8.2% CP and 63% aNDFom. Eight primiparous (584 ± 21 kg of BW and 105 ± 11 DIM) and 16 multiparous (710 ± 57 kg of BW and 105 ± 18 DIM) Holstein cows were randomly assigned to 1 of 4 diets in a replicated 4 × 4 Latin square design with 21-d periods. Diets included BS or SDS triticale silage with dietary forage inclusion of 54 (high-forage; HF) or 36% (low-forage; LF). Dry matter intake was measured individually using a Calan gate system (7 last d of each period). Cows were milked twice daily (1:00 a.m. and 1:00 p.m.). Milk composition was determined on 4 consecutive samples collected on d 15 and 16. Performance was evaluated using the MIXED procedure of SAS, and the statistical model included the fixed effects of square, harvesting time, forage inclusion, and their interaction and the random effects of cow and period. Feeding LF diets increased DMI (27.7 vs. 26.5 kg/d; $P < 0.01$) but harvesting time did not affect DMI. Feeding BS diets (42.6 vs. 41.5 kg/d; $P < 0.05$) and LF diets (42.9 vs. 41.1 kg/d; $P < 0.01$) increased milk yield. Feeding SDS diets tended to increase milk fat concentration (4.64 vs. 4.49%; $P < 0.10$) but forage inclusion did not affect milk fat concentration. Feeding LF diets increased milk protein concentration (3.12 vs. 3.06%; $P < 0.01$) but harvesting time did not affect milk protein concentration. Feeding LF diets increased fat-corrected milk yield (49.6 vs. 46.9 kg/d; $P < 0.01$) but harvesting time did not affect fat-corrected milk yield. None of the main factors affected feed efficiency ($P > 0.36$). In conclusion, under the conditions of this study, harvesting triticale at SDS relative to BS increased fat concentration but fat-corrected milk yield and feed efficiency did not differ.

Key Words: small grain, boot stage, soft-dough stage

1661W Organic Matter Digestibility Index (OMDI) for evaluation of corn silage quality for dairy cows.

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The nutritive value of corn silage (CS) is determined by digestibility of its main nutrients. The Organic Matter Digestibility Index (OMDI) was developed to assist nutrition consultants and dairy producers in evaluating corn silage hybrids quality based on common laboratory analyses, such as neutral-detergent fiber (NDFD) and starch digestibilities. Objectives of the current study were to determine the relationship of CS OMDI with (1) ruminal in situ (IS) degradability of dry matter (DM) and (2) milk production variables (IV) from experiments in which CS was the main forage in the diets of lactating dairy cows. For the IS experiment, 5 CS hybrids were ensiled for 0, 30, 60, 120, and 150 d and incubated in the rumen of 3 rumen-cannulated dairy cows for up to 72 h. Samples were analyzed for IS effective degradability of DM (DMED) assuming a rate of passage of 0.06/h. For the IV evaluation, a database from 25 dairy cow experiments (n = 1,113 cows; 119 ± 46 d in milk; and 43 ± 9 milk yield, MY) in which CS was fed at 40 to 51% of dietary DM was compiled and the relationships between CS OMDI and production variables were investigated using a mixed model regression analysis with random study effect. There was a relatively strong relationship ($P < 0.01$) of CS OMDI with ruminal DMED of CS hybrids (DMED % $= -23.2 + 1.20 \times$ OMDI; $R^2 = 0.76$). Corn silage OMDI also related positively ($P < 0.01$) with in vivo DM intake (DMI kg/d $= 12.9 + 0.22 \times$ OMDI; $R^2 = 0.74$; RMSE = 0.48) and MY (MY kg/d $= 23.5 + 0.35 \times$ OMDI; $R^2 = 0.77$; RMSE 0.70), but not energy-corrected MY (ECM; $P > 0.05$). Relationship of CS digestible OM and NDF intakes (calculated based on OMDI and NDFD) with ECM was high and positive ($P = 0.01$; $R^2 = 0.76$; RMSE = 1.31 and 1.05, respectively) but confounded with CS intake and overall DMI. There was no relationship of CS digestible starch intake with MY or ECM. Although further verification is needed, the developed OMDI relates positively with ruminal DM degradability and in vivo DMI and MY and may be a useful tool in assessing the nutritive value of CS for dairy cows.

Key Words: corn silage, organic matter digestibility index, dairy cow

1662W Different forages for lactating Murciano-Granadina dairy goats.

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It is important to optimize forage choices for improved milk production and goat health. Our hypothesis was that alfalfa hay (AH), corn silage (CS), and wheat straw (WS) can be utilized by lactating Murciano-Granadina goats toward efficient milk production and that feed intake and efficiency and milk quantity and quality responses as well as metabolic parameters would differ among the forage sources. The objective was to determine the effects of feeding different major forages on feed intake, feeding behavior, milk production and composition, and circulating blood metabolites in lactating Murciano-Granadina goats. Thirty lactating goats were used in a completely randomized design study with 3 treatments including diets containing 1) WS, 2) AH, or 3) CS at 40.3% of diet dry matter. Treatment diets were formulated to be isoenergetic (ME) and isonitrogenous (CP). Ten goats were assigned to each treatment. Forage source affected ($P < 0.01$) dry matter intake (DMI), such that the highest intake was for CS (1,904 g/d) and the lowest intake was for WS (1,406 g/d) with AH being intermediate (1,674 g/d). The yields of raw and fat-corrected milk, milk protein, lactose, and solids-nonfat were greater ($P < 0.01$) for CS than for other 2 forages. Milk contents of fat, total solids, urea nitrogen, unsaturated fatty acids, polyunsaturated fatty acids, and blood concentrations of total proteins were greater ($P < 0.05$) for AH than for other forages. Milk somatic cell counts tended to be lower ($P < 0.10$) for CS than for AH and WS. Treatments did not affect ($P > 0.10$) times spent standing, lying and ruminating, and blood concentrations of glucose, albumin, and nonesterified fatty acids. Total protein concentrations in serum were, however, greater ($P < 0.05$) for AH than for CS and WS. In conclusion, different forage choices can be fed to lactating Murciano-Granadina goats with different aims; CS for increased milk yield, AH for improved milk fat content, and WS for lower feed cost. Changes in forage cost and availability, production systems and strategies, and consumers' demand will determine how to optimize forage choices for lactating goats.

Key Words: feeding behavior, forage, metabolite

1663W Dietary neutral detergent fiber influence on fecal bacterial mass in sheep.

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Examining how dietary NDF affects fecal bacterial mass in sheep was our objective. Dry matter digestibility is a function of dry matter intake and fecal output which consists of undigested plant material, bacterial mass, and endogenous losses. Knowing the proportion of fecal bacterial mass to undigested feed matter may improve digestibility estimates for different diets. Sheep were offered one of 3 diets (n = 24), where daily NDF intakes ranged from: 0.64 kg NDF/day ± 0.19 (Hi), 0.55 kg NDF/day ± 0.13 (Med), and 0.16 kg NDF/day ± 0.00 (Lo). Sheep were fed assigned diet for at least 2 weeks before fecal collection. Fecal samples were collected from individual sheep, freeze-dried, and ground. Samples were run in triplicate. A common method for determining bacterial mass in human feces was used where a 0.5 g subsample was weighed into a 50 mL centrifuge tube, shaken for 10 min with 40 mL formylsaline (NaCl 0.9% wt/vol and formalin 1% vol/vol), and 0.5 mL of 10% sodium lauryl sulfate. Mixture was filtered through a nylon mesh (aperture size: 150 µm) under vacuum and residue left on the filter was washed an additional 3 more times. Residue was dried for 48 h at 105°C and weighed. Remaining filtrate was filtered through a second filter (aperture size: <2 µm) and dried for 48 h 105°C. Statistical analyses were performed in R using a one-way AVOVA where diet was the independent variable and fecal bacterial percentage was the dependent variable. Tukey's HSD post-hoc test was performed for pairwise analysis. Sheep on the Hi diet had a greater proportion of fecal bacteria vs. Med and Lo diets (33.6 ± 4.7% vs. 25.1 ± 3.9% and 17.1 ± 2.9%, $P = < 0.01$). Microscopic observations revealed undigested plant material contamination within the bacterial pellet, demonstrating that further method development is needed to successfully detach and separate the fecal bacteria from the undigested plant material. In summary, currently used methods to determine fecal bacteria do not account for NDF in sheep diets regardless of level.

Key Words: fecal bacteria, NDF, sheep

1664W Evaluation of an in-field near infrared device for dry matter determination.

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The objective was to compare DM predictions by a portable near infrared (NIR) device (SCiO Cup, Consumer Physics, St. Cloud, MN) to conventional oven drying on alfalfa haylage (AH), corn silage (CS), and fresh hay (FH). In Experiment (EXP) 1, AH (n = 34), CS (n = 30), and FH (n = 30) were scanned 3 times at room temperature (20°C, RT) in 5 Cups. The entire Cup contents were dried for 24 h at 60°C in a forced air oven to determine DM. Bias, standard error of prediction (SEP), and R^2 were calculated for each Cup. Data were averaged across the 5 Cups and regression analysis was used to evaluate deviation of slopes from 1 and intercepts from 0. In EXP 2, 13 AH and 10 CS were divided into 3 representative subsamples to compare DM predictions in cold (4°C) and warm (39°C) conditions to RT. Bias, SEP, and R^2 were calculated for each temperature. Regression analysis was used as described for EXP 1 to determine deviation of slopes from 1 and intercepts from 0. The DM for AH, CS, and FH in EXP 1 ranged from 29.4–54.0, 29.5–47.7, and 11.1–62.5%, respectively. The greatest biases among the 5 Cups were 1.78, 1.37, and 1.53 for AH, CS, and FH, respectively. Slope did not differ from 1 nor intercept from 0 for AH ($P > 0.10$, $R^2 = 0.98$, SEP = 0.99). For CS, the intercept was not different than 0 ($P = 0.18$) but slope tended to be greater than 1 ($P = 0.08$, $R^2 = 0.95$, SEP = 1.52). The slope and intercept deviated from 1 and 0, respectively, for FH ($P < 0.05$, $R^2 = 0.97$, SEP = 2.14). In EXP 2, the DM of AH ranged from 29.7–52.7% and DM of CS from 29.2–36.8%. When scanned at 4°C, the slope for AH was not different from 1 ($P = 0.19$) but intercept tended to differ from 0 ($P = 0.06$, $R^2 = 0.97$, SEP = 1.93). At 39°C, slope tended to differ from 1 and intercept from 0 ($P > 0.10$, $R^2 = 0.97$, SEP = 1.13). The CS slope deviated from 1 and intercept from 0 ($P < 0.05$, $R^2 = 0.89$, SEP = 2.31) at 4°C but slope and intercept did not differ from 1 or 0, respectively, for CS scanned at 39°C ($P > 0.10$, $R^2 = 0.52$, SEP = 1.81). The SCiO Cup is effective for measuring DM within target harvest ranges. Optimal performance can be obtained by allowing samples to equilibrate to room temperature before scanning.

Key Words: moisture

1665W Occurrence of major mycotoxins in maize silage from 2022 harvest around the globe.

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Maize silage is widely used as a major source of fiber and energy for dairy cows in many countries around the globe, often representing around half of their dry matter intake. One of the main factors determining silage quality is the mycotoxin contamination, which can occur not only during storage but also during the development of the maize in the fields. The objective of this survey was to measure the prevalence and concentration of contamination of maize silage from harvest 2022, by the 6 major mycotoxins. Within the DSM Mycotoxin Survey, the longest running survey, 947 samples of maize silage from the global harvest of 2022 (September 2022 to February 2023) have been collected. The distribution of samples from region of the world were 97 from Asia and Oceania, 523 from Europe, 4 from Africa, 218 from North America (US and Canada) and 95 from South America (Mexico to Argentina). The methods of analysis were LC:MS/MS and HPLC. Deoxynivalenol (DON) was the most common mycotoxin with 66% prevalence and average of positives 821 ppb (n = 927, all concentrations expressed on dry matter basis), followed by zearalenone (ZEN, 55%, 188 ppb; n = 930), fumonisins (FUM, 41%, 856 ppb; n = 772), aflatoxins (Afla, 5%, 15 ppb; n = 778), T-2 toxin (2%, 38 ppb; n = 710), and ochratoxin (OTA, 3%, 5 ppb; n = 718). Globally, 59% of the samples analyzed for more than 3 mycotoxins, contained also more than one mycotoxin. This shows that co-occurrence is common, and that maize silage can be a source of multiple mycotoxins. The profiles of contamination vary between the regions. The main mycotoxins of risk in Asia and Oceania are ZEN (62%, 897 ppb), DON (55%, 4,116 ppb) and Afla (14%, 84 ppb), in Europe are DON (72%, 695 ppb) and ZEN (59%, 167 ppb), in North America DON (74%, 2,484 ppb) and ZEN (40%, 609 ppb) and finally in South America ZEN (60%, 174 ppb) and DON (30%, 1,469 ppb). The results of this survey underline that the risk of mycotoxin contamination when using maize silage as the base of a diet for dairy cows is high, as they are commonly contaminated by not only one mycotoxin but combinations of them, which adds up to the risk of health and performance losses.

Key Words: maize silage, mycotoxin, contamination

1666W Effect of defoliation frequency around flowering time on the nutritive value of orchardgrass.

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Determining the optimal defoliation frequency is essential to increase the productivity of pasture-based dairy systems. An experiment was carried out to study the effect of the defoliation regimen around flowering time on the evolution of chemical composition and nutritional value of orchardgrass. The experiment was conducted on a 4-year-old orchardgrass (*Dactylis glomerata* cv 'INIA Perseo') pasture at the experimental station "La Estanzuela" (INIA, Uruguay). Three defoliation regimens cut at 2 leaf-stage (L2), 4 leaf-stage (L4), or 2 pre-flowering and at 4 leaf-stage afterward (L2–4) were studied. Defoliation treatments were arranged in a completely randomized block design with 5 repetitions (blocks). The DM, NDF, CP and in vitro digestible NDF (NDFD; Ankom Technology Corp., Macedon, NY) production (kg of DM/ha) were determined for each defoliation event and treatment through the spring season (08/28/2019 to 11/25/2019). The evolution during the spring of each variable was studied through linear regression including the block as a random effect, the treatment as an independent categorical variable, and the days of biomass accumulation as a continuous independent variable. The slopes of the regressions were compared using the "ESTIMATE" statement of the MIXED procedure. There were 3 harvest events for L2 and L2–4 and 2 for L4 treatments. The DM accumulation rate did not show differences ($P > 0.1$) between treatments (71 kg DM/ha/d on average for the 3 treatments). The CP content of the pasture decreased (−0.250%/d average of 3 treatments; $P < 0.01$) and NDF content increased (0.191%/d average of 3 treatments; $P < 0.01$) along the spring without differences between treatments for both fractions. The DNDF decreased in all treatments ($P < 0.01$), being higher for L2–4 than for L2 or L4 (−0.402%/d vs. −0.199%/d and −0.272%/d, $P < 0.01$, respectively). Defoliation frequency, however, had no effect ($P > 0.1$) on total DNDF production (26.7 kg/ha/d, average for the 3 treatments). Defoliation frequency of an orchardgrass pasture in spring did not significantly modify its nutritional value.

Key Words: orchardgrass, defoliation regime, nutritive value

1667W Ruminal fermentation, *in vitro* digestibility, gas production parameters and chemical composition of some cool season grasses.

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Table 1 (Abstr. 1668W). Effect of harvesting time, post 48 h of soaking and 2% of hypochlorite on the quality of HGF

	Nutritional analysis							
Harvest	DM (kg/m^2)	CP (%)	Ash (%)	EE (%)	ADF (%)	NDF (%)	Ca (%)	P (%)
8 days	4.65^b	10.35^c	1.55^c	2.00^b	12.59^b	18.64^c	0.00452^{bc}	0.342^a
12 days	4.93^b	11.75^b	1.86^b	2.08^b	15.03^{ab}	21.70^{cb}	0.00604^a	0.375^a
14 days (25 cm length)	4.99^b	12.26^{ab}	1.99^{ab}	2.45^a	15.25^{ab}	24.15^{ab}	0.00476^b	0.378^a
15 days	6.44^a	13.22^a	2.22^a	2.47^a	18.74^a	27.98^a	0.00437^c	0.389^a
P-value	≤0.01	≤0.01	≤0.01	≤0.05	≤0.10	≤0.05	<0.001	>0.05
R^2	0.85	0.84	0.86	0.76	0.88	0.93	0.96	0.54
CV (%)	8.02	5.28	7.41	8.14	8.40	6.73	3.47	7.08

$^{a–c}$Different letters in the same column represent statistical differences between harvesting days.

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An experiment was conducted to investigate the ruminal fermentation, *in vitro* digestibility, gas production parameters and chemical composition of selected genotypes of 4 cool season grasses (including: *Festuca arundinacea*, *Bromus inermis*, *Dactylis glomerata*, and *Phalaris aquatica*). For this propose seeds of 4 grasses were planted as randomized complete block design with 4 treatments and 3 replications in research farm of Agricultural Biotechnology Research Institute of Iran (ABRII), Tabriz. Samples were taken from the mentioned species in the vegetative stage. The fermentability and gas production potential of samples were measured by gas test method with 200 mg of each sample at 2, 6, 8, 12, 24, 48, 72 and 96 h after incubation. Fermentation parameters and digestibility were calculated using the produced gas at 24 h after incubation. Data analysis was performed as Duncan test (α = 0.05). Based on the results, the tested grasses showed significant differences for all measured traits ($P < 0.05$). Dry forage yield was high in *Dactylis glomerata* and there was no significant in other species ($P < 0.05$). The crud protein percentage was higher in *Phalaris aquatica* and lower in *Festuca arundinacea* ($P < 0.05$). The percentage of neutral detergent fiber and acid detergent fiber were the highest in *Festuca arundinacea* and the lowest in *Phalaris aquatica* ($P < 0.05$). The amount of short chain fatty acid, non-fiber carbohydrates, organic matter digestibility, and microbial protein synthesis content in *Bromus inermis* were better than other 3 species ($P < 0.05$). The amount of digestible energy and dry matter intake were the highest in *Phalaris aquatica* and the lowest in *Festuca arundinacea* ($P < 0.05$). The highest of GP at 24 and 48 h was in the *Bromus inermis* and the lowest in *Festuca arundinacea* and *Dactylis glomerata* ($P < 0.05$). Based on the results, *Bromus inermis* in comparison with other species had better nutritional value.

Key Words: chemical composition, digestibility, farm

1668W Development of a protocol to produce low-technology hydroponic green fodder of sorghum (*Sorghum bicolor*). D. Redrovan*[1,4], P. Melendez[2], A. Sierra[3,4], and M. Moncada[4], [1]*Texas Tech University, Amarillo, TX,* [2]*City University of Hong Kong, Hong Kong, China,* [3]*University of Florida, Gainesville, FL,* [4]*Zamorano University, Francisco Morazan, Honduras.*

The development of strategies that minimize land use for grazing is a priority, especially in the tropic areas. Hydroponic Green Fodder (HGF) is a promising technology to produce biomass of high nutritional quality, without soil and less water; however, the procedures and techniques have not been well standardized. The objective of this study was to design a low-technology protocol by evaluating factors that influence the quality and yield of HGF sorghum (*Sorghum bicolor*) for adaptation by small-scale producers in dry tropical areas of Latin America and the Caribbean. This study was conducted at Zamorano University in Honduras, using a factorial completely randomized design, testing 4 concentrations of sodium hypochlorite, 3 times of soaking, and 4 d of harvesting. A standardized operating procedure was developed and adapted to the crop needs and low-technology infrastructure; consequently, the optimum relative humidity and temperature for HGF were 74.31% and 24.5°C, respectively. Results revealed that 2% sodium hypochlorite decreased seed mold growth by 98.34% ($P \leq 0.01$). Soaking for 48 h led to 95.4% germination ($P \leq 0.01$). Nutritional analyses of sorghum HGF showed greater DM yield, CP, EE, ADF, NDF, and ash with time, and maximal output at 15 d after sowing (Table 1). In summary, we developed a reproducible protocol for hydroponic sorghum that could potentially be scaled up for feeding livestock using low technology for small-producers with minimal land and water use.

Key Words: hydroponic, sorghum, Central America

1669W *In situ* degradability of diets with forage corn (*Zea mays*) silage and banana residues (*Musa paradisiaca*). I. Espinoza*[1], A. Sánchez[1], A. Barrera[1], D. Romero[1], M. Medina[1], E. Torres[1], A. García[2], C. Barba[2], and G. Alvarez[1], [1]*Universidad Técnica Estatal de Quevedo, Quevedo, Los Ríos, Ecuador,* [2]*Universidad de Córdoba, Córdoba, España.*

The objective of the work was to determine the in situ rumen degradability of forage corn silage and banana residue in different proportions. The treatments were T1, 100% corn silage and 0% banana residues; T2 corn silage 90% and banana residues 10%; T3 corn silage 80% and banana residues 20%; T4 corn silage 70% and banana residues 30%; and T5 corn silage 60% and banana residues 40%. A randomized block design was applied with 5 treatments and 4 repetitions (cows with fistulated rumen) and 7 incubation times (0, 3, 6, 12, 24, 48, and 72 h) for the differences in the means, the Tukey test ($P > 0.05$) was used. Maize forage harvested at 90 d and stored together with the banana residue in plastic bags of 40 kg capacity was used. The fermentation process lasted 30 d, the samples were dried in a forced-air oven at 65°C for 48 h, and ground in a Thomas Wiley mill with a 2-µm sieve. For the in situ digestibility of the dry matter, a 10 × 21 cm nylon bag with a 5 µm pore size was used, with 10 g of ground sample, with 0, 3, 6, 12, 24, 48, and 72 h of incubation. The in situ digestibility of dry matter increased ($P < 0.05$) with T4 with 40% inclusion of banana residue (62.38%) in relation to T1, T2, T3, and T4 (55.14, 56.46, 57.88, and 59.73% respectively). The in situ ruminal degradability parameters of the dry matter of forage corn silage with banana residues, is a viable alternative using 40% of due to the dynamics of rumen dry matter degradation.

Key Words: diet, fermentation, agricultural residue

Growth and Development 2

1670W Reproductive performance and milk yield in the first lactation of Holstein calves in northern Mexico fed in the pre-weaning stage with milk at 15% solids. E. Carrillo-Moreno*[1], E. Carrillo[2], E. Perez-Rebolloso[3], D. Carrillo-Moreno[1], M. Mellado[1], and F. G. Veliz[1], *[1]Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, México, [2]Instituto Tecnológico de Torreón, Torreón, Coahuila, México, [3]Centro de Bachillerato Tecnológico Agropecuario No. 47, León Guzmán, Durango, México.*

The objective of this study was to evaluate the effect of feeding at the pre-weaning stage (0–60 d of age) on reproductive parameters (age at gestation, services per conception and age at first calving) and productive (milk 305 ME and total lactation milk) from Holstein calves fed whole milk with 15% total solids milk replacer (15% TS) or only milk (11% TS). Seventy-eight calves were completely randomly divided into 2 feeding groups (n = 39/group) 15% TS or 11% TS (5–8 L/day); Treatments included starter and water ad libitum. Subsequently, at weaning, both groups were treated under the same feeding and management scheme. Variables were analyzed using PROC MIXED at SAS (SAS Institute Inc., Cary, NC). The age at gestation was lower in calves fed at 15% ($P < 0.02$) than in calves fed at 11% (409 ± 21 vs. 425 ± 31 d), the number of services per conception was lower ($P < 0.01$) for calves fed at 15% than calves fed at 11% (1.44 ± 0.82 vs. 2.15 ± 1.20 no. services). There were no differences in the weight of the calves at 13 mo, but if a higher trend was marked in the calves fed at 15%, they were 12 kg heavier (433 ± 25 vs. 421 ± 30 kg), which is explained by the average daily gains (ADG) since at that age the calves fed at 15% show a higher ADG ($P < 0.02$) than the calves fed at 11% (989 ± 50 vs. 954 ± 61 gr). Age at first calving was lower ($P < 0.05$) for 15%-fed calves than for 11%-fed calves (685 ± 22 vs. 700 ± 32 d). Milk production of calves fed 15% was 700 kg higher ($P < 0.02$), both in total production (9,750 ± 1,339 vs. 9,028 ± 1,119 kg) and milk at 305 ME (12,607 ± 1,268 vs. 11,831 ± 1,218 kg) than calves fed at 11%. In conclusion, this study showed the benefits and scope of a feeding program that supplies milk at 15% TS in the pre-weaning stage to improve the general growth of the animals and the development of the mammary gland parenchyma (PAR), the scope of these effects is long-term, and permanently, because it also stands out that it improves the reproductive and productive parameters of the calves.

Key Words: growth, pre-weaning, milk yield

1671W Performance of the growth and development of F_1 Holstein cattle in northern Mexico fed at the pre-weaning stage with a high volume of milk at 14% solids. E. Carrillo-Moreno*[1], E. Carrillo[2], E. Perez-Rebolloso[1], M. Mellado[3], D. Carrillo-Moreno[1], and F. G. Veliz[1], *[1]Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, México, [2]Instituto Tecnológico de Torreón, Torreón, Coahuila, México, [3]Centro de Bachillerato Tecnológico Agropecuario No. 47, León Guzmán, Durango, México.*

This study aimed to evaluate the effect of feeding F_1 Holstein × beef breeds (beef production scheme in intensive dairy systems) milk with 14% total solids (14% TS; enriched with the addition of milk replacer) or milk only (11% TS) on growth parameters. During the pre-weaning stage (0–60 d of age) both groups received a high volume of milk (450 L/60 d). Eighty calves were randomly allotted into 2 groups which were fed milk containing 14 or 11% TS. These 2 groups were subdivided by sex (males and females) forming 4 groups (n = 20/group). Data were analyzed by ANOVA which included the group × sex term (Proc GLM of SAS). At the end of the pre-weaning stage (60 d), calves in the 14% TS group were 6.6 kg heavier ($P < 0.01$) than the 11% TS group (99.45 vs. 93.28 kg). Average daily gain (ADG) was greater ($P < 0.01$) for the 14% TS group than the 11% TS group (989 vs. 850 g/d); the group × sex interaction was not significant. Preweaning weight gain did not differ for males and females in the 14% TS group (100.6 vs. 98.3 kg for males and females) and males and females in the 11% TS group (95.8 vs. 90.8 kg for males and females, respectively). Postweaning ADG for 14% TS calves was 992 g for males and 986 g for females with no significant difference. Also, in the 11% TS group, postweaning daily weight gain did not differ between males (848 g/d) and females (854 g/d). At 72 d of age, body weight was higher ($P < 0.01$) for the 14% TS than the 11% TS calves (105.8 ± 9.5 vs. 100.3 ± 13.4 kg, respectively). At this age, ADG did not differ between the groups (499 ± 39 vs. 452 ± 56 g/day for 14% TS and 11% TS, respectively). At 105 d of age, body weight was higher ($P < 0.05$) for the 14% ST group (136 ± 14) than the 11% TS group (128 ± 19 kg). Similarly, ADG was greater ($P < 0.01$) for the 14% TS group (915 ± 11) than for the 11% TS group (821 ± 16 g) than the 11% TS group. This study demonstrated the benefits of using a high volume of milk feeding program adjusted to 14% TS on the growth of F_1 Holstein × beef breeds and depicts a feasible nutrition practice to markedly improve profitability for the dairy industry, combining milk yield and meat production.

Key Words: beef on dairy, growth, pre-weaning

1672W Variation in calf starter intake before weaning: Long-term consequences on growth and production. E. R. Russell*, M. A. G. von Keyserlingk, and D. M. Weary, *University of British Columbia, Vancouver, British Columbia, Canada.*

The transition from milk to solid feed can be difficult for some calves, especially when weaned from higher milk allowances. Calves vary in their solid feed intake before weaning, but it is unclear if higher intakes benefit growth and production over the longer term. The aim of this study was to describe the variation in calf starter intake and ADG of calves weaned from high milk allowances (20% BW) and to explore associations between these measures and age at first calving and first lactation milk yield. We conducted a meta-analysis of 2 published weaning studies (n = 25, n = 47 calves, respectively). Calves were offered 12 L/d of whole pasteurized milk from an automated milk feeding system until d 30 of age. At d 31 of age a range of gradual weaning treatments were applied, intended to result in different intakes of solid feed before the calves were fully weaned from milk by d 70 of age. Calves had *ad libitum* access to a calf starter via an automated feeder that also measured daily intake. Starter intake over the weaning period (d 31–69 of age) averaged 0.69 ± 0.44 kg/d, ranging among calves from 0.01 to 2.06 kg/d. Similarly, ADG averaged 0.67 ± 0.24 kg/d, and ranged from 0.03 to 1.13 kg/d. For every 1 kg increase in starter intake, ADG increased by 1.3 ± 0.3 kg/d. Moreover, for every 1 kg increase in ADG during the weaning period, heifers freshened 42 ± 17 d earlier. We found no effect of ADG before weaning on 305-d milk yield in the first lactation. Our results illustrate that starter intake and ADG both vary widely before weaning. In addition, low intakes relate to low ADG before weaning, and that low ADG before weaning delays age at first calving. These results reinforce the importance of understanding the effects of different feeding strategies to improve solid feed intakes during the weaning phase.

Key Words: calf, weaning, ADG

1673W Effects of a *Megasphaera elsdenii* oral capsule on reticulorumen volatile fatty acid dynamics and papillae development in dairy-beef calves.

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This study evaluated the effect of an oral probiotic capsule (*Megasphaera elsdenii* NCIMB 41125; Lactipro FLX Calf, MS Biotec) on reticulorumen VFA dynamics and papillae development in calves. Holstein × Angus calves (n = 31; 45.3 ± 7.1 kg; 8.2 ± 2.0 d old) were enrolled in a 76 d randomized trial in 2 blocks. Calves were assigned to one of 3 treatments: placebo (ME_0), a single dose of probiotic on d 15 (ME_{15}), or probiotic administration on d 15 and 39 (ME_{15+39}). Calves were individually housed and had unlimited access to water and calf starter (18% CP, 2.5% fat). Calves were fed 7 L/d of milk replacer (Cows Match Warm Front, Land O Lakes; 150 g/L, 2x/d) until d 41. On d 42, milk was reduced in half until weaning on d 56. Reticulorumen fluid was collected by esophageal tube on d 14, 35, 49, 58, and 70. Calves were harvested starting on d 77. At harvest, reticulorumen fluid and tissue were collected from the ventral sac. Reticulorumen fluid was analyzed for VFA concentration by gas chromatography. Reticulorumen papillae length, width, surface area, and thickness of the strata were recorded using an image software. Probiotic effects on reticulorumen VFA and papillae development were determined using a mixed model. Treatment tended to affect total VFA ($ME_0 = 86.8 \pm 21.7$, $ME_{15} = 102.2 \pm 21.7$, $ME_{15+39} = 106.5 \pm 21.5$ mmol; $P = 0.07$) and acetate ($ME_0 = 51.8 \pm 16.0$, $ME_{15} = 63.0 \pm 15.9$, $ME_{15+39} = 64.9 \pm 15.9$ mmol; $P = 0.08$) concentrations during the trial. Also, treatment affected propionate concentration ($ME_0 = 25.6 \pm 3.4$, $ME_{15} = 28.2 \pm 3.5$, $ME_{15+39} = 30.7 \pm 3.3$ mmol; $P = 0.04$) during the trial. Treatment did not affect VFA dynamics at harvest. However, it affected papillae length ($ME_0 = 2.5 \pm 0.4$, $ME_{15} = 3.6 \pm 0.4$, $ME_{15+39} = 2.7 \pm 0.3$ mm; $P = 0.01$), width ($ME_0 = 1.8 \pm 0.1$, $ME_{15} = 2.0 \pm 0.1$, $ME_{15+39} = 1.8 \pm 0.1$ mm; $P = 0.04$), and surface area ($ME_0 = 14.8 \pm 1.5$, $ME_{15} = 19.3 \pm 1.6$, $ME_{15+39} = 16.1 \pm 1.4$ mm^2; $P = 0.01$). Probiotic treatment did not affect the papillae epithelium strata. Overall, intervention with *M. elsdenii* tended to positively affect VFA dynamics and positively affected papillae development of dairy-beef calves. Future research should investigate the effects of *M. elsdenii* on reticulorumen microbiome.

Key Words: beef on dairy, probiotic, acidosis

1674W Supplementation of tyndallized *Lactobacillus helveticus* paraprobiotic to Holstein dairy calves on health and performance around weaning.

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The objective of this study was to determine how tyndallized *Lactobacillus helveticus* (TLH) supplementation impacts the health and performance of newborn calves that are exposed to an abrupt weaning model (d 35–42). A total of 44 newborn bull calves were randomly assigned to 1 of 2 treatments and were fed at a rate of 2.5 g of premix/feeding containing either Control (CON, n = 22) consisted in a carrier with no paraprobiotic, and treatment tyndallized *Lactobacillus helveticus* (TLH, n = 22) providing 2×10^9 cfu/d/calf of TLH. Treatments were added to the milk replacer (MR) at the morning and evening feedings and were fed until d 42. Calves received 6 L/d of MR (150 g powder/L) daily, which was increased to 9 L/d one week after arrival and fed split over 3 meals. Starter feed was offered ad libitum starting on d 28. Calves were weighed weekly from d 1–56 (BW), and intake of MR and starter feed were recorded daily. Calf health scores were recorded daily, based on the Wisconsin calf health scoring system, and included fecal consistency (0–1 = normal; 2–3 = abnormal or diarrhea), rectal temperature, and respiratory score (considered respiratory disease with a score of ≥5). Additionally, fecal samples were collected weekly to evaluate fecal dry matter. Data were analyzed using a generalized linear mixed model in SAS with fixed effect of treatment, day, its interaction, and random effect on group. A binary distribution was used for the health scores data. No treatment differences were detected for BW, MR intake before weaning and ADG during the postweaning period ($P = 0.60$, $P = 0.51$, and $P = 0.72$, respectively). There was a treatment by day interaction for starter intake, wherein TLH calves had higher intake postweaning, from d 44 to 55 ($P = 0.01$). No differences were detected for abnormal fecal consistency ($P = 0.50$), respiratory score ≥5 ($P = 0.80$), or rectal temperature ($P = 0.53$). No differences were detected for fecal dry matter between treatments ($P = 0.57$). In conclusion, supplementing TLH did not help with growth or health, but may have improved starter intake after weaning.

Key Words: paraprobiotic, health and performance, weaning

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1675W The effect of parity on insulin, lactoferrin and insulin-like growth factor-1 in colostrum and transition milk. S. L. Cartwright*[1], A. J. Fischer-Tlustos[1], K. Hare[1], M. Toradès[2], M. Terré[2], A. Aris[2], E. Garcia-Fruitós[2], and M. A. Steele[1], *[1]Department of Animal Biosciences University of Guelph, Guelph, Ontario, Canada, [2]Ruminant Production, Institute of Agrifood Research and Technology, Caldes de Montbui, Spain.*

Relatively few studies have evaluated if differences between parities exist for the concentration of bioactive molecules in colostrum and transition milk. Therefore, the objective of this study was to determine the effect of parity on the concentration of insulin, lactoferrin and insulin-like growth factor-1 (IGF-1) in colostrum and transition milk. Colostrum and milk samples were collected from 10 multiparous (MP) and 10 primiparous (PP) Holstein dairy cattle. Cattle were milked twice per day and colostrum samples were taken within 12 h after calving with calves removed immediately, followed by samples collected at milkings 2 through 5 and 12. Concentrations of insulin, lactoferrin and IGF-1 in all samples were determined using commercial enzyme linked immunosorbent assay kits validated for colostrum and milk. Differences between parities for the concentration of each bioactive molecule were analyzed using the GLIMMIX procedure in SAS OnDemand, with the models including the fixed effects of parity, milking, and the interaction between parity and milking. Results showed PP cows had greater concentrations of insulin in colostrum (PP = 55.12 ng/mL SE = 9.85 vs. MP = 12.8 ng/mL SE = 2.42; $P < 0.01$) and milking 2 (PP = 22.26 ng/mL SE = 5.63 vs. MP = 8.4 ng/mL SE = 1.37; $P = 0.04$) and greater concentrations of IGF-1 in milking 12 (PP = 18.83 SE = 3.18 ng/mL vs. MP 11.1 ng/mL SE = 0.89; $P = 0.04$) relative to MP cows. Conversely, MP cows had greater lactoferrin concentration in colostrum (MP = 1.21 mg/mL SE = 0.53 vs. PP = 0.26 mg/mL SE = 0.04; $P = 0.07$), milking 2 (MP = 0.62 mg/mL SE = 0.3 vs. PP = 0.13 mg/mL SE = 0.03; $P = 0.10$), milking 3 (MP = 0.41 mg/mL SE = 0.16 vs. PP 0.10 mg/mL SE = 0.01; $P = 0.05$), milking 4 (MP = 0.18 mg/mL SE = 0.03 vs. PP = 0.09 mg/mL SE = 0.01; $P = 0.01$), milking 5 (MP = 0.18 mg/mL SE = 0.04 vs. PP = 0.07 mg/mL SE = 0.01; $P = 0.01$) and milking 12 (MP = 0.11 mg/mL SE = 0.01 vs. PP = 0.05 mg/mL SE = 0.01; $P < 0.01$) relative to PP cows. These results suggest that concentrations of bioactive molecules in colostrum and transition milk differ between parities. These may have implications for neonatal calf health when feeding.

Key Words: bioactive molecule, parity, transition milk

1676W Effect of palmitic, stearic, and oleic acid on lipogenic genes in bovine mammary epithelial cell culture. A. Haile*[1], A. Lisuzzo[2], E. Fiore[2], and K. Harvatine[1], *[1]Pennsylvania State University, State College, PA, [2]University of Padua, Viale dell'Universita, Padua, Italy.*

Palmitic (PA; C16:0), stearic (SA; C18:0), and oleic (OA; *cis*-9 18:1) acid are the 3 most abundant fatty acids (FA) in dietary supplements and duodenal flow in dairy cows and have different effects on milk fat yield. We hypothesized that PA, SA, and OA have specific and unique regulatory roles impacting mammary lipogenic pathways. Our objective was to determine the direct effect of PA, SA, and OA on mammary lipogenic gene expression. Most in vitro work is done in a FA free media, but to better mimic plasma, DMEM media was supplemented with 500 μM of a cocktail of FA (30% PA, 30% SA, 30% OA, 5% linoleic and 5% linolenic). A bovine mammary cell line (MAC-T) was grown to 80% confluence on plastic, serum starved for 18 h, and treatments applied for 24 h. Key lipogenic enzymes and regulators were analyzed by quantitative RT Real-time PCR. Treatments were FA free media, the basal FA cocktail, and the cocktail plus 50, 100, 250, and 500 μm of PA, SA, and OA. Data were analyzed using JMP Pro 16 and means separated using a protected LSD with significance declared at $P < 0.05$. The basal FA cocktail drastically upregulated *CD36* (+10987%), but reduced other lipogenic genes including *SCD* (−240%), *FASN* (−166%), and *SREBP1C* (−286%) and had no effects on *AGPAT6*, and *DGAT1*. Compared with the FA cocktail alone, the addition of 500 μM PA increased *SCD* +31%, quadratically decreased *FASN* up to −53% at 250 μM and decreased *CD36* up to −35% at 250 and 500 μM. Addition of SA decreased *SCD* −47% and *FASN* −80% at 500 μM, decreased *SREBP1C* up to −60% at 250 and 500 μM, and increased *CD36* +21% at 100 μM and reduced it −68% at 500 μM. Increasing OA reduced *FASN* −58% at 500 μM, quadratically decreased *SREBP1c* with maximal reduction of −44% at 250 μM, ($P = 0.05$), and reduced *CD36* up to −34% at 500 μM. In conclusion, *CD36* was strongly stimulated by the FA cocktail. Using a FA cocktail may allow a better representation of plasma FA supply, but a lower concentration may better mimic post-peak cows. In general, SA more potentially inhibited genes related to de novo FA synthesis compared with PA.

Key Words: milk fat, lipogenesis, saturated fat

1677W Evaluation of prepartum body condition score and its changes after calving on the milking performance, ruminal fermentation, and blood parameters in dairy cows. Y. Hao*[1,2], T. Ouyang[1], W. Wang[1], Y. Wang[1], Z. Cao[1], H. Yang[1], L. Guan[2], and S. Li[1], *[1]China Agricultural University, Beijing, China, [2]University of Alberta, Edmonton, Alberta, Canada.*

Body condition score (BCS) has been recognized as one of the important factors affecting dairy cows' milking performance and longevity. Here, we evaluated whether prepartum BCS (PBCS) and BCS change patterns (BCSCP) from prepartum to 21 d in milk (DIM) were associated with the milking performance, rumen fermentation, and blood parameters of dairy cows. Seventy-three multiparous Holstein dairy cows with similar parity and expected date of parturition were enrolled in this study, and BCS was assessed at 21 d before expected calving and 21 DIM. Rumen fluid and blood samples were collected on −21, 21, and 60 DIM, and the milk samples were collected on 21 and 60 DIM. All the data were analyzed using a mixed model with PBCS, BCSCP, DIM, and their interaction as fixed effects, and cow as random effects, and an ANOVA Post hoc test via Tukey pairwise multiple comparisons. The BCS of cows were categorized as High (3.75–4.5); Medium (3.0–3.5); and Low (2.75) prepartum as well as High (3.25–3.5); Medium (2.75–3.0); and Low (2.25–2.75) postpartum. After calving, 5 BCS change patterns were observed for 73 cows, namely: H to H group (n = 19), H to M group (n = 17), M to M group (n = 6), M to L group (n = 23), and L to L group (n = 7). Milk fat yield, milk total solids yield, fat-corrected milk yield, ruminal acetate proportion, ruminal propionate proportion, and blood glucose concentration were affected ($P < 0.05$) by the interaction among PBCS, BCSCP, and DIM. Milk protein yield was affected ($P < 0.05$) by DIM and the interaction between PBCS and BCSCP. Milk fat and fat-corrected milk yield in M to L group were higher ($P < 0.05$) than those in H to L, and M to M groups, while H to H and L to L groups had no difference from the other groups. Blood triglyceride and β-hydroxybutyrate were affected by the interaction between PBCS and BCSCP. Our study suggested that the interactions among PBCS, BCSCP, and DIM could be the important factor affecting cows milking performance, rumen fermentation, and blood parameter. Uncovering their interactive mechanisms warrant further study.

Key Words: BCS, transition cow, milking performance

1678W Carry-over effects of maternal late gestation heat stress on granddaughter's growth and mammary gland development.

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Maternal (F_0) exposure to late gestation heat stress reduces daughter's (F_1) mammary gland parenchyma (mPAR) and fat pad mass (mFP), epithelial cell proliferation, and milk yield at maturity. In addition, granddaughters (F_2) born to F_1 produce less milk in their first lactation. Herein, we investigated the effect of maternal late gestation heat stress on growth and mammary development of granddaughters. Multiparous F_0 cows had access to heat abatement (n = 41, shade, fans, water soakers) or not (n = 41, shade only) for the last 54 ± 5 d of gestation. F_1 daughters born to these cows were heat stressed (HT_{F1}, n = 36) or cooled (CL_{F1}, n = 37) in utero, and raised as an identically managed cohort until first calving. F_2 granddaughters born to HT_{F1} (HT_{F2}, n = 12) and CL_{F1} (CL_{F2}, n = 17) were raised as a cohort until 70d of age. Body weight, hip height, wither height, chest girth, and head circumference were measured at birth, d 7, d 21, d 35, d 49, and d 70. Mammary gland teat length (TL) and the left-right and front-rear teat distances (TD) were measured at d 21, d 35, d 49, and d 70. Average daily gain (ADG) was calculated over the course of the pre-weaned period (49 d). At d 70, mammary ultrasounds were performed (n = 9 heifers/group) on the developing rear left and right quarters at 2.8 depth to quantify mPAR and mFP areas. Data was analyzed using PROC MIXED in SAS. Heifer growth from birth to d49 was similar between CL_{F2} and HT_{F2} (all, $P > 0.2$). There was a treatment by day effect, whereby CL_{F2} TD was shorter relative to HT_{F2} ($P = 0.03$). Teat length was similar across the pre-weaning period between groups (all, $P > 0.4$). On d70, CL_{F2} tended to have greater left mPAR area than HT_{F2} (37,619 vs. 27,665 pixels^2, respectively, $P = 0.08$), and the average mPAR (right and left quarters) was greater in CL_{F2} relative to HT_{F2} (36,925 vs. 28,123 pixels^2, respectively, $P = 0.05$). Although the left mFP was smaller in HT_{F2} (42,091 vs. 58,100 pixels^2, respectively, $P = 0.05$) relative to CL_{F2}, the average mFP was not different ($P = 0.22$). In summary, HT_{F2} heifers grow at an identical rate to CL_{F2} heifers, however they have less mPAR area with similar mFP after weaning.

Key Words: in utero, multigenerational, ultrasound

1679W Mineral and trace mineral concentrations in colostrum, transition, and mature milk of primiparous and multiparous Holstein dairy cattle.

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Previous literature has justified that mineral concentrations are greater in colostrum compared with whole milk (WM); however, their concentrations in transition milk have not yet been characterized. Therefore, the objective of this study was to determine macro and trace mineral concentrations in colostrum and transition milk (TM) of primiparous (PP) and multiparous (MP) Holstein cows. On a commercial dairy farm, 15 MP and 6 PP dairy cows were fed the same pre- and postpartum rations. Dams and calves were separated before suckling could occur. Colostrum (milking [M1]) was collected within 12 h after calving followed by the collection of M2, M3 (TM), and M14 (WM). Macro minerals were quantified by inductively coupled plasma (ICP)-optical emission spectroscopy and trace minerals were determined by ICP-mass spectrometry. Data was evaluated using PROC GLIMMIX considering the fixed effects of milking number, parity, and their interaction, and the random effect of cow. The most abundant mineral in the colostrum and WM was Ca ($P < 0.001$; 10.61 ± 0.269 g/kg) and K ($P < 0.04$; 11.40 ± 0.52 g/kg), respectively. Zinc was the most abundant trace mineral in colostrum ($P < 0.0001$; 114.7 ± 11.38 mg/kg and WM ($P < 0.01$; 40.2 ± 4.45 mg/kg). Concentrations of all macro- ($P \leq 0.02$) and trace minerals ($P \leq 0.078$) were higher in colostrum compared with M14, except for Ca ($P = 0.95$), Na ($P = 0.99$), and Cu ($P = 0.97$). In terms of parity, Mg ($P = 0.06$; 1.20 ± 0.038 vs. 1.07 ± 0.06 g/kg) and Se ($P = 0.002$; 0.73 ± 0.018 vs. 0.61 ± 0.028 mg/kg) concentrations were greater in MP than PP cows over the sampling period, while Cu ($P = 0.0032$; 0.73 ± 0.057 vs. 1.07 ± 0.092 mg/kg) and Mo ($P = 0.07$; 0.16 ± 0.012 vs. 0.21 ± 0.021 mg/kg were lower in MP cows compared with PP cows. In addition, MP cows had greater S ($P = 0.0036$; 7.00 ± 0.158 vs. 5.84 ± 0.24 g/kg) than PP cows in colostrum. The results suggest that macro- and trace mineral concentrations decrease gradually after colostrum, and that parity differentially affects the concentrations of minerals produced over the first week of lactation.

Key Words: colostrum, transition milk, macro and trace minerals

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1680W Differences in post-absorptive lipid metabolism may contribute to variation in feed efficiency in dairy cows. S. R. Naughton*[1], M. J. Vandehaar[1], H. M. White[2], and Z. Zhou[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Department of Animal and Dairy Science, University of Wisconsin–Madison, Madison, WI.*

Our objective was to determine if differences in post-absorptive energy metabolite use might contribute to differences in residual feed intake (RFI) of lactating cows. We compared expression of key genes regulating liver fatty acid oxidation and circulating profile of fatty acid oxidation intermediates (acylcarnitines) in cows with high and low RFI. Mid-lactation primi- (n = 42) and multi-parous (n = 74) Holstein cows were enrolled for a 42-d study in 7 cohort groups, each with its own diet. At the end of the experimental period, RFI for each cow was calculated as the deviation of her actual intake from her expected intake, based on energy sinks and days postpartum. Cows with RFI values in the top and bottom 30% from each cohort were deemed most feed efficient (low-RFI) and least feed efficient (high-RFI). Eight low-RFI (−1.6 ± 0.7 kg/d) and 8 high-RFI (1.74 ± 1.0) multiparous cows with similar days postpartum (125 ± 12 d) were selected for subsequent liver and blood sampling. Liver tissue was harvested via biopsy for mRNA abundance of genes regulating lipid metabolism. Blood samples were collected from coccygeal vein for quantification of 18 acylcarnitines (fatty acid oxidation intermediates) with LC-MS. Data were analyzed using PROC MIXED in SAS. Although mRNA abundance of peroxisome proliferator activated receptor alpha and carnitine palmitoyltransferase 1A were similar in high- and low-RFI cows ($P > 0.05$), a tendency toward greater ($P = 0.10$) expression of Acyl-CoA oxidase 1 in low-RFI cows was observed compared with high-RFI cows. Expression of Acyl-CoA dehydrogenases medium chain also tended to be greater ($P = 0.10$) in low-RFI cows compared with high-RFI cows, suggesting enhanced hepatic fatty acid oxidation in more efficient (low-RFI) cows. These changes could be associated with changes in insulin sensitivity in extra-hepatic tissues, as we found a tendency toward greater ($P = 0.10$) plasma concentration of acylcarnitine (16:1n7) in low- compared with high-RFI cows. Overall, these results indicate that differences in lipid metabolism could contribute to individual cow variation in feed efficiency.

Key Words: RFI, feed efficiency, lipid metabolism

1681W Abomasal infusion of branched-chain amino acids or branched-chain keto-acids alter liver lipid metabolism in early lactation dairy cows. G. Ahmad*, C. Collings, I. Bernstein, K. Gallagher, M. J. VandeHaar, and Z. Zhou, *Department of Animal Science, Michigan State University, East Lansing, MI.*

The capacity of fatty acid oxidation in the liver is crucial for minimizing the negative impact of negative energy balance in dairy cows during early lactation. The objective of this study was to assess the extent to which BCAAs and BCKAs alter liver lipid metabolism during early lactation. Thirty-six multiparous Holstein cows were used in a randomized block design experiment. Cows were blocked according to expected calving date and were abomasally infused for 21 d after parturition with solutions of saline (CON, n = 12); BCAA (n = 12) including 67 g valine, 50 g leucine, and 34 g isoleucine; and BCKA (n = 12) including 77 g ketovaline, 57 g ketoleucine, and 39 g ketoisoleucine. All cows received the same diet. Liver tissue was harvested at 1, 7, 14, and 21 d relative to calving for ^{14}C-palmitate oxidation assays and mRNA abundance of genes regulating lipid metabolism. Blood samples were taken at 0, 3, 7, 14 and 21 d postpartum for quantification of 18 acylcarnitines (fatty acid oxidation intermediates) with LC-MS. Data were analyzed using PROC MIXED in SAS. Cows receiving BCKA increased oxidation of palmitate to CO_2 compared with CON ($P = 0.05$). Similarly, oxidation of palmitate to CO_2 also tended to be greater in cows receiving abomasal BCAA infusion during early lactation ($P = 0.10$). In agreement with these results, plasma concentration of acylcarnitine (8:0), a medium chain acylcarnitine metabolized to CO_2 and water in mitochondria, was also greater in BCKA cows compared with CON ($P < 0.01$). Although mRNA abundance of peroxisome proliferator activated receptor alpha, acyl-CoA oxidase 1, carnitine palmitoyltransferase 1A, and acyl-CoA dehydrogenases were not changed in response to BCAA or BCKA infusion ($P > 0.05$), expression of uncoupling protein 2 was greater in BCAA cows compared with CON ($P = 0.03$), suggesting enhanced fatty acid oxidation in the mitochondria. Overall, results indicate that abomasal BCAA or BCKA infusion enhanced fatty acid oxidation in the liver of dairy cow during early lactation. Further work is required to understand the underlying mechanisms to the changes observed in this study.

Key Words: BCAA, BCKA, lipid metabolism

1682W Supplementing *Saccharomyces cerevisiae* fermentation products improves performance, metabolism, and immune status of dairy calves. M. Sfulcini[1], V. Lopreiato[2], L. Cattaneo[1], F. Piccioli-Cappelli[1], A. Zontini[3], I. Yoon[4], E. Trevisi[1], and A. Minuti*[1], [1]*Department of Animal Science, Food and Nutrition (DIANA) Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Department of Veterinary Sciences, Università di Messina, Messina, Italy,* [3]*Cargill Animal Nutrition and Health, Fiorenzuola D'Arda, Italy,* [4]*Diamond V Inc., Cedar Rapids, IA.*

This study aimed to evaluate the effect of supplementing *Saccharomyces cerevisiae* fermentation products (SCFP) on performance, metabolism, and immune status of calves from birth to 160 d of age. After 3 L of colostrum administration within 4 h after birth, 18 Holstein heifer calves were enrolled in the study and allocated to 2 groups of 9 calves each: control (CTR; orally dosed with 10 g/d of grounded starter, from 5 to 70 d) or supplemented group (SCFP; 1 g/d of SmartCare, Diamond V, in milk replacer plus oral dose of 5 g/d of NutriTek Diamond V and 5 g/d of grounded starter from 5 to 70 d as solid feed). Calves were housed in individual hutches and received milk replacer until weaning (60 d) and *ad libitum* starter from 5 to 70 d. Then, they were moved to multiple boxes and received 2–3 kg of concentrate and *ad libitum* ryegrass and alfalfa hay mix until 160 d. Body weight (BW) and feed intake were recorded. Blood samples were collected throughout the study for the assessment of metabolic profile and phagocytosis of polymorphonuclear cells (PMN) with flow cytometry. Data were analyzed by repeated measures mixed models (proc GLIMMIX of SAS). No difference in body weight or daily gain were observed from 0 to 70 d period, however, SCFP had higher average daily gain from 70 to 100 d (590 vs. 929 ± 94.0 g/d in CTR and SCFP, respectively; $P < 0.01$) and higher BW from 100 to 160 d (about 10 kg more). SCFP tended to have greater plasma concentration of β-hydroxybutyrate at 60 d (0.27 vs. 0.32 ± 0.03 mmol/L in CTR and SCFP; $P = 0.06$) and urea at 70 d (4.33 vs. 4.89 ± 0.32 mmol/L; $P = 0.08$). Plasma myeloperoxidase was higher in SCFP compared with CTR at 70 d (275 vs. 305 ± 37.3 U/L in CTR and SCFP; $P = 0.04$) and PMN phagocytosis was higher in SCFP compared with CTR at 60 and 70 d with average values of 67.4 vs. 76.8% in CTR and SCFP ($P = 0.03$). Overall, these data indicated a beneficial effect of supplementing SCFP in the diet of dairy calves, reducing weaning stress through improved rumen activity (higher blood concentration of β-hydroxybutyrate and urea) and immune system functionality (greater PMN phagocytosis and higher myeloperoxidase concentration), which eventually resulted in increased post-weaning growth.

Key Words: calf, *Saccharomyces cerevisiae* fermentation product, metabolic profile

1683W Plasma fatty acids differ by residual feed intake group in mid-lactation cows and can improve performance of predictive models of dry matter intake.

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Previous work indicated differences in fatty acid (FA) metabolism by feed efficiency status, but the circulating profile of individual FA has not been characterized. The objectives were to identify differential FA and metabolites by residual feed intake (RFI) group and consider their inclusion in DMI prediction models. Mid-lactation Holstein cows (n = 124) had access to Insentec feeders (2 replications; 45 d). Data included metabolic BW, BCS, and milk yield and components. SMARTBOW sensors recorded behavior and location data. Blood metabolites included glucose, NEFA, BHB, triglycerides, ALT, AST, albumin, creatinine, lactate, and FA profile. A model search was performed using MuMIN in R (v. 4.2.1) and a leave-one-out cross validation was used to evaluate models of DMI based on RMSE, r^2, and concordance correlation coefficient (CCC). Residual feed intake was calculated by regressing DMI on milk energy (parity), DIM (parity), metabolic BW (parity), ΔBW (parity), and study. The top and bottom 25% were selected as LowRFI and HighRFI. The effect of RFI group on blood metabolites was determined using PROC GLIMMIX (SAS 9.4) with fixed effects of group, study, and parity, accounting for heterogeneity of variance. Results are reported as (mean [95% CI]). LowRFI had greater ($P < 0.05$) creatinine (0.71 [0.67, 0.75] vs. 0.66 [0.63, 0.69]) and NEFA (0.13 [0.12, 0.15] vs. 0.11 [0.10, 0.12]) compared with HighRFI. LowRFI cows tended ($P < 0.10$) to have lower C15:0 (0.27 [0.26, 0.28] vs. 0.28 [0.27, 0.30]) and C20:3 (1.31 [1.27, 1.36] vs. 1.37 [1.32, 1.42]), whereas C16:1 tended to be greater in LowRFI compared with HighRFI (0.69 [0.67, 0.71] vs. 0.66 [0.64, 0.68]). Without blood variables, the best model had a RMSE = 1.59, $r^2 = 0.82$, and CCC = 0.90. When all variables were offered, model performance improved (RMSE = 1.40; $r^2 = 0.86$; CCC = 0.93). When only blood variables were considered, the best model achieved a RMSE = 2.52, $r^2 = 0.55$, and CCC = 0.72. Differences in individual FA may indicate preferential use or altered metabolism of FA by RFI group. Additionally, inclusion of blood metabolites in predictive models of DMI improved predictive performance.

Key Words: feed efficiency, metabolite

1684W Effect of bovine pyruvate carboxylase and phosphoenolpyruvate carboxykinase knockdown in BFH12 cells.

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Pyruvate carboxylase (PC) replenishes the oxaloacetate (OAA) pool in the tricarboxylic acid (TCA) cycle and increases oxidative capacity, while phosphoenolpyruvate carboxykinase (PCK) utilizes and depletes the OAA pool for gluconeogenesis. The objectives of our study were to optimize an siRNA knockdown (KD) for PC and PCK and to determine if KD would affect cellular metabolism in BFH12 cells, an in vitro immortalized bovine fetal hepatocyte cell line. Hepatocytes were cultured in monolayer and at least 80% confluent after 24 h when cells were randomly assigned to treatment in triplicate with PC (siPC1, siPC2, siPC3) or PCK (siPCK1, siPCK2, siPCK3) targeted siRNA at either 20, 60, or 80 µ*M*, or a nonspecific-siRNA control (siNON). Media was replenished after 4 h and cells incubated for 20 h before harvest in TRIzol for subsequent protein isolation. Protein abundances (arbitrary units; AU) were determined by Western blot and normalized to total lane protein. Media was collected at harvest and glucose concentrations quantified for KD impact (Catachem Well-T Autoanalyzer). Data were analyzed (PROC MIXED, SAS 9.4) with fixed effect of siRNA and concentration. Differences were declared at $P \leq 0.05$; tendencies at $0.05 < P \leq 0.10$ and preplanned linear contrasts explored if $P \leq 0.10$. Knockdown of PC was successful ($P = 0.01$) with all 3 siRNA. The most effective KD was with siPC2 and siPC3 resulting in an average 25% and 44% reduction in PC abundance across concentrations, respectively, with siPC3, but not siPC2, resulting in a reduction in media glucose ($P = 0.04$; 139.1 vs. 124.9 ± 5.3 mg/dL). Increasing concentrations of siPC2 resulted in linear reductions ($P = 0.05$; 0.78 vs. 0.65, 0.58, 0.51 ± 0.07 AU), and siPC3 tended to result in linear reductions ($P = 0.07$; 0.78 vs. 0.52, 0.45, 0.37 ± 0.14 AU) of PC abundance compared with siNON. Knockdown of PCK was not successful ($P = 0.27$; 1.27 vs. 0.66 ± 0.51 AU) and did not change media glucose concentrations ($P = 0.74$). The linear knockdown of PC in the BFH12 cell line could be a valuable research model, but further investigation to ensure that gluconeogenic and oxidative pathways are consistent with lactating cow physiology is needed.

Key Words: pyruvate carboxylase, TCA cycle, metabolism

1685W Alterations to fatty acid composition in plasma and leukocytes following systemic inflammation.

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Our objectives were to investigate the changes in fatty acids (FA) composition in peripheral blood plasma and leukocytes (PBL) following an intravenous (IV) infusion of lipopolysaccharide (LPS) in early postpartum cows. At 14 DIM, cows were blocked by parity and BCS and randomly assigned to one of 2 IV infusions: LPS at 0.0625 µg/kg of BW diluted in saline (LPS; n = 8) or saline only (CON; n = 8). Infusions were completed over 1 h using an automatic infusion pump. Blood samples were collected at 0 (basal) and 6 h (1.5 h after peak temperature) to harvest plasma and PBL in which total FA methyl esters in plasma and FA composition of the PBL phospholipid fraction were quantified by gas chromatography. Data were expressed as a percentage of total FA. For each cow, the relative change in FA was calculated as: (h 6 − h 0)/h 0 × 100. Relative changes were analyzed by ANOVA using GLIMMIX of SAS assessing the effects of treatment, parity, BCS category, and their interactions. Body temperature did not change in CON, but LPS induced systemic inflammation and hyperthermia for a mean of 5.8 h (50 to 400 min after onset of infusion). Compared with CON, LPS cows had an increase in the percentage of MUFA (−5.4 ± 1.7 vs. 10.1 ± 1.7%; $P < 0.01$) and a decrease in the percentage of PUFA (3.4 ± 0.9 vs. −2.0 ± 0.9%; $P < 0.01$) in plasma, with no differences in the dynamics of SFA (−2.2 ± 0.8 vs. −2.0 ± 0.8; $P = 0.90$). Within PUFA, both n3 and n6 FA decreased, with no difference in the n6:n3 ratio (−1.3 ± 0.9 vs. −2.3 ± 0.9; $P = 0.48$). Regarding individual FA in plasma, LPS increased the percentages of 12:0, 16:0, 16:1 c9, 18:1 c9, and decreased the proportions of 18:0, 18:2 n6, 18:3 n3, 20:0, and 20:4 n6, compared with CON. In PBL phospholipids, there were no differences in FA classes, but LPS increased the proportions of 18:3 n3, 22:0, 22:5 n6, 24:0, and 24:1 and decreased the proportions of 18:1, 20:3 n6, 20:4 n6, 22:4 n6 compared with CON. In conclusion, the FA profile in the peripheral blood of early postpartum cows is influenced by systemic inflammation, which might reflect the utilization of FA for oxylipin synthesis or other adaptive responses.

Key Words: inflammation, transition cow, fatty acid

1686W Effect of time of exposure to prepartum anionic salts on peripartum serum calcium and nonesterified fatty acid concentrations.

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Forty-seven cows (19 Holsteins, 28 Jerseys) of 2nd (22) or 3rd (25) parity were enrolled in an experiment to evaluate the effects of length of time of exposure to prepartum anionic salts on peripartum serum metabolite concentrations. Cows were moved to a common close-up pen either 24 ($n = 16$), 17 ($n = 18$), or 10 ($n = 13$) days before expected calving date; $CaCl_2$ was added to the close-up diet to maintain urine pH (monitored twice weekly) within the range of 6.0−7.0. Blood and urine samples were obtained immediately before the close-up pen move; thereafter urine and blood were sampled twice weekly until parturition and blood was also sampled on the day of and following parturition, and then twice weekly for 30 d. Urine pH was determined immediately and samples were then acidified and frozen. Urinary Ca excretion (UCE) was estimated from subsequent analysis of Ca and creatinine from the last urine sample taken prepartum. Blood serum samples were frozen for subsequent analysis of Ca and nonesterified fatty acids (NEFA). Serum metabolite concentrations were analyzed as a mixed linear model including the effects of breed, lactation, and their interaction, with day as a repeated factor. Serum Ca and NEFA concentrations were affected by day ($P < 0.001$); maximum NEFA and minimum Ca concentrations were obtained on the day of and the day following parturition, respectively. Data for these maximum and minimum concentrations were then analyzed by ANOVA. The model included breed, parity, and their interaction as categorical covariates and the linear and quadratic effects of prepartum urine pH and UCE, colostrum yield, and days of exposure to anionic salts as continuous covariates. Minimum serum Ca concentrations were lower ($P = 0.01$) for 3rd vs. 2nd parity, decreased ($P \leq 0.05$) with increasing colostrum yield or increased time of exposure to anionic salts, and increased with increasing UCE. Maximum serum NEFA concentrations were unaffected ($P \geq 0.10$) by any continuous covariate. Data suggest that the effectiveness of prepartum anionic salts may be enhanced by reducing the time of exposure below the standard practice of 21 d.

Key Words: dairy cow, anionic salt, hypocalcemia

1687W Direct effects of heat stress on mitochondrial structure and energy metabolism in lactating dairy cows.

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Heat stress negatively affects energy production through reduced mitochondrial integrity and function in vitro. However, effects on in vivo mitochondrial parameters are unknown. We hypothesized that heat stress directly affects energy metabolism through altered mito-nuclear signaling pathways and a compromised mitochondrial structure. Mid-lactation (94 ± 6 d in milk) multiparous Holstein cows ($n = 23$) were blocked by previous 305-d milk yield (MY) and parity and assigned to a heat-stressed (HS) or pair-fed thermoneutral (PFTN) group. Cows were housed in individual tie-stalls and acclimated for 3 d before experimental periods (P1 and P2). During P1 (7 d), all cows were thermoneutral and fed ad libitum. During P2 (10 d), HS cows were fitted with heat blankets and fed ad libitum whereas PFTN cows were maintained under thermoneutral conditions and pair-fed to match the reduction in feed intake of HS cows. Rectal temperature (RT), respiration rate (RR), and MY were recorded twice daily during P1 and P2. Blood samples were collected on d 1, 5, 6 of P1 and d 1, 5, 9 of P2 for cortisol quantification. On d 10 of P2, liver and mammary biopsies were taken from all cows. The ATP, ADP, and the expression of genes involved in energy metabolism (e.g., glucocorticoid and adrenergic receptors) were quantified by qRT-PCR. Mitochondria were imaged through transmission electron microscopy and mitochondrial number, area, and aspect ratio quantified. Data were analyzed using *t*-tests or mixed models with fixed effects of period, treatment, and their interaction and cow ID as a random effect. The HS cows had an average of 39.0°C rectal temperature during P2. THI of the barn was 58 ± 5 across the study. Both RT and RR were higher for HS compared with PFTN during P2 ($P < 0.01$) but not P1. The MY was similar between groups in P1, but was lower for HS group compared with PFTN in P2 ($P = 0.004$). There tended to be fewer mammary mitochondria in HS cows relative to PFTN ($P = 0.08$); however, mitochondrial area and aspect ratio were similar between groups. The ATP, ADP, and cortisol concentrations were similar between groups. Our results suggest our heat stress challenge had limited effects on energy metabolism and mitochondrial morphology.

Key Words: adenosine triphosphate, bioenergetics, histology

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1688W Geographic trends in automatic milking systems: A scoping review. T. Marques*[1,2], C. Lage[3], D. Bruno[4], M. Endres[5], F. Ferreira[1], and F. Lima[1], [1]*Department of Population Health and Reproduction, University of California, Davis, CA,* [2]*Federal Institute Goiano, Rio Verde, Goias, Brazil,* [3]*Cornell Cooperative Extension, Cornell University, Bath, NY,* [4]*Cooperative Extension, University of California Agriculture and Natural Resources, Fresno, CA,* [5]*Department of Animal Science, University of Minnesota, Saint Paul, MN.*

Increase in labor costs and reduced workforce availability are contributing to greater adoption of automatic milking systems (AMS) in the US. Our aim was to review the literature investigating AMS on dairy farms and future research opportunities. Studies published from January 2000 to September 2022, in full text in English, of at least 500 words related to AMS in dairy farms were searched in 6 electronic databases: Biosis, CAB Abstracts, Medline, PubAg, AGRIS, and Scopus. A total of 536 studies were included after screening 4,292 titles and abstracts. The studies were conducted in Europe (73.5% of the studies), followed by North America (16.2%), Asia (5.4%), Australia/Oceania (4.3%), South America (0.4%), and Africa (0.2%). Canada contributed 10.1% of the total AMS studies, while the US contributed 6.1%. Most studies were performed in commercial herds (67.9%), consisting mostly of Holstein cows (57.7%), using Lely or DeLaval brands (45.4% vs. 39.7%), and free flow cow traffic (52.7%). The main topics explored in Europe and other countries were milk production, milk composition, and AMS efficiency (39.9% of the studies), behavior and welfare (14.7%), mastitis (13.4%), nutrition (7.8%), and health disorders (5.3%). In the US, trends were similar except for nutrition which was no studies. Since 2016, there has been increased interest in energy and water consumption, technological development, environment, reproduction, genetics, and longevity/culling. However, available studies in those areas are in initial stage of trials and further research is needed to establish optimum best practices and indexes under this system.

Key Words: dairy farming, voluntary milking, robotic dairy

1689W Estimation of udder emptying based on milk fat content of strip samples after milking. B. Jenni, O. Wellnitz, and R. M. Bruckmaier*, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Incomplete udder emptying (UE) at machine milking can be caused by various stressors, and is also reported in cow-calf contact rearing systems in dairy cows. We have tested the hypothesis that UE can be estimated by different contents of milk constituents in strip milk (SM) after cluster detachment. Ten Holstein dairy cows were used for the experiment. Different degrees of udder emptying were achieved by either normal routine milking (trt 1), or i.v. administration of the oxytocin (OT) receptor blocking agent atosiban (12 µg/kg BW) either before (trt 2) or immediately after a 1-min manual prestimulation (trt 3). After cessation of spontaneous milk flow the cluster was detached and a SM sample was taken, followed by i.v. injection of 10 IU of OT and cluster re-attachment to empty the udder completely in all treatments (assumed as 100%). Statistical analyses of treatment effects were conducted by MIXED procedures (SAS) with Tukey's *t*-test. Total milk yield (including residual milk obtained after OT injection) was similar in trt 1, 2, and 3 (12.0 ± 0.8, 11.9 ± 0.4, and 12.6 ± 0.6 kg, resp.). Spontaneously removed milk (before OT injection) differed significantly ($P < 0.05$) among the 3 treatments and was 11.0 ± 0.7 kg (91.4 ± 1.9%) in trt 1, 2.3 ± 0.7 kg (18.4 ± 4.9%) in trt 2, and 5.8 ± 0.8 kg (46.0 ± 5.2%) in trt 3, indicating normal milk ejection, removal of only cisternal milk, and partial milk ejection, resp. SM gross composition (fat, protein, lactose, urea) was measured by Mid-IR technology (Foss Milkoscan). Among all measured parameters most pronounced differences among treatments and hence degree of UE were found for the fat content of SM which was higher in trt 1 (6.36 ± 0.35%) than trt 2 (2.78 ± 0.24%) and trt 3 (3.35 ± 0.38%) ($P < 0.05$). We have therefore used a quadratic regression analysis of SM relative to UE independent of the treatments. The resulting equation was $\text{Fat}\,\% = 3.33 - 0.054 \times \text{UE} + 0.001 \times \text{UE}^2$ ($R^2 = 0.82$). Due to the low regression coefficients an estimation of UE below 60% was not reliable. At UE >60% the fat content of SM increased considerably, thus allowing a reliable prediction of UE.

Key Words: udder emptying, estimate, strip milk

1690W Birth season affects cow longevity. I. M. Toledo*[1], L. Cattaneo[2], J. E. P. Santos[1], and G. E. Dahl[1], [1]*University of Florida, Gainesville, FL,* [2]*Università Cattolica del Sacro Cuore, Milan, Italy.*

Dairy cow longevity is an important economic trait for producers. In modern dairy farming, longevity of dairy cows is determined by culling decisions, which are the result of several risk factors, including diseases, reproductive and productive performance. Previous studies have documented that seasonal changes affect health, behavior, and performance of dairy cows throughout their life cycle. Increasing cow comfort by making management adjustments to decrease exposure to high temperatures during the hot months give farmers the opportunity to decrease culling risk factors and possibly increase cow longevity. In the present study, we analyzed the relationship between birth season and longevity of Holstein dairy cows in Florida (n = 1,282) and in California (n = 712), to test the hypothesis that birth in a cool season would increase the length of herd life. Longevity analysis included cows that remained in the herd for more than 5 lactations. The birth seasons were "cool" (CL- cows born in December, January, February, and March) and "hot" (HS- cows born in June, July, August, and September). Results showed that in Florida, most cows that remained in the herd for more than 5 lactations (between 5 and 8 lactations) were born during CL compared with cows born during HS (74 vs. 26%; $P = 0.04$). The same observations were made in California, where greater longevity (between 5 and 9 lactations) was attributed to cows born in CL compared with HS cows (69 vs. 31%; $P < 0.01$). Cows with more than 5 lactations represented 22% of the total herd in Florida and 9.8% of the total herd in California. These results may help farmers create opportunities to make management adjustments related to birth season, or alter negative seasonal factors (i.e., heat stress) to possibly increase cow longevity in dairy herds.

Key Words: birth season, longevity

1691W Evaluation of nutrient composition variation of grocery food waste fed to dairy cattle in Upstate New York. J. M. Steele, H. Hu*, and K. F. Reed, *Cornell University, Ithaca, NY.*

In the US, over 30% of the human-edible food is estimated to end up as waste, decomposition of which generates methane (CH_4), contributing to global warming. Recycling food waste as livestock (e.g., dairy cattle) feed can mitigate environmental impacts from the decomposition process and reduce feed costs. New York law requires businesses generating food waste 2 tons per week and up to recycle it locally starting 2022. Large grocery and produce vendors must find alternative methods for managing their heterogeneous mixture of food waste (e.g., fruits, vegetables, and bakery products). Due to high variation in nutrient composition and potential for high fat, sugar, and starch concentrations, feeding food waste products can be challenging and even detrimental to dairy cattle health and production. The objective of this study was to characterize and evaluate nutrient variation of grocery food waste fed to dairy cattle at a farm in Upstate New York. Three independent samples of grocery food waste were collected twice a week for 7 weeks and duplicate subsamples were sent to DairyOne for chemical composition analysis. Nutrient variations were quantified and partitioned into 3 parts: week-to-week, sampling, and analytical and residual using linear mixed-effects models. Results show that dry matter (DM) ranged from 12.1 to 23.9% on an as fed basis. Average chemical composition (%DM [±SD]) was ether extract (EE): 12.9% (±3.9), starch: 16.0% (±7.3), sugars: 32.4% (±7.2), neutral detergent fiber (NDF): 11.7% (±4.3), and crude protein (CP): 11.3% (±1.0). Most of the observed variation (>80%) for starch, EE, and CP was attributed to sampling variation. Conversely, week-to-week variation accounted for 76.6% and 50.5% of total variation for sugars and NDF respectively. The SD of the combined analytical and residual variation was below 1.0 except for NDF and sugars. Future work will evaluate the impact of this feed source on productivity and environment, assess the potential for higher inclusion rates, and expand data collection over a longer period for seasonal analysis.

Key Words: food waste, nutrient composition, variation

1692W Effect of Kernza straw in forage-based diets on nitrogen balance and blood urea nitrogen in dairy heifers.

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Kernza Intermediate wheatgrass is a novel perennial grass used as a dual-purpose crop (i.e., grain for human and forage as a low-cost feed for ruminants). Straw can be used to reduce energy density in the diet of heifers and their dry matter intake (DMI) to avoid over-conditioning. Our goal was to determine the effects of increasing dietary levels of Kernza straw on N excretion, N balance, and blood urea-N. The study was performed as a completely randomized design using 24 Holstein heifers. Eight heifers were fed 1 of 3 diets including (DM basis) either 0, 20 or 40% Kernza straw, a 40:60 mixture of corn silage and alfalfa haylage, and urea (0, 0.7, and 1.4%, respectively) for 12 weeks. On wk 9, DMI was measured daily, bodyweight (BW, 577 ± 20 kg) was measured on 2 consecutive days, and fecal and spot urine samples were collected on 4 consecutive days (d 1 800 h; d 2 1100 h; d 3 1700 h, and d 4 1400 h). Urine output was estimated using a creatinine excretion coefficient of 26.8 mg/kg BW. Fecal output was estimated using indigested NDF. Heifer was the experimental unit, and the model included the fixed effect of diet and the error term. On wk 9, the 0, 20 and 40% Kernza straw diet contained 37.7, 42.0 and 50.6% NDF, and 15.6, 14.7 and 14.1% CP (DM basis). For heifers fed diets with 0, 20 and 40% Kernza straw, DMI was 12.5, 11.2, 11.3 kg/d ($P = 0.03$), N intake was 356, 312 and 301 g/d ($P < 0.01$), Fecal N excretion was 77.8, 59.5 and 64.9 g/d ($P < 0.01$), urine output was 30.1, 33.1 and 27.5 L/d ($P = 0.35$), urinary N excretion was 226, 215 and 211 g/d ($P = 0.68$), N balance was 52.7, 37.4 and 24.3 g/d ($P = 0.10$), urinary urea-N excretion was 157, 153 and 140 g/d ($P = 0.10$), and blood urea-N was 15.0, 16.3 and 16.7 mg/dL ($P = 0.06$), respectively. Inclusion of Kernza straw reduced DMI, N intake and fecal N excretion. There was no treatment effect on urine volume and urine N excretion, but tendencies for a reduction in N balance, a decrease in urinary urea-N excretion and an increase in blood urea-N. In this study, the high levels of urinary N excretion, urinary urea-N excretion, and blood urea-N might be associated with excess of rumen-degradable N.

Key Words: nitrogen, heifer, Kernza

1693W Late-gestation heat stress alters hair length and skin morphology of granddaughters.

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Hair coat and skin play important functions in homeostasis and thermoregulation. In utero hyperthermia triggers fetal hair and skin adaptations that impact thermoregulation in postnatal life. Herein, we investigated the long-term effects of late gestation heat stress on hair and skin characteristics of the second generation. Pregnant dams (F_0) were heat stressed (access to shade, n = 41) or cooled (access to shade, fans, and water soakers, n = 41) for the last ~56 d of gestation on a commercial farm in Florida. They gave birth to the first generation of heifers in September and October 2020 (F_1) which were in utero heat stressed (HT_{F1}, n = 36) or in utero cooled (CL_{F1}, n = 37), respectively. All F_1's were raised as a cohort from birth to first calving and managed identically. From August to October 2022, 30 granddaughters (F_2) were born to HT_{F1} (HT_{F2}, n = 12) and CL_{F1} (CL_{F2}, n = 18) and managed identically from birth to 70 d of age. Hair samples and skin biopsies were collected from the neck on d 70, from a subset of F_2's (n = 6/treatment). Skin biopsy tissue was fixed for 16 h in 10% formalin, dehydrated, paraffin embedded, sectioned, and stained with hematoxylin and eosin to visualize morphology. Hair length and diameter, stratum corneum (SC) area, epidermis thickness, sweat gland (SWG) number, area, and average size, sebaceous gland (SBG) number, area, and average size, and SWG depth were assessed. Hair and skin measurements were performed using the ImageJ software. Data were analyzed using PROC MIXED in SAS. Hair tended to be shorter (15.7 vs. 19.6 ± 1.4 mm, $P = 0.08$; HT_{F2} vs. CL_{F2}, respectively) and thicker (0.35 vs. 0.29 ± 0.01 mm, $P = 0.002$) in HT_{F2}, compared with CL_{F2}. The HT_{F2} had significantly smaller SC area (39.5 vs. 53.4 ± 3.6 μm^2, $P = 0.02$) and tended to have a thinner epidermis (0.01 vs. 0.009 ± 0.0006 mm, $P = 0.09$). There were no differences (all $P \geq 0.17$) in SWG number, area, average size, or depth. Relative to CL_{F2}, HT_{F2} heifers had more SBG (21 vs. 14 ± 2 glands, $P = 0.05$) but smaller average SBG size (0.81 vs. 1.31 ± 0.09 μm^2, $P = 0.004$). Overall, late gestation heat stress modifies F_2 phenotypes, suggesting a multigenerational impact on thermoregulatory ability.

Key Words: hyperthermia, sebaceous, sweat gland

1694W Effects of total-mixed ration moisture content on its yeast, total bacteria, and clostridial population growth over time.

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Microorganisms reported in feed are known to increase heat and may cause spoilage, ultimately diminishing feed's nutrient value. The aim of this study was to determine the effects of water inclusion on microbial growth of a dry cow total-mixed ration (TMR) with addition of wheat straw (37% of total dietary DM). A total of 6 (n = 6) gallon size buckets were randomly divided into 2 treatment groups: NW (TMR with no water added; 54.4 ± 1.9% dietary DM) and WW (TMR with 7.3 kg of water added, 0.03% of DM; 46.7 ± 1.9% dietary DM]. The TMR samples were collected at 0, 6, 12, 18, and 24 h after the mixture was prepared and placed into buckets. Three samples per treatment were composited at each time point and the composites were plated for yeast, total bacteria, and clostridial counts. Yeast populations were enumerated through serial dilutions using potato dextrose agar (39 g/1 L; MP Biomedicals, Solon, OH) and incubated at room temperature (25 to 28°C) for 5 d. Total bacteria populations were enumerated through serial dilutions using tryptic soy agar (40 g/1 L; Remel, Lenexa, KS) and incubated at 37°C for 24 h. Clostridia were enumerated through serial dilutions using tryptose sulfite cycloserine agar (41 g/1 L; Neogen, Lansing, MI) and incubated at 37°C for 24 h inside an anaerobic container (Mitsubishi Gas Chemical, Tokyo, Japan). Statistical analysis was performed using the MIXED procedure in SAS. The NW had higher yeast counts than WW (7,924 vs. 5,578 ± 3,406 cfu/g, respectively; $P = 0.03$). A time effect (h) was observed ($P = 0.05$) for yeast with increased counts as time elapsed. There was no treatment effect for total bacteria (NW = 420,367 ± 126,197 cfu/g and WW = 448,667 ± 126,197 cfu/g; $P = 0.71$; however, total bacteria counts increased with time (304,167 vs. 664,167 ± 142,245 cfu/g; $P = 0.04$). There was no treatment effect for clostridia count ($P = 0.49$). In conclusion, water included in a dry cow TMR decreased yeast counts over time but did not impact total bacteria or clostridia count.

Key Words: yeast, total bacteria, *Clostridium*

1695W Impact of feeding frequency on vaginal temperature and production outcomes of lactating dairy cattle during the summer months.

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The objective of this study was to determine the effect of feed delivery frequency on vaginal temperature (VT), behavior and production of dairy cows during hot weather. Lactating Holstein cows (n = 12), housed in a tie-stall system, were enrolled in this study with a 2 × 2 Latin square design with 23-d adaptation and 5-d data collection. Two feed management treatments were imposed: Control (CON, 2× daily feed delivery) and Feed Frequency (FF, 3× daily feed delivery). Cows in the CON group were given 110% of their as fed intake, for each feeding (6AM; 6PM). Cows in the FF group were given 110% of their daily as fed intake, divided into equal meals (6AM; 2PM; 10PM). Individual dry matter intake (DMI), sorting behavior, milk production, rumination, activity, lying behavior, VT, and THI were recorded over the 5-d collection period and summarized by day. Data was analyzed in SAS using linear mixed models with cow as the experimental unit, day within treatment as the repeated measure and cow, block, and sequence as random effects. The mean, maximum, and minimum (±SD) daily THI was 69.0 ± 5.1, 75.1 ± 4.9, 61.0 ± 5.5. The FF group had lower mean VT (38.6 ± 0.05 vs. 38.7 ± 0.05°C; $P = 0.03$) and lower minimum VT (38.1 ± 0.04 vs. 38.2 ± 0.04°C; $P = 0.05$) than the CON group; but no effect on maximum VT. Cows within the FF group produced fewer liters of milk (40.5 ± 1.3 vs. 41.6 ± 1.3 kg; $P = 0.02$) and tended to have lower DMI (31.8 ± 0.5 vs. 30.4 ± 0.5 kg; $P = 0.08$) but no impact was found on fat production (1.5 ± 0.05 vs. 1.5 ± 0.05 kg; $P = 0.29$). Treatment was associated with changes in sorting behavior (top screen of shaker box: 93.4 ± 2.9 vs. 89.4 ± 2.9%; $P = 0.04$; Pan: 96.3 ± 13.4 vs. 97.5 ± 13.3%; $P = 0.05$) and the FF group had greater rumination (500 ± 13 vs. 478 ± 13 min/d; $P = 0.04$). The FF group tended to have lower activity (316 ± 15 vs. 330 ± 14 min/d; $P = 0.09$) and had fewer lying bouts (10.9 ± 0.8 vs. 12.5 ± 0.8 bouts; $P = 0.02$). In conclusion, feeding 3 equal-sized meals was associated with reduced VT, increased rumination, and less restlessness. Although milk quantity was slightly reduced, the overall production of fat was unaffected.

Key Words: feed management, heat stress, vaginal temperature

1696W Effect of supplementing Holstein cows with a mixture of essential oils on enteric methane production and milk production.

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The objective of this study was to determine the effect of supplementing dairy cow diets with a mixture of essential oils (MEO) on enteric methane emissions and milk production of Holstein cows. Sixteen multiparous lactating cows (DIM = 95 ± 12.9; milk yield = 47.3 ± 2.78 kg) were used in a completely randomized design. The experimental period consisted of 12 weeks preceded with 3 week of covariate. Cows were fed (ad libitum) either a total mixed ration not supplemented (Control), or supplemented with 1 g/d of MEO (coriander seed oil, eugenol, and geranyl acetate). The TMR consisted of 69% forages (corn silage, alfalfa silage, hay) and 31% concentrates, on dry matter (DM) basis. During the experiment (including the covariate period), feed intake and milk production were measured daily and averaged weekly for statistical analyses. Milk was sampled weekly (once during covariate period) over 8 consecutive milkings to determine milk composition. Enteric CH_4 was determined over 4 consecutive days using respiration chambers once during the covariate period and at wk 1, 4, 8, and 12 after the covariate period. Data were analyzed (MIXED Procedure, SAS) using the repeated statement and adjusted for the covariate. Significant effect of MEO or MEO × sampling week interaction was declared when $P \leq 0.05$. The interaction between the experimental treatment and sampling week was not significant for CH_4 production, DM intake (DMI), milk production and composition. Intake, energy-corrected milk (ECM) yield, and feed production efficiency averaged (±SEM) 27.4 (±0.18) kg/d, 45.8 (±0.47) kg/d, and 1.67 (±0.021) kg ECM/kg DMI, respectively, and were not affected by MEO addition. No effect of MEO was observed on daily CH_4 production, CH_4 emission yield or intensity, which averaged 507 (±17.4) g/d, 18.8 (±0.50) g/kg DMI, and 11.2 g/kg (±0.33) ECM. This study shows that the blend of coriander seed oil, eugenol, and geranyl acetate did not influence milk performance (production, composition), feed efficiency or CH_4 emissions when fed to dairy cows at the rate of 1 g/d.

Key Words: enteric methane, milk production, essential oil

1697W Iodoform supplementation in total mixed rations reduces enteric methane emission from dairy cows without affecting dry matter intake.

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A previous in vivo study showed that the halomethane iodoform (CHI_3) was a potent methane (CH_4) inhibitor when pulse-dosed intra-ruminally to dairy cows twice daily. However, the study also found large reductions in dry matter intake (DMI) with increased inhibition of methanogenesis. Hence, the aim of this pilot study was to investigate whether reductions in enteric CH_4 emission could be obtained without affecting the DMI when iodoform instead was supplied continuously throughout the day by mixing it into the total mixed rations (TMRs). The experiment consisted of 4 periods of 14 d. In the first period, no iodoform was added to the TMRs of 4 Danish Holstein dairy cows, while either 8, 16, or 20 mg iodoform/kg DM was added to the TMRs in the 3 remaining periods in a crossover design. Milk production, DMI, and gas exchange were measured for the last 2 d in each period using 4 individual transparent respiration chambers. Data were analyzed using the mixed procedure of R. Milk production ($P = 0.24$) and DMI ($P = 0.32$) were unaffected by the addition of iodoform to the TMRs. A linear decrease and increase in daily CH_4 ($P = 0.04$) and hydrogen (H_2) ($P = 0.03$) emissions, respectively, were observed with increasing iodoform supplementation. Similarly, CH_4 and H_2 yield (g/kg DMI) also linearly decreased and increased, respectively ($P = < 0.01$ and $P = 0.04$), with increasing iodoform dose. Moreover, CH_4 and H_2 yield were significantly lower and higher, respectively, for 16 and 20 mg iodoform/kg DM compared with 0 mg iodoform/kg DM. Hence, CH_4 yield decreased by 23.8% and H_2 yield increased by 1620% with the addition of 20 mg iodoform/kg DM compared with 0 mg iodoform/kg DM. In conclusion, this pilot study indicated that reductions in enteric CH_4 emission can be obtained by the addition of iodoform in the diets of dairy cows without affecting DMI.

Key Words: halomethane, feed additive, hydrogen

1698W Assessment of methane emissions with a laser methane detector in Mediterranean buffaloes in two different seasons. L. Lanzoni*[1], M. G. G. Chagunda[2], M. Giammarco[1], M. Chincarini[1], I. Fusaro[1], M. Podaliri[3], A. S. Atzori[4], and G. Vignola[1], [1]*Department of Veterinary Medicine, University of Teramo, Teramo, Italy,* [2]*Department of Animal Breeding and Husbandry in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany,* [3]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy,* [4]*Department of Agriculture, University of Sassari, Sassari, Italy.*

The laser methane detector (LMD) is a portable, smart, non-invasive tool to measure CH_4 emissions from animals. Its application requires a robust protocol that considers all the variables such as the season of recording. Seasonal variations can act as a physiological stressor for the animals, triggering a range of behavioral and biological responses. The present study aimed to assess the variability of CH_4 emissions from Italian Mediterranean buffaloes (IMB) between the summer and winter seasons. Direct CH_4 emissions of 20 non-productive IMB, under the same feeding regimen, were monitored daily for 12 d in summer and winter with an LMD. Each measurement lasted 4 min and the distance of the LMD from the animal was 1 m. The daily temperature-humidity index (THI) and the dry matter intake (DMI) were recorded during the trial. The LMD output data were divided for each day and each subject into CH_4 from eructation (CH_E), breathing (CH_B), and overall average (CH_{AV}). Linear and linear mixed models were used to analyze the effect of the variables. The average CH_E, CH_B, and CH_{AV} measured during the trial period were 115.76 ± 56.38, 18.33 ± 7.41, and 34.16 ± 14.35, respectively. A linear mixed model showed a statistically significant influence of the season on CH_E, CH_B and CH_{AV} ($P < 0.001$). Higher emission values were recorded during winter (summer: $CH_E = 84.37 \pm 42.62$, $CH_B = 11.34 \pm 3.45$, $CH_{AV} = 20.32 \pm 6.38$; winter: $CH_E = 130.20 \pm 40.98$, $CH_B = 21.60 \pm 5.07$, $CH_{AV} = 40.62 \pm 9.83$). A significantly lower % of DMI on live weight ($P < 0.001$) and higher THI ($P < 0.001$) was observed in summer (summer: %DMI = 1.8, THI = 74.5 ± 1.9; winter: %DMI = 2.2, THI = 49.6 ± 4.9). The linear regression confirmed that a reduction in THI was significantly ($P < 0.001$) associated with an increase in CH_E ($R^2 = 0.69$), CH_B ($R^2 = 0.83$) and CH_{AV} ($R^2 = 0.85$). The variation of CH_4 between the 2 seasons could therefore be attributable to THI changes, which also influence the feed intake. In conclusion, our results suggest the need to account for the season of assessment in LMD protocols. Supported by "European Cattle Associations Young Scientist Dairy Travel award."

Key Words: sustainability, greenhouse gas emissions, non-invasive tool

1699W Prepartum supplementation of pasture-based dairy cows with inorganic selenium, organic selenium or rumen-protected choline does not affect lactation performance. F. McDermott*[1], E. Kennedy[2], S. A. Hogan[1], L. Brennan[3], and T. F. O'Callaghan[4], [1]*Teagasc Moorepark, Teagasc Food Research, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*Teagasc Moorepark, Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland,* [3]*University College Dublin, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [4]*University College Cork, School of Food and Nutritional Sciences, University College Cork, Co. Cork, Ireland.*

Calving and early lactation are physiologically stressful periods for dairy cows. During the dry period, it is recommended to provide mineral supplements 6 weeks before calving to ensure accumulation of a strong mineral reserves, to prevent mineral deficiencies or metabolic disorders during these stressful periods. A dearth of information exists on the effects of selenium or rumen-protected choline supplementation in dairy cows managed on pasture-based systems. The objective of this study was to evaluate the effects of prepartum supplementation of cows with mineral supplements containing 1) inorganic selenium (sodium selenite [INORG]), 2) combined inorganic and organic selenium (sodium selenite and selenium yeast [ORG]) or 3) rumen-protected choline (RPC), on milk yield and milk composition over the subsequent lactation. In total, 84 (18 primiparous and 66 multiparous cows) Holstein-Friesian (HF) and HF × Jersey (JE) were supplemented daily from 52 ± 17.1 d before calving until day of calving. Each treatment group consisted of 28 cows, the INORG treatment group was considered the control group as this treatment group as this mineral blend supplied is typical of those given to dairy cows during the prepartum period in a commercial setting on a pasture-based seasonal production system. Post-parturition, milk yield was recorded daily and milk macronutrients determined weekly for the subsequent 37 weeks of lactation. All statistical analyses were conducted using SAS (version 9.4). The effects of prepartum supplementation on milk yield and milk composition were analyzed using linear mixed models (PROC MIXED), with week of lactation as a repeated measure. Prepartum supplementation with INORG produced milk with a higher concentration of fat ($P = 0.05$) over the entire experimental period (+0.06% higher fat than RPC). Primiparous animals in the ORG group produced 0.25 kg/day higher milk solids than INORG primiparous animals. Prepartum supplementation had a significant effect on daily milk yield ($P = 0.05$) from wk 9 to 12, ORG and RPC diets resulted in a slower reduction in milk yield following peak production. Supplementation with RPC also reduced somatic cell count. Despite the trends observed, supplementation with INORG, ORG or RPC during the prepartum period does not improve lactation performance in the subsequent lactation.

Key Words: selenium, rumen-protected choline, milk yield

1700W Effects of methanogenic inhibitors on individual methanogens in the rumen of dairy cattle. K. Narayan*[1], N. Indugu[1], J. Bender[1], A. Hristov[2], and D. Pitta[1], [1]*University of Pennsylvania, Kennett Square, PA,* [2]*The Pennsylvania State University, University Park, PA.*

Enteric methane (CH_4) emissions from dairy cows contribute to 30% of total methane emissions and are considered a net energy loss to the animal. In the rumen, methanogens (hydrogenotrophic, methylotrophic, and methylaminotrophic) reduce carbon dioxide (CO_2), methanol, and methylamines, respectively, to generate CH_4 via different mechanisms. Although several methanogenic inhibitors have been investigated to reduce CH_4 production, their effect on individual methanogens in vivo is unknown. Thus, this study investigated the effect of either 3-nitrooxypropanol (3-NOP) or *Asparagopsis taxiformis* (AT) on individual methanogens in rumen samples of dairy cows from 2 different experiments.

First, we identified 13 ruminal methanogens which represented the 3 major substrates for methanogenesis along with the commonly present 16S rRNA archaeal gene. The copy number of individual methanogens was quantified using sequence specific primers using quantitative PCR (qPCR). These data were then compared with the 16S rRNA gene-based archaeal diversity retrieved via Illumina Mi-seq platform. The copy number of the 16S rRNA did not differ with either 3-NOP or AT, however, the individual methanogens showed differences among the 2 inhibitors. The copy numbers of hydrogenotrophic methanogens such as *M. ruminantium*, *M. smithii*, *M. millarae*, *M. olleyae*, and *M.* sp. *YE315* were reduced ($P < 0.05$) with 3-NOP and AT, whereas *M.* sp. *AbM4* and *M. formicimum* were reduced ($P < 0.05$) only with AT supplementation. Among methylotrophic methanogens, *M. intistilis* increased ($P < 0.05$) with 3-NOP whereas *M. archaeon* ISO4-H5 was reduced ($P < 0.05$) with AT. Among methanol utilizing methanogens, *M. stadtmanae* were completely reduced ($P < 0.05$) with AT and *M. mazei* increased ($P < 0.05$) with 3-NOP. The 16S rRNA analysis revealed that 3-NOP is more inhibitory on *Methanobrevibacter* and AT is more specific to inhibit *Methanosphaera*. This comparative study revealed novel information on the effects of 3-NOP and AT on individual methanogens. Further studies are required to understand the action mechanisms of these inhibitors on individual methanogens.

Key Words: enteric methane, methanogens, 3-NOP

1701W Application of ML algorithm to identify phenotypic responses from precision livestock data.

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Precision technologies along with high-throughput sequencing have the potential to identify novel phenotypes for important responses in livestock. Rumination sensors provide data on rumination time (RT), a moderately heritable (heritability = 0.34) trait that can be linked to animal phenotypic responses. Attempts to link RT to animal responses has limited success. Here we applied dynamic time warping (DTW), a ML algorithm to compare paths traversed by 2 time series sequences to find the relationship between the sequences. Our hypothesis is that the path traversed by RT and eating time (ET) is constant for a cow but differ between cows thus allowing for grouping cows as “High” and “Low” RT phenotypes. To test our objective, a total of 49 lactation dairy cows ranging from 2 to 97 d in milk were selected from a herd. The input data for DTW consisted of hourly measurement of both RT and ET for 60 d for each cow. Hierarchical clustering was computed with DTW distance matrix. The clusters were visualized using principal components analysis (PCA). The DTW clustering based on RT and ET data resulted 2 optimal clusters named as C1 and C2. Based on PCA analysis these 2 clusters had 24.6% of variance was explained by the first 2 principal components (15.4 and 9.2%). We used Silhouette index to select the most homogenous cows within the cluster, this resulted in 10 cows form each cluster. The stage of lactation in C1 and in C2 were distributed similarly. Interestingly, animal performance variables such as days in milk (44.5 vs. 63.9), average milk yield (82.6 vs. 97.3), total milk yield (67.6 vs. 102.7), RT (467.8 vs. 554.9), and ET (298.4 vs. 372.5) were higher in C2 compared with C1. Difference in fermentation profiles such as acetate (63.9 vs. 58.9) and propionate (19.5 vs. 22.8) were observed between 2 clusters. Further, generalized additive models (GAM) results revealed that low-rumination and low eating cluster (C1) had a significant ($P < 0.001$) reduction in intraday variation compared with high-rumination and high eating cows (C2). These data reveal that DTW algorithm may be a reliable tool to link precision data to phenotypic responses.

Key Words: enteric fermentation, precision technology, time series data

1702W Occurrence of mycotoxins in 2022 US corn and corn by-product feed ingredients.

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Mycotoxins have been identified in a variety of feed ingredients across the US and have shown to contribute to reduced animal health and performance. This study compiled data from 2022 US corn grain and corn by-product feed ingredients and reported on the occurrence and contamination levels of mycotoxins including: aflatoxins (AFLA), type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A. Corn samples labeled as 2022 harvest and corn by-products submitted from October 2022 through February 2023 were screened via the LC-MS/MS method. Samples that were above the limit of detection for each mycotoxin group were analyzed using the GLIMMIX procedure of SAS (SAS 9.4, Cary, NC) to test the effect of harvest year on average contamination level. Results are presented in Table 1. To date, 260 corn samples and 66 corn by-products from the 2022 harvest have been evaluated. Mean B-Trich, ZEN, FUM and AFLA levels (parts per billion, ppb) for corn grain samples were similar in 2022 as 2021 and 2020. Preliminary results of this survey suggest mycotoxin occurrence and contamination remains similar to previous harvest years except for greater FUM levels in corn-byproducts.

Key Words: by-product, corn, mycotoxin

1703W Mycotoxin occurrence in total mixed rations from the US and Canada: 2019–2023.

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Many types of feed ingredients can be contaminated with mycotoxins which can affect animal health and performance. The complexity of dairy total mixed rations (TMR) provides multiple potential sources of such fungal metabolites. Mycotoxin occurrence and contamination levels in TMR samples from the US and Canada were characterized by year of submission from 2019 through 2023. Fifty-five to over 110 samples were submitted for analysis per year in each country. Samples were screened via liquid chromatography with tandem mass spectrometry method (LC-MS/MS) for 6 major mycotoxin groups: aflatoxins, type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A. The presence of clinical health or performance concerns on-farm was noted for each sample. Effect of clinical concern (yes/no) and harvest year on contamination levels were analyzed using LSMeans statement with Tukey’s adjustment in the generalized linear mixed model procedure of SAS (v9.4). There were differences between clinical concerns for B-Trich in each country; therefore, samples noted with clinical signs were excluded and harvest year was used as main effect. The most frequently detected mycotoxin in both countries across the survey was B-Trich, occurring in more than 94% of samples each year. The second most frequently detected mycotoxin each year was ZEN except for Canada which reported greater occurrence of FUM in 2021 and more A-Trich in 2020. During the survey, the highest mean concentrations (dry matter basis) within each country ($P < 0.05$) of B-Trich in the US were in 2020 (2,643 ppb) and in Canada in 2019 (1,139 ppb). Initial results for 2023 include 7 samples from US and 18 samples from Canada. For the US, B-Trich were detected in 100% of samples at a mean concentration of 2,490 ppb and maximum of 4,027 ppb. For Canada, B-Trich were detected in

Table 1 (Abstr. 1702W). Mycotoxin contamination in US corn-based feed ingredients

Corn grain						Pooled SEM	*P*
Year	2018	2019	2020	2021	2022		
Total N	437	329	303	306	260		
Positive N	337	267	194	211	234		
B-Trich						128.3	
Prevalence, %	77	81	58	69	65		0.107
Mean, ppb	1,316	869	1,102	899	1,004		
ZEN						94.3	
Prevalence, %	46	46	20	28	28		0.962
Mean, ppb	333	412	340	384	394		
FUM						419.7	
Prevalence, %	77	78	64	64	75		
Mean, ppb	2,140	3,341	2,804	2,980	3,884		0.077
AFLA							
Prevalence, %	9	3	5	7	10	53.4	0.170
Mean, ppb	200	13	120	57	51		
Corn by-products						SEM	*P*
Year	2018	2019	2020	2021	2022		
Total N	67	55	96	89	66		
Positive N	63	55	94	88	66		
B-Trich						331.9	
Prevalence, %	94	100	98	99	97		0.038
Mean, ppb	2,167^{ab}	3,518^{a}	2,512^{ab}	2,440^{b}	2,012^{ab}		
ZEN							
Prevalence, %	78	95	85	88	73	48.0	<0.001
Mean, ppb	323^{b}	632^{a}	322^{b}	263^{b}	239^{ab}		
FUM							
Prevalence, %	90	98	97	99	100	301.5	<0.001
Mean, ppb	1,047^{bc}	2,441^{a}	1,951^{ab}	809^{c}	2,156^{ab}		
AFLA							
Prevalence, %	3	2	4	2	5	1.23	<0.001
Mean, ppb	3^{b}	36^{a}	4^{b}	2^{b}	2^{b}		

$^{a–c}$Values across rows are different ($P < 0.05$).

94% of samples at a mean concentration of 598 ppb and maximum of 2,380 ppb. Although feed ingredient sources and ration formulations vary widely across North America, survey results indicate B-Trich occur most often with frequent co-contamination of ZEN in US and Canada TMR samples since 2019.

Key Words: mycotoxin, TMR, trichothecene

1704W Using computer vision to predict cyclicity of dairy cows during the transition period through 3D body surface images. R. E. P. Ferreira*[1], T. Bresolin[2], P. L. J. Monteiro[1], M. C. Wiltbank[1], and J. R. R. Dorea[1], *[1]University of Wisconsin–Madison, Madison, WI, [2]University of Illinois Urbana-Champaign, Champaign, IL.*

During the transition period, dairy cows can experience severe negative energy balance, which may negatively impact the normality of estrus cyclicity and the success of subsequent insemination. Early detection of cyclicity during the transition period can thus improve the reproductive management of a dairy herd. However, measuring blood progesterone (P4) to accurately assess cyclicity status can be costly and stressful for the animals, impeding its implementation on a large scale. The use of computer vision systems (CVS) could be an alternative to monitor body shape changes during the transition period and potentially early detect cyclicity in dairy cows. The aim of this study was to develop a CVS to predict cows' cyclicity at 21 and 35 d in milk (DIM). A total of 16,750 depth images were acquired from 67 Holstein cows once a week, from −21 to +15 DIM. For each image, 2,048 features were extracted from a neural network (Xception) previously trained to predict body condition score. Those features were then averaged by cow and used to train machine learning (ML) algorithms to predict the cyclicity of each cow at 21 and 35 DIM. P4 was used as gold standard to indicate cyclicity. P4 was measured once a week from 7 to 49 DIM, and cows were considered cyclic starting at the first week when circulating P4 concentration above 1.0 ng/mL was detected. Out of the 67 cows, 24 and 45 were cyclic at 21 and 35 DIM, respectively. The ML algorithms were trained using a nested 5-fold cross-validation and the best performing algorithm was logistic regression with LASSO regularization, achieving F1-scores of 0.36 and 0.80, and accuracies of 0.61 and 0.73 for predicting cyclicity at 21 and 35 DIM, respectively. The results of this study indicate that body shape changes captured by depth images during the transition period are associated with cow's cyclicity and the developed system can accurately predict cyclicity at 35 DIM. This technology has the potential to provide a non-invasive, cost-effective, and efficient way to monitor and improve reproductive management practices in dairy farming.

Key Words: computer vision, cyclicity, transition period

1705W Optimizing training sets for individual identification of dairy cows. R. E. P. Ferreira*, M. C. Ferris, and J. R. R. Dorea, *University of Wisconsin–Madison, Madison, WI.*

Recent studies explored the use of deep learning techniques to perform individual identification of dairy cows. Such techniques require a large and diverse set of annotated images to perform well, and images are usually randomly selected for annotation. However, annotating large image data sets can be time-consuming and require the expertise of trained individuals for some domain-specific tasks such as animal identification. The objective of this study was to evaluate a method for selecting the optimal set of images to be annotated among an initially unlabeled image data set, comparing the performance of neural networks trained using the images selected from such method with those trained using randomly selected images. For each of the 14,199 images containing one of 16 cows in diverse angles and positions, we extracted the output of the second-to-last layer of a neural network (Xception architecture) pre-trained on the large ImageNet data set, resulting in 2,048 features per image. The ImageNet data set contains more than a million images of diverse objects, so deep neural networks trained using that data set are able to extract general and useful features from images taken on the natural world. Then, we executed K-means clustering to separate the images into different clusters according to their feature space representation, with the number of clusters coinciding with the number of images desired in the final reduced training set. When compared with randomly selecting an equally sized set of images to annotate and train the neural networks, the largest improvements were achieved when the final training set contained between 160 and 352 images (between 6.1 and 9.5 percentage points). When the training set sizes were set to a number below 160 or above 352 (up to 5,120), the benefits of using the proposed method were reduced to between 1.2 and 4.5 percentage points. These results show that the proposed technique can guide the annotation of image data sets, achieving better performance than simply selecting a random set of images to annotate and train neural networks for dairy cattle identification.

Key Words: computer vision, optimization, identification

1706W Factors influencing Ontario dairy producers' management and care of down dairy cattle. J. Brindle*, C. Winder, and D. Renaud, *Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada.*

The objective of this cross-sectional study was to identify factors associated with how Ontario dairy producers manage down cows on their farms. An on-line survey was distributed to all dairy producers in Ontario, Canada (n = 3,367) and was available from November 2020 to March 2021. All dairy producers were identified through the provincial dairy organization and contacted via email and social media. The full survey was comprised of 134 questions, 31 of which were related to down cow management. Descriptive statistics were evaluated and 2 logistic regression models were generated using STATA17, exploring factors associated with 1) relocating down cows with hip lifters and 2) assisting cows to stand, within an hour after going down. A total of 226 farmers responded, which was a response rate of 7.4%. Participants were predominantly male (68%), farm owners (78%) and between 30 and 39 years old (29%). The first model found that smaller farms (<57 cows) were less likely to move down cows with hip lifters, compared with larger farms (>129 cows) (odds ratio [OR] = 0.33; $P = 0.04$; 95% CI 0.11–0.97). In addition, farms that used hip lifters to lift cows had a higher odds of moving down cows with hip lifters [DH1] (OR = 12.96; $P < 0.001$; 95% CI 2.92–57.49). The second model identified that farms that used hip lifters to move cows had a higher odds of assisting a cow to stand within an hour following recumbency (13.04; $P = < 0.001$; 95% CI 3.23–52.65). Additionally, producers who waited longer to relocate a down cow (<1 h vs. >1 h) had a lower odds of assisting the cow to stand within one hour of finding them down (OR = 0.03; $P < 0.001$; 95% CI 0.006–0.129). Data from this study will be helpful in designing future research that further explores the barriers and motivations of producers when implementing evidence-based management plans to care for down dairy cows.

Key Words: non-ambulatory, management practice, epidemiology

1707W Effect of Holstein genotype on lying and movement activity, feed intake, and milk production. E. Jacobson*, W. Weber, E. Shepley, G. Cramer, B. Crooker, and I. Salfer, *University of Minnesota, St. Paul, MN.*

Genetic selection of Holsteins has led to immense increases in milk production over the past 50 years, but the effects on behavior are unknown. The University of Minnesota has maintained a herd of unselected (UH) Holsteins with genetic merit equivalent to Holsteins in the mid-1960s which can be used to compare with contemporary Holsteins (CH) to determine effects of genetic selection since the mid-1960s. The objective of this preliminary experiment was to determine if lying time, motion index, feed intake and milk production differ between UH and CH. Eleven UH and 11 CH were blocked by calving date and parity group (primiparous, n = 6 vs. multiparous, n = 16) and enrolled over a 6-mo period. Cows were grouped into 3 cohorts based on DIM: early lactation (0 to 28 DIM, n = 22 cows), peak lactation (28 to 90 DIM, n = 20), and mid-lactation (90 to 150 DIM, n = 16). Lying time and motion index were measured using pedometers placed on the rear leg of the cow (IceQube, IceRobotics Ltd.). Milk production and DMI data were collected daily. Data were analyzed a mixed effects model in with the main effect of genotype, fixed effect of parity, the random effect of block and the repeated effect of day. In early lactation, CH spent more time lying than UH (670 vs. 643 min/d; $P = 0.01$). Additionally, UH cows had a 45% greater motion index score compared with CH cows in early lactation ($P < 0.01$), indicating greater activity. In mid-lactation, CH spent more time lying than UH (773 vs. 742; $P < 0.01$). Mid-lactation UH cows had a 24% greater motion index than CH cows ($P < 0.01$). Furthermore, early lactation CH cows produced 83% more milk ($P = 0.01$) and consumed 24% more feed than UH ($P < 0.01$).

Key Words: Holstein genotype, lying time, activity

1708W Associations of dietary, genetic, management and ambience hallmarks with a farm success index. J. H. Carneiro*[1], B. J. A. Villar[2], M. Poczynek[1], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil.*

The aim of this study was to correlate a farm success index with dietary, genetic, milk solids and management characteristics in commercial dairy farms in Brazil. Annual data from a group of 10 Brazilian farms located in the south and center of Brazil were used. The farms raise Holstein cattle in an intensive system totaling 7,945 cows, a total of 314,698 kg of milk per day, and 39.7 kg/d ECM. All farms are attended by the same nutritionist and had their cow's genome evaluated. Farm success index was built with variables that are easy to measure, reliable, and that impact financial profitability. The following merits were selected and given a weight in index calculation depending on farm location within the group average. 1: Milk yield—the highest score was given to the highest production of the group; somatic cell count: farms with SCC average

≤200,000 received maximum score. Farms with SCC average 200–400 received intermediate, and farms with SCC average ≥400 received minimum score; days in milk: farms with average ≤160 received maximum score; farms with average 160–180 received intermediate; and farms with ≥180 received minimum score. Percentage of non-pregnant cows after 150 DIM: the farm with lower number within the group average received maximum score; cow culling rate: the farm with lower number within the group average received maximum score; young stock culling rate followed the same pattern as the culling rate. To build the correlation matrix dietary, the tested traits were corn silage starch content and 48-h NDF digestibility, employee turnover rate, percentage of cows with one or more diseases after calving, milking cows' stocking rate, rBST use, percentage of diet forage, fat/protein ratio, MUN, production difference between hot and cold seasons, PTAmilk, PTADPR, and PTAPL. The more impacting trait was employee turnover rate ($P = 0.01$) with $r^2 = 54.51$, followed by PTAPL ($P = 0.02$; $r^2 = 14.93$), then corn silage starch content ($P = 0.01$; $r^2 = 13.82$), and finally % of cows with one or more diseases ($P = 0.05$; $r^2 = 1.70$). We also found a tendency for corn silage 48-h NDF digestibility ($P = 0.06$; $r^2 = 10.17$), percentage of diet forage ($P = 0.08$; $r^2 = 1.76$) and milking cows' stocking rate ($P = 0.08$; $r^2 = 0.77$). In conclusion, within the variables tested, the employee turnover rate was the most impacting, showing the importance of a high-quality team in dairy farms.

Key Words: employee turnover rate, PTA

1709W Quantifying the impact of canola meal on enteric methane emissions of lactating dairy cows.

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Dry matter intake (DMI), dietary fat (EE), and digestible NDF (NDFd) concentrations can largely determine enteric methane (CH_4) emissions of dairy cows. Recent research indicates the potential to mitigate the emissions by manipulating relative inclusion rates of protein concentrates in the diet. The objective of this study was to quantify the effect of increasing the dietary canola meal content (CM) relative to soybean meal content (SM) on enteric CH_4 emissions (g/d) of lactating dairy cows through meta-regression analyses. Eight published studies offering 30 treatment means of enteric CH_4 emissions (g/d) were used. All treatment diets included CM and SBM representing together 3.2 to 24.1% of dietary DM (mean = 11.4%). The CM to SM ratio (CM: SM) ranged from 0.03 to 0.96 with a mean of 0.58. The CH_4 emissions, DMI, EE, CP, and NDFd varied between 149 and 505, 14.7 and 24.4, 2.1 and 8.4, 14.4 and 20.9, and 11.7 and 29.1 with the means of 372 g/d, 19.4 kg/d, 4.5% of DM, 16.6% of DM, and 18.3% of DM, respectively. Most of the diets (80%) included grass silage as the main forage source. Multilevel meta-regression analyses were conducted with the *metafor* package of the R software to estimate the effect of CM:SM on CH_4 emissions when adjusted to the effects of DMI, EE, and NDFd. A random-effect model created to estimate the total heterogeneity of CH_4 emissions was extended to mixed-effect models by including the fixed effects of DMI, EE, NDFd, and CM:SM. As expected, DMI and NDFd had positive relationships ($P < 0.001$), whereas EE had a negative relationship ($P < 0.001$) with CH_4 emissions. The model including those 3 variables explained 88% of the heterogeneity of CH_4 emissions. When the CM:SM effect was added, the model explained 93% of the heterogeneity. The CM:SM had a negative association with the CH_4 emission, and a unit increase in CM:SM (e.g., 0:1 to 1:1) was associated with a 46 g/d CH_4 emission decrease (95% confidence interval = −10.0 to −83.3 g/d; $P = 0.013$). The results support the notion that compared with SM, the inclusion of CM in the diet may reduce the carbon footprint of dairy production.

Key Words: canola meal, dairy cow, methane emission

1710W Agro-pastoralist farmers' preferences for dairy cattle breeds and their attributes in arid and semi-arid areas of Benin (West Africa).

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To understand farmers' preferences for dairy cattle breed attributes and their breeding practices, including selection criteria, 150 agro-pastoralists in arid and semi-arid areas of Benin were interviewed in a cross-sectional survey. The sample was selected using the snowball sampling approach. Respondents scored their preference for cattle breeds, traits, and selection criteria of breeding stock on a scale of 1 (most preferred) to 5 (least preferred). Rank means (RM) and relative importance (RI) of preferred traits associated with cattle breeds were calculated and compared between farm types (sedentary or transhumant). The non-parametric Mann-Whitney U test was used for means comparison between farm types (sedentary or transhumant). Results showed that the Gudali breed (RM: 1.09) was most preferred, followed by Yakana (RM: 2.14), while the indigenous Borgou (RM: 3.34) was least desired. Zebu Yakana breed was preferred by transhumant ($P < 0.01$), while sedentary farmers preferred Zebu Gudali. The relative importance given by respondents to the different trait preferences revealed that the Gudali breed was preferred for its high milk production (RI: 4.93) and big body size (RI: 4.35). Zebu M'bororo was preferred only for its high milk production (RI: 3.01). The Yakana breed was chosen for its big body size (RI: 3.73), its adaptability to harsh environments (RI: 4.62), and its low feed requirement (RI: 4.01). However, the indigenous Borgou was the only breed preferred for its high fertility (RI: 2.51). Breeding practices were similar in replacement strategies, uncontrolled mating, and no record keeping. The common criteria mentioned by farmers for the selection of breeding females were the milk yield (RI: 3.03), the calves' survival in the first 3 mo (RI: 2.05), and the earlier sexual maturity (RI: 1.94). All farmers had a least one bull, that age at first service was 4.07 years old. Transhumant herds used Yakana bull, while sedentary farmers preferred Yakana or Gudali bull. Replacing the indigenous Borgou breed in its original belt with more milk-producing breeds calls for this breed conservation. Community-based breeding programs for pure Borgou cattle and crossbreds aiming to improve body size and milk production could be implemented.

Key Words: Gudali, trait preference, small-scale farming

1711W Development of an auto recovery system to calibrate GreenFeed.

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GreenFeed is an automated feeding station that measures the amount of methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2) emitted by ruminant animals while they are visiting the station. To ensure accuracy of the system, the sensors must be regularly calibrated. The objective of this research was to design and test the accuracy of a new automatic gas recovery system (AGRS). The AGRS was constructed of a dual-stage high pressure regulator, gas flow controller, gas tubing, a secondary controller, and a gas distribution release nozzle. During operation, the AGRS was connected to a single high-pressure gas tank with gas concentrations of 3.5% CH_4, 11.5% CO_2, and 400 ppm H_2, with the balance of N_2. The AGRS flow controller was pre-set to provide an outflow rate of 10 L/min to produce known release rates of 3,246 g/d, 361 g/d, and 0.5 g/d for CH_4, CO_2, and H_2, respectively. After each use of the AGRS, the measured release rate of gases was calculated using the concentration increase from background just after the release and multiplying by the GreenFeed airflow rate, and ideal gas laws were applied for temperature and pressure corrections. Percent recovery rate was calculated as the GreenFeed measured release rate divided by the known release rate. A 100% recovery indicates a perfect sensor response. Field tests were performed using 5 independent GreenFeed units that were housed both inside and outside, under varying climate conditions. Based on 55 releases spread over 41 d, average recovery rate was 100.2% (SD = 2.45%) and 101.6% (SD = 3.13%) for CH_4 and CO_2, respectively. These recovery rates are consistent with those achieved with manual recoveries but only required one high pressure gas bottle and no human intervention. The AGRS provided an accurate and repeatable method to calibrate and assess accuracy of GreenFeed.

Key Words: methane, dairy cow, GreenFeed

1712W DairyPrint model: Paving pathways for dairy farmers towards higher sustainability.

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Quantifying greenhouse gas (GHG) emissions (i.e., methane, nitrous oxide, and carbon dioxide) from all significant sources in dairy farms is difficult and prohibitively expensive. The same applies to nutrient balance and management. Therefore, farmers must rely on mathematical models to achieve this. Available models are cumbersome and overwhelming to use. Thus, our objective is to develop the DairyPrint Model: a simple, minimalistic, user-friendly, and scientifically sound whole-farm decision support model to assess environmental tradeoffs of dairy farming. The DairyPrint Model is composed of herd, manure, crop, and economic modules. In the herd module, based on inputs such as total number of cows, calving interval, and culling rate the model runs the simulations performing herd dynamics in monthly steps, outputting annual herd demographics. Moreover, milk yield curves and milk composition, body weight, dry matter intake, and feed efficiency are estimated. These variables, in turn, are used to estimate the total excreted manure, urine, feces, nitrogen, phosphorus, and potassium, in addition to enteric methane emissions. From the herd module, there is a distribution of the produced outputs into other modules, in addition to the specific user inputs for each module. The barn module receives all the manure produced and from it, along with information from weather data and type of facility (free-stall or tie-stall), methane, ammonia, and nitrous oxide emissions are estimated. Once the manure in the barn is transferred to the manure module, the manure is handled and processed according to the practices adopted by the farm. After manure processing, the processed material is distributed to the crop fields in the crop module. In the crop module, all GHG emissions are accounted for due to the application of manure, fertilizers, and limestone. Additionally, nutrient balances are estimated. Therefore, the DairyPrint Model is capable of helping farmers move toward higher sustainability, providing a user-friendly and intuitive graphical user interface allowing the user to respond to "what-if" questions.

Key Words: greenhouse gas, nutrient, whole-farm

1713W Effect of anti-mycotoxin feed additives on the reduction of mycotoxins in milk and urine of dairy cows fed multi-mycotoxin–contaminated diets.

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Our aim was to evaluate the effects of different anti-mycotoxin feed additives on the excretion of mycotoxins in milk and urine of dairy cows fed diets contaminated with multiple mycotoxins. Twelve Holstein cows were blocked according to parity, milk yield, and DIM and used in a 4 × 4 Latin square design with four 21-d periods. Treatments were control (CON), basal diet (BD) without anti-mycotoxin feed additives; adsorbent (AD), adding hydrated sodium calcium aluminosilicate 25 g/cow/day to the BD; mycotoxin deactivator 1 (MD1), adding mycotoxin deactivator (Mycofix 5.0, DSM Nutritional Products) 15 g/cow/day to the BD; and mycotoxin deactivator 2 (MD2), adding the same product at 30 g/cow/day to the BD. A daily total of 400 µg of aflatoxins (AFs), 5,000 µg of deoxynivalenol (DON), 8,000 µg of fumonisins (FBs), 200 µg of T2 toxin (T2), and 2,000 µg of zearalenone (ZEN) was administered and mixed with the BD between d 14 to 21 in each period. Milk and urine samples were taken on d 21 of each period. Multi-mycotoxin determination was done by ultra-performance liquid chromatography system coupled to a mass spectrometer. Statistical significance was considered at $P \leq 0.05$. Anti-mycotoxin feed additives reduced AFM1 secretion in milk, ranging from 39.6% (AD) and 94.3% (MD1) to 97.8% (MD2), and in urine, ranging from 10.8% (AD) and 17.8% (MD1) to 26.1% (MD2). In addition, MD1 and MD2 prevented the secretion of other mycotoxins (DON, FBs, T2, and ZEN) in milk (levels were below the detection limits, LOD), with no difference between the AD treatment and CON. In urine, MD1 and MD2 reduced DON and FBs, to levels below LOD, but no differences were observed between AD and CON. α-Zearalenol and β-zearalenol excreted in urine were reduced by 97.5% and 92.4% (respectively) for MD1 treatment, residues were not detected for MD2 treatment (below LOD), while no differences were observed between CON and AD. In conclusion, the mycotoxin-deactivator MD was effective in reducing multi-mycotoxins in milk and urine in the 2 doses evaluated.

Key Words: decontamination, mycotoxin, urine

1714W Silvopastoral systems as a heat abatement recourse for grazing dairy cows.

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Heat abatement is a hot topic for the dairy industry. This study aimed to estimate the thermal comfort of dairy cattle during the summer in a silvopastoral system compared with a treeless pasture. Our study did not focus on direct measurements on animals, but on estimating the thermal comfort based on microclimate data. We evaluate 2 pasture systems: treeless pasture (TLP) and silvopastoral system (SPS) with trees on the border fence. In both pasture systems, microclimatic variables of air temperature, relative humidity, wind speed, and black globe temperature were measured during the summer (December 2020 and February 2021) in southern Brazil. With the microclimatic data, we determined the radiant heat load (RHL, W/m^2) as an indicator of thermal comfort. All data were analyzed using a mixed model with the pasture systems as a fixed factor, and days and hours as random effects through the R statistical software. There was an influence ($P < 0.05$) of the pasture system on the thermal comfort indicators. Overall, the SPS had 16% odds to show lower ($P < 0.01$) RHL values than TLP. The TLP showed the highest average (±SD) values ($P < 0.001$) of the RHL (TLP: 599.1 ± 55.1 W/m^2; SPS: 520.6 ± 85.6 W/m^2). Furthermore, there was an influence ($P < 0.001$) of the areas on RHL; in the shaded areas, the tree canopy provided on average a thermal heat reduction of 25%. Our findings highlight that areas without heat abatement can be challenging for dairy cows in pasture-based systems of subtropical climates. Under the conditions of this study, the SPS promoted a better thermal environment for animals raised on pasture compared with the TLP, indicating its potential as a heat abatement resource during summer.

Key Words: biometeorology, thermal comfort, heat stress

1715W Use of sensors for the detection and genetic evaluation of heat stress in dairy cattle.

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Reaction of milk production traits is generally used to evaluate heat stress (HS) in dairy cattle. However, milk recording data are in most cases only obtained once per month which drastically limits the number of records obtained during hot days. A solution could be to add sensor information that is obtained daily. In addition, sensors are already used for estrus detection in heifers and cows, but also for health and welfare of animals. The objectives of this study were thus to evaluate the usability of sensor data to detect HS and to assess the possible gain for HS genetic evaluation. Daily SenseHub collar records for activity, rumination and eating time were obtained from 2019 to 2022 for 453 Walloon Holstein cows. Meteorological data, and fat- and protein-corrected milk (FPCM) were also obtained from 2015 to 2022 for 1,740 cows from the same herds. The thresholds at which the different traits start to be affected by HS were estimated at a temperature-humidity index (THI) of 37, 58, 62, and 66, respectively, for FPCM, eating, activity and rumination time. The thresholds were clear for sensors traits while FPCM decreased all along the THI scale. Variation of the sensors traits with the THI were higher in comparison with FPCM. A genetic random regression reaction norm model using extracted pedigree information was performed with these different thresholds to fit at the maximum the trait reaction to THI. Regarding the heat tolerance genetic reaction norm effect, sensor traits showed positive or slightly negative genetic correlations with FPCM (activity time: 0.32 ± 0.54, rumination time: 0.05 ± 0.41, eating time: −0.15 ± 0.36). In addition, these traits respectively presented heritability values of 0.14 ± 0.06, 0.18 ± 0.05, and 0.14 ± 0.05 at the thresholds and these values were similar at high THI. In conclusion, sensors data could be valuable tools to detect HS because they react well and their THI thresholds were easy to detect. They could also be useful in genetic evaluation for HS because their daily records provides information for all HS events. Sensor traits were heritable including at high THI and the genetic correlation of the effects on activity and on FPCM was positive. On this basis, activity time seems to be the most interesting sensor trait for genetic evaluation of HS.

Key Words: heat stress, sensor, threshold

1716W Accuracy of prediction of future milk production with an empirical Bayes method.

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Accurate and precise prediction early in lactation of milk production in the remainder of lactation is important for insemination and culling decisions. Tools for accurate future milk production are not readily available. The objective of this study was to evaluate the accuracy of an empirical Bayes method to predict milk production in the remainder of the lactation. The empirical Bayes method selects lactations from an historical data set of completed lactations based on similarity of early lactation milk weights with the lactation in progress. We used lactations completed at the University of Florida Dairy Unit between 2015 and 2017. We deleted lactations with more than 30 d of missing milk weights. Local regression was used to complete remaining missing milk weights and reduce daily variation. The resulting data set consisted of 214,761 milk weights in 723 lactations of 656 unique cows. These data were split into lactation 1 (n = 272), lactation 2 (n = 234), and lactation 3+ (n = 217). Within each lactation group, 80% of the lactations were put into the historical data set and 20% into the predictor data set of lactations in progress. Each lactation in the historical database was fit with the Milkbot model to obtain a parametric lactation curve. For each cow in the predictor database, her milk weights from 10 to 70 DIM and the empirical Bayes method were used to predict the lactation curve for the remainder of the lactation (71 to 305 d). Because each cow in the predictor data set had also completed her lactation, we evaluated goodness of fit as the root mean squared error (RMSE) of local actual vs. the predicted daily milk weights. The RMSE of daily weights was 5.1 ± 2.5 kg for all lactations (lactation 1, 4.6 ± 2.4 kg; lactation 2, 5.3 ± 2.6 kg; and lactation 3+, 5.7 ± 2.6 kg). These RSME are similar to published results from more complex methods. We concluded that the empirical Bayes method is an attractive method to predict future milk production. Further work should include extension of the historical data set with other traits such as season and health events to improve accuracy.

Key Words: yield, prediction, accuracy

Reproduction 3

1718W Fertility of lactating Jersey cows submitted to a Double-Ovsynch protocol for timed artificial insemination or artificial insemination after a synchronized estrus based on synchrony and expression of estrus. M. R. Lauber* and P. M. Fricke, *University of Wisconsin–Madison, Madison, WI.*

We hypothesized that lactating Jersey cows submitted to a Double-Ovsynch protocol for timed artificial insemination (TAI) at 1st service would have more pregnancies per AI (P/AI), with no difference based on expression of estrus, than cows artificially inseminated (AI) after a detected estrus regardless of synchronization rate. Lactating Jersey cows (n = 1,138) from a commercial dairy farm were randomized within parity to 1 of 2 treatments for 1st service: 1) a Double-Ovsynch protocol [DO, n = 712 ; d 0 GnRH; d 7 $PGF_{2\alpha}$; d 10 GnRH; d 17 GnRH; d 24 and d 25 $PGF_{2\alpha}$; 32 h GnRH (d 26); 16 h TAI (d 27)] or 2) a protocol for synchronization of estrus with twice-daily detection of estrus based on rubbed tail chalk from d 21 to 32 [EDAI, n = 426 ; d 3 GnRH; d 10 $PGF_{2\alpha}$; d 24 and d 25 $PGF_{2\alpha}$]. Expression of estrus for DO cows was recorded from 24 to 27 d and was classified as: 1) > 24 h before or 2) at TAI. The synchronization rate to the protocols was assessed by serial blood sample collections (24, 27, and 34 d) and synchrony was defined as high progesterone (≥0.5 ng/mL) at 24 and 34 d and low progesterone (<0.5 ng/mL) at 27 d. Binomial variables were analyzed by logistic regression using the GLIMMIX procedure of SAS with the fixed effects of treatment and parity. Overall, the synchronization rate was greater ($P < 0.01$) for DO than for EDAI cows (91.9 vs. 79.0%). For synchronized cows, DO cows had more ($P = 0.04$) P/AI than EDAI cows (54.5 vs. 48.7%). Expression of estrus for DO cows between 24 and 27 d was 28.2% with 4.2% and 25.1% of cows in estrus >24 h before and at TAI, respectively. Overall, P/AI did not differ ($P = 0.20$) for DO cows that expressed or did not express estrus at TAI (59.5 vs. 53.8%, respectively). In conclusion and in support of our hypothesis, lactating Jersey cows submitted to a Double-Ovsynch protocol for TAI yielded more P/AI, with no difference based on expression of estrus, than cows AI to a detected estrus regardless of synchronization rate. Supported by NIFA USDA CARE project 2021–68008–34105.

Key Words: synchrony, expression of estrus, pregnancies per artificial insemination

1719W Associations of early postpartum metabolic and inflammatory markers with time to onset of cyclicity in clinically healthy dairy cows. T. C. Bruinjé*, E. I. Morrison, and S. J. LeBlanc, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Our objective was to investigate associations of early postpartum metabolic and inflammatory markers with time to onset of cyclicity in clinically healthy dairy cows. Holstein cows (n = 1,424) from 2 herds in Ontario, Canada were examined for these markers and clinical disorders. Total Ca, haptoglobin (Hp), and nonesterified fatty acids (NEFA) were measured in serum at 2 and 6 ± 2 DIM, blood β-hydroxybutyrate (BHB) at 4, 8, 11, and 15 ± 2 DIM, and endometrial cytology was assessed by cytobrush at 35 ± 3 DIM. Serum progesterone (P4) concentrations was measured at 21, 35, 49, and 63 ± 3 DIM. Cows diagnosed with difficult calving, twin birth, retained placenta, metritis, displaced abomasum, mastitis, lameness, or purulent vaginal discharge were excluded (n = 701; 49% of the total). Markers were categorized based on ROC cutpoints associated with pregnancy at first AI, and data were analyzed with multivariable logistic or Cox-proportional hazard regression. Thirty-nine, 71, 88, and 94% of cows resumed cyclicity (first P4 ≥ 1 ng/mL) by 21, 35, 49, and 63 DIM, respectively. The adjusted odds ratio (AOR; 95% CI) of onset of cyclicity by 21 DIM was decreased in cows with elevated NEFA at 2 DIM (≥0.73 m*M* in 20% of cows; AOR: 0.65; 0.43 to 0.97), elevated Hp at 6 DIM (≥0.62 g/L in 28% of cows; AOR: 0.69; 0.49 to 0.99), or hyperketonemia at 11 DIM (≥0.7 m*M* in 49% of cows; AOR: 0.66; 0.48 to 0.91). Cows with elevated Hp at 2 DIM (≥1.54 g/L in 10% of cows) had reduced adjusted hazard rate (AHR; 95% CI) of onset of cyclicity by 63 DIM (AHR: 0.74; 0.57 to 0.96), and those with hypocalcemia at 2 DIM (≤2.09 m*M* in 43% of cows; AHR: 0.76; 0.60 to 0.97) or hyperketonemia at 11 DIM (AHR: 0.73; 0.57 to 0.93) had reduced rate of onset of cyclicity by 21 DIM, but not beyond 35 DIM. Alterations in early postpartum metabolic and inflammatory markers are likely indicators of subclinical disorders that interfere with early resumption of cyclicity postpartum.

Key Words: anestrus, fertility, transition health

1720W Association between earlier emergence of large follicles and subsequent fertility in postpartum dairy cows. M. Sakaguchi*[1], T. Yamazaki[2], and H. Kusaka[1], [1]*School of Veterinary Medicine, Kitasato University, Towada, Aomori, Japan,* [2]*Hokkaido Agricultural Research Center, NARO, Sapporo, Hokkaido, Japan.*

Postpartum resumption of ovarian activity plays an important role in the subsequent fertility of lactating dairy cows. In our previous study, lactating dairy cows experienced postpartum first ovulation at 31 d on average after parturition, and before that, one or more large follicles (LF) could develop but some of the follicles became atretic. Only a few studies have focused on the influence of early ovarian activities on subsequent fertility, concerning the earlier emergence of LF. The main objective of our study was to evaluate the impact of the earlier emergence of LF on subsequent reproductive performance. Data were collected from 499 lactations (188 primiparous and 311 multiparous) of 241 Holstein dairy cows in an experimental herd. The emergence of LFs with ≥15 mm in diameter (XLFs) was confirmed in 161 lactations (32.3%) at 10 d postpartum by ultrasonography. A multivariate logistic regression model was used to calculate the odds ratio for various risk factors for the emergence of XLFs. The selection of explanatory variables included calving season (spring, summer, autumn, and winter), parity (1, 2, 3, and ≥4), BCS (<2.50, 2.50 to 3.00, and >3.00 at D10), and calving year (1999 to 2002, 2003 to 2005, 2006 to 2008, and 2009 to 2012). Survival curves were generated for the time to pregnancy between cows with and without the XLFs using Kaplan-Meier analysis. A generalized Wilcoxon test was used to compare the survival curves. Compared with the animals that calved in summer, the emergence of XLFs was significantly lower in the animals that calved in spring and winter (41.0% vs. 26.5% and 29.0%, $P < 0.05$). The cows in the second parity had 2.5 times higher emergence of XLFs compared with those in more than the fourth parity (39.1% vs. 21.7%, $P < 0.05$). Survival analysis indicated that the proportion of non-pregnant cows tended to significantly differ between cows with and without XLFs; the cows with XLF were pregnant earlier than that without ($P = 0.09$). In conclusion, earlier emergence of follicles with ≥15 mm diameter could be a good indicator of subsequent pregnancy rates in lactating dairy cows.

Key Words: fertility, postpartum cow, ovarian activity

1721W Peripartum supplementation of omega-3 fatty acids modulates uterine proteome towards enriched metabolic and inflammatory pathways in dairy cows. J. R. Daddam[1], G. Kra[1,2], U. Moallem[1], H. Kamer[1], and M. Zachut*[1], [1]*Agriculture Research Organization, Volcani Center, Rishon LeZion, Israel,* [2]*Faculty of Agriculture, the Hebrew University, Jerusalem, Israel.*

Omega-3 (n-3) fatty acids can modulate inflammation and the endocannabinoid system (ECS). We examined the effects of peripartum n-3 supplementation on the uterine proteome in dairy cows. Thirty-five 256-d pregnant multiparous Israeli-Holstein cows were individually fed from 21 d prepartum: i) CTL – prepartum and postpartum (PP) common diets; ii) FLX – prepartum diet containing 700 g/d/cow of extruded flaxseed supplement containing C18:3n-3 (Valomega 160, Valorex, France), and PP supplemented at 6.4% of diet (DM basis). Following estrous cycle synchronization, uterine biopsies were performed at 47–66 DIM (average of 58.5 DIM). Proteomic analysis of uterine tissues (n = 5 from each treatment) were performed using LC-MS/MS, and Ingenuity Pathway Analysis (Qiagen) was used for canonical pathways, networks and functions. A total of 2,325 proteins were identified; from which 32 proteins were differential by *t*-test in FLX vs. CTL [$P \leq 0.05$ and fold change ± 1.5]. The abundances of mucin-1, histone H2B, and gamma-interferon-inducible-lysosomal-thiol-reductase were lower in uterus of FLX vs. CTL, while fibronectin-type-III-domain-containing-1, several MHC class 1 antigens, and lipopolysaccharide (LPS)-binding protein were increased in FLX than in CTL. Top canonical pathways enriched in FLX uterus included: taurine biosynthesis, sulfate activation for sulfonation, CLEAR signaling, LPS-stimulated MAPK signaling and oxidative phosphorylation, eNOS signaling and acute phase response signaling. Peripartum supplementation of n-3 affected the uterine proteome and may have anti-inflammatory effects, possibly related to modulation of the ECS by n-3 in dairy cows.

Key Words: proteomics, uterus, reproduction

1722W Effects of prepartum acetylsalicylic acid administration on reproductive microbiome in postpartum Holstein dairy cattle. J. Lection*[1,2], E. Jimenez[3], P. Zarei[3], S. Bierly[2], J. Spring[3], M. Martinez[3], A. A. Barragan[3], and E. Ganda[2,4], [1]*Intercollege Graduate Degree Program in Integrative and Biomedical Physiology, The Pennsylvania State University, University Park, PA,* [2]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [3]*Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA,* [4]*Microbiome Center, The Pennsylvania State University, University Park, PA.*

Modulating inflammation in the periparturient period has been associated with decreased incidence of uterine diseases; however, the impact on the postpartum reproductive microbiome is still unknown. The objective was to assess the association of prepartum acetylsalicylic acid [ASA] and postpartum reproductive microbial diversity. Animals were blocked by parity 14 ± 3 d before expected calving date and randomly allocated to one of 2 treatments: ASA (n = 41): one oral administration of acetylsalicylic acid (125 g/d), or PLC (n = 40): one oral administration of water-filled gelatin capsules. Vaginal discharge was collected from enrolled cows at 7 ± 3, 14 ± 3, and 21 ± 3 d in milk (DIM) with a Metricheck device with a sterile cup. Clinical metritis was diagnosed based on presence of reddish-brown watery discharge (ASA n = 16, PLC n = 20). Vaginal discharge samples underwent DNA extraction, 16S rRNA gene PCR amplification, and sequencing. Data were analyzed using R with Welch's *t*-test for α diversity and PERMANOVA for β-diversity. There was no difference in α diversity (the richness/evenness of the microbial population within each sample) between treatment groups among healthy cows. However, richness and evenness were lower in ASA-treated cows with metritis as compared with placebo-treated cows with metritis at 7 ± 3 DIM ($P = 0.047$) and 14 ± 3 DIM ($P = 0.044$). Beta diversity (comparison of microbial communities between cows) was not different between treatment groups among both healthy and metritis cows. Differential abundance testing revealed increased relative abundance of the taxonomic phylum *Actinomycetota* ($P = 0.044$) in metritis cows receiving the placebo. These results suggest that prepartum ASA may have no effect on the reproductive microbiome of non-metritic postpartum cows but may decrease α diversity in metritic cows. Overall, acetylsalicylic acid may have a beneficial effect on cows with metritis as the phylum *Actinomycetota*, which contains metritis-associated pathogens such as *Trueperella pyogenes,* was less relatively abundant in addition to potentially reducing antibiotic usage.

Key Words: acetylsalicylic acid, clinical metritis, reproductive microbiome

1723W Effect of human chorionic gonadotropin on pregnancy outcomes in multiparous lactating Jersey cows receiving an IVF beef embryo after a synchronized estrus versus a synchronized ovulation. N. Hincapie*[1], M. R. Lauber[1], T. Valdes-Arciniega[1], P. Carvalho[1], R. Faber[2], R. Farruggio[3], and P. M. Fricke[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Reprovider LLC, Janesville, WI,* [3]*Jefferson Veterinary Clinic, S.C., Jefferson, WI.*

To determine the effect of recipient type and hCG treatment on pregnancy outcomes after IVF embryo transfer (ET), lactating multiparous Jersey cows were randomized to a 2x2 factorial arrangement of treatments to test the main effect of recipient type (Double-Ovsynch [DO; n = 169] vs. a synchronized estrus [ED; n = 180]) and the main effect of treatment with 2,500 IU of human chorionic gonadotropin (hCG, n = 148 vs. control, n = 147) at ET. Grade 1, stage 7, IVF beef embryos were produced from black Angus-based dams using proven high fertility Angus sires and were frozen for direct transfer. Only cows that had a CL ≥13 mm on the day of transfer received ET, and ET occurred 8 d after the last GnRH treatment of the DO protocol or 7 or 8 d after estrus. All ED cows not detected in estrus received ET 8 d after an Ovsynch protocol (OV, n = 90). The effect of treatments and their interaction were analyzed statistically using a mixed effect logistic regression model. Overall, more ($P < 0.001$) DO cows (93%) received ET than ED+OV cows (76%), and ovulatory response to hCG based on an accessory CL 7 d later was 85% and did not differ between recipient types. Plasma progesterone (P4) concentrations at ET were greater ($P = 0.04$) for ED than for DO cows (3.3 ± 0.1 vs. 2.8 ± 0.1 ng/mL), and P4 concentrations 7 d after ET were greater ($P < 0.001$) for DO and ED cows, respectively, treated with hCG (8.9 ± 0.4 and 8.3 ± 0.5 ng/mL) than control cows (5.3 ± 0.3 and 7.3 ± 0.5 ng/mL). There was a tendency ($P = 0.1$) for a treatment interaction on pregnancies per ET (P/ET) at 33 d in which treatment with hCG increased P/ET for DO cows (26% vs. 41%), but not for ED+OV cows (25% vs. 24%). Pregnancy loss from 33 to 61 d did not differ among treatments. In conclusion, multiparous lactating Jersey cows submitted to a DO protocol and treated with 2,500 IU of hCG at ET had increased P4 concentrations 7 d after ET and tended to have more P/ET than untreated cows or ED+OV cows. Supported by WI Experiment Station Hatch project WIS02096.

Key Words: IVF embryo transfer, human chorionic gonadotropin, recipient synchronization

Table 1 (Abstr. 1725W). Effect of increasing supplementation of sodium pyruvate (SP) on sperm parameters

Item	0	5	10	15	20	SEM
Total motility,¶ %	54.0	65.9	63.1	57.6	53.2	3.2
Progressive motility,¶ %	48.4	60.1	57.3	51.1	48.2	3.4
Curvilinear velocity,* %	81.0	91.5	84.4	75.9	75.3	4.8
Viability,¶ %	62.3	72.3	69.7	64.2	63.2	3.1
Plasma membrane integrity,*¶ %	36.8	42.8	43.7	41.2	34.7	4.6
Acrosomal integrity,*¶ %	86.7	90.5	92.0	90.3	86.2	1.3
Catalase,*¶ KU/L	1.47	12.07	8.93	4.70	2.20	1.89
Malondialdehyde,$^{*\varphi¶}$ µ*M*/L	2.38	1.93	1.95	2.02	2.10	0.08

$P \le 0.05$: Effect of SP (*); linear (φ) or quadratic (¶) effects of SP.

1724W Effect of the interval between the last PGF$_{2\alpha}$ and GnRH on ovulatory dynamics of dairy heifers submitted to a modified 6-d synchronization protocol.

I. M. R. Leão*, F. P. J. da Silva Junior, M. P. Zutz, T. Valdes-Arciniega, E. Anta-Galvan, and J. P. N. Martins, *University of Wisconsin–Madison, Madison, WI.*

We aimed to determine the effect of 2 intervals between the last PGF$_{2\alpha}$ and GnRH on final ovulation time in a modified 6-d synchronization protocol for dairy heifers. All heifers (n = 185) were pre-synchronized with a PGF$_{2\alpha}$ (0.5 mg of cloprostenol)on d-2 and started the protocol on d 0 with a GnRH (100 µg). Heifers received PGF$_{2\alpha}$ on d 6 and 7. Estrus was detected once daily by tail chalk from d 7 to 9. Heifers detected in estrus on d 7 and 8 were inseminated on the same day and did not receive a final GnRH. Heifers not detected in estrus were randomly assigned to either receive a final GnRH (G2) on d8 and timed-AI (TAI) on d9 (P2G) or to receive G2 and TAI on d9 (P3G). Ovaries were scanned on d 0, 6, 8, 9, 10, and 12 to evaluate follicular and luteal dynamics. Continuous and binary variables were analyzed by linear and logistic regression, respectively. Of all heifers, 38.0% were detected in estrus and inseminated on d8, resulting in a P/AI 32 d post-AI of 66.2%. The final ovulatory response of these heifers was 92.8% (47.6% ovulated from d 8 to 9, 36.5% from d 9 to 10, and 15.9% from d 10 to 12). For heifers that received G2, treatment did not affect the proportion of heifers in estrus on d9 (P2G: 50.0% vs. P3G: 63.0%, $P = 0.17$), final ovulatory response (P2G: 89.3% vs. P3G: 87.0%, $P = 0.77$) nor ovulatory time distribution ($P = 0.68$; from d 8 to 9: 17.5%; d 9 to 10: 67.0%; and d 10 to 12: 14.4%). Additionally, estrus on d9 affected ovulatory time distribution ($P < 0.01$; from d 8 to 9: 17.7% vs. 17.7%; d 9 to 10: 75.8% vs. 52.8%; and d 10 to 12: 6.5% vs. 29.4% for estrus vs. no estrus, respectively). Ovulatory follicle size tended to be greater for P3G than P2G (15.5 ± 0.3 mm vs. 14.8 ± 0.3 mm, $P = 0.06$) and larger for heifers detected in estrus on d9 than not detected (15.7 ± 0.3 mm vs. 14.0 ± 0.3 mm, $P < 0.01$). Treatment did not affect P/AI 32 d post-AI (P2G: 43.6% vs. P3G: 40.7%, $P = 0.43$). However, P/AI of heifers in estrus on d 9 was increased than heifers not in estrus (57.4% vs. 22.9%, $P < 0.01$). In summary, estrus had a greater impact on ovulatory dynamics than the interval between the last PGF$_{2\alpha}$ and GnRH.

Key Words: heifers, estrus, ovulation

1725W Effects of supplementing sodium pyruvate on post-thaw semen evaluation in Nili-Ravi buffalo bulls.

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Buffalo spermatozoa gather increased content of polyunsaturated fatty acids in their plasma membrane, which increase the lipid peroxidation and compromise their survival. Objectives were to determine the effects of supplementing increasing amounts of sodium pyruvate (SP) as an antioxidant on sperm functions in buffalo bulls (n = 4). Semen was collected twice a week and a total of 8 replicates were performed. Each ejaculate of each bull was evaluated, and ejaculates having at least 1 mL of semen with 65% progressive motility and 0.5 billion/mL sperms were included and pooled. The pooled semen was extended with Tris-egg yolk extender to achieve the concentration of 50×10^6 spermatozoa/mL. The extended semen was divided into 5 tubes equally, and assigned randomly to receive 0, 5, 10, 15, and 20 m*M* of SP. The extended semen representing each treatment was subjected for cryopreservation, and 5 semen straws from each treatment were evaluated. Data were analyzed by ANOVA with linear mixed-models using the MIXED procedure of SAS. The statistical models included the fixed effects of treatment, and the random effect of replicate. Orthogonal contrasts evaluated the effect of supplementing SP (0 vs. 1/4·5 + 1/4·10 + 1/4·15 + 1/4·20 m*M*), and polynomial contrasts investigated the linear (3/4·5 + 1/4·10 vs. 1/4·15 + 3/4·20), quadratic (1/2·5 + 1/2·10 vs. 1/2·15 + 1/2·20), and cubic effects (1/4·5 + 3/4·10 vs. 3/4·15 + 1/4·20) of increasing supplementation of SP from 5 to 20 m*M*. Data are presented in Table 1. Supplementing 5- or 10-m*M* SP improved the sperm quality and reduced the oxidative damage, which might be beneficial to optimize semen extender during semen cryopreservation in buffaloes.

Key Words: Nili-Ravi buffalo, sodium pyruvate, sperm

Ruminant Nutrition: Calves and Heifers 3

1726W Effect of β-casein A1 or A2 milk on visceral adipose tissue in dairy calves. R. Kappes[1,2], V. Schneider[2], H. Schweizer[2], S. Nüske[2], D. A. Knob[3], A. Thaler Neto[1], and A. Scholz*[2], *[1]Centro de Ciências Agroveterinárias-Universidade do Estado de Santa Catarina, Lages, Santa Catarina, Brazil, [2]Lehr- und Versuchsgut Oberschleissheim- Ludwig-Maximilians-Universität München, Oberschleissheim, Bavaria, Germany, [3]Justus-Liebig University Giessen, Giessen, Hessen, Germany.*

Because of the postulated absence of β-casomorphin 7 in calves fed homozygous A2 milk, we assumed differences in the amount of visceral adipose tissue (VAT) and in vivo body composition (BC) in dairy calves fed either homozygous β-casein A1 or homozygous A2 milk on d 15 of life. Calves were offspring of Holstein (n = 10), Simmental (n = 15), and Holstein x Simmental (n = 3) cows. Calves were weighed after birth and received colostrum ad libitum. On the second day, calves were housed alternately in pairs in double-igloo systems according to their random birth order and received either A1 (n = 14; 9 female/5 male) or A2 milk (n = 14; 7 female/7 male). They were offered 7.5 L/day. Actual total milk intake (TMI) was recorded. On d 15, VAT volume was assessed by open magnetic resonance imaging (Siemens Magnetom C! - MRI) and dual energy x-ray absorptiometry (GE; Core Scan Mode - DXA). In addition, fat (FM) and lean (LM) mass as well as bone mineral content (BMC) and bone mineral density (BMD) were determined by DXA. A mixed model (SAS 9.4) was used with fixed effects β-casein milk, gender, milk x gender, and random effect breed. The covariate DXA body weight was included for BC traits. There was no significant BC difference between the 2 milk types. However, female calves had more VAT than male calves (Table 1). Thus, we conclude that A1 or A2 milk has no effect on calf BC. However, A2 calves had a lower TMI than A1 calves, which resulted in significantly higher daily gains (ADG) in A1 calves.

Key Words: body composition, visceral fat, A2 milk

1727W Milk-derived bioactive peptide XPP alleviates colitis by inhibiting NF-κB and other inflammatory protein pathways and restoring intestinal flora. J. Hou*, X. Wang, W. Du, and Q. Xu, *Huazhong Agricultural University, Huazhong Agricultural University, Wuhan, Hubei, China.*

Limited milk consumption shows a higher incidence of intestinal inflammatory diseases in calves before and after weaning than unrestricted milk consumption, resulting in significant economic losses on a global scale each year. Milk-derived bioactive peptide XPP (MBP) is the product of casein in milk degraded by gastrointestinal protease, which accounts for a high proportion and has absolute bioavailability. The MBP has been reported to inhibit inflammation related signaling pathways, but the specific mechanism of MBP in alleviating intestinal inflammation is still unclear. The experimental mice were C57BL/6, male mice, 18–20 g, housed in SPF environment with a constant temperature of 37°C, 12 h light and 12 h dark cycle, and all mice had free access to food and water. All experimental animals were given at least 1 week to acclimatise to laboratory conditions. All experimental animals were randomly divided into 3 groups: Control group, DSS group and MBP group (DSS + MBP), 8 mice in each group. In Control group, normal saline (0.2 mL/d) was given by gavage from d 1 to d 17. In DSS group, normal saline (0.2 mL/d) was given by gavage from d 1 to d 17, and the drinking water was replaced by 3% DSS solution from d 8 to d 14. In MBP group, XPP solution (100 mg/kg BW) was given by gavage from d 1 to d 17, and drinking water was replaced by 3% DSS solution from d 8 to d 14. Our results showed that MBP could alleviate the symptoms of inflammatory bowel disease ($P = 0.0119$), increase the level of short-chain fatty acids in intestinal contents ($P = 0.0293$), inhibit the expression of MAPK ($P = 0.0317$) and COX2 ($P = 0.0357$) in colon tissues, increased the expression of tight junction proteins ($P = 0.0303$) in colon tissues, increased the abundance of AKK bacteria ($P = 0.0079$), and level of IAA, a metabolite of colonic flora ($P = 0.0047$). In conclusion, MBP could reduce intestinal inflammation by inhibiting the expression of inflammatory pathway proteins, restoring intestinal flora and increasing the abundance of AKK bacteria and IAA metabolites. The MBP may become a new strategy for clinical treatment of intestinal inflammatory diseases.

Key Words: milk-derived bioactive peptide, colitis, NF-κB

1728W Assessment of blood and fecal oxidative stress markers in neonatal dairy calves with diarrhea. Z. L. Fu[1,2], Y. Yang[1,4], N. Malmuthuge[3], L. Ma[1], L. L. Guan[2], and D. P. Bu*[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, [3]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta,*

Table 1 (Abstr. 1726W). Body weight (BW), TMI, ADG, and body composition in dairy calves fed either homozygous β-casein A1 (A1) or homozygous A2 milk (A2)

Item	A1	A2	Female	Male
Birth BW (kg)	40.1 ± 2.6	41.6 ± 2.6	40.2 ± 2.5	41.5 ± 2.7
TMI (L)	100.13 ± 2.4[a]	91.35 ± 2.3[b]	96.47 ± 2.2	95.00 ± 2.5
ADG (g/d)	798 ± 65[a]	543 ± 63[b]	668 ± 45	673 ± 68
MRI VAT (cm^3)	419 ± 41	381 ± 43	443 ± 41[a]	358 ± 45[b]
DXA VAT (cm^3)	353 ± 36	343 ± 33	370 ± 33	325 ± 39
DXA FM (g)	3,169 ± 103	3,100 ± 104	3,208 ± 98	3,061 ± 114
DXA LM (g)	47,092 ± 118	47,176 ± 120	47,064 ± 113	47,205 ± 130
DXA BMC (g)	1,662 ± 33	1,666 ± 30	1,674 ± 30	1,654 ± 36
DXA BMD (g/cm^2)	0.811 ± 0.02	0.802 ± 0.01	0.814 ± 0.01	0.799 ± 0.02

[a,b]Different letters between effect groups indicate significance ($P < 0.05$).

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Oxidative stress is the imbalanced redox status, which has been reported to induce intestinal diseases, with abnormal glandular secretion, intestinal injury and increased intestinal mucosal permeability. However, it is unclear whether neonatal calves are under oxidative stress when they develop diarrhea. This study assessed the oxidative stress in neonatal calves during diarrhea by measuring the dynamics of reactive oxygen species (ROS), malondialdehyde (MDA), and H_2O_2 in blood and feces. Calves were enrolled in the study at d 21 after birth (D21) and monitored 7 d for diarrhea by evaluating fecal score. At d 28 (D28) calves were grouped based on their health status as follows; continuous diarrhea from D21 to D28 (DD, n = 15), diarrhea at D21 but recovered at D28 (DH, n = 19), healthy at D21 but diarrhea at D28 (HD, n = 14), and no diarrhea/healthy from D21 to D28 (HH, n = 16). The blood and fecal samples were collected on D21 and D28 to measure oxidative stress markers, including ROS, MDA and H_2O_2, using fluorescence, chromatography and biochemistry methods, respectively. All the data were analyzed using a repeated measurement design in nlme package of R project (version 4.1.0). The results showed that all indicators measured were stable in DD and HH as calves grew from D21 to D28. The concentration of both serum and fecal ROS and MDA became higher in HD as calves had diarrhea ($P < 0.05$), while became lower in DH as calves recovered from diarrhea ($P < 0.05$). In contrast, serum H_2O_2 became higher in DH from D21 to D28 ($P < 0.05$), while lower in HD calves from D21 to D28 ($P < 0.05$). In conclusion, both circulatory (blood) and intestinal (fecal) indicators revealed the oxidative stress occurred when calves developed diarrhea. The shift of serum H_2O_2, one species of ROS, with health dynamics warrants further study to understand its vital role in physiological change in calves with diarrhea.

Key Words: reactive oxygen species, oxidative stress, calf diarrhea

1729W Evaluation of differing interventions (including pork plasma) at the onset of neonatal calf diarrhea. D. W. Wood*[1], A. J. Keunen[2], B. W. Keunen[2], R. M. Blome[1], L. C. Ribeiro[2], and D. R. Renaud[3], *[1]Animix, Juneau, WI, [2]Mapleview Agri, Palmerston, ON, Canada, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of the study was to investigate strategies to treat diarrhea. Farm and auction-sourced male calves (n = 160; 140 Holstein, 20 crossbred) arrived at a research facility in 2 batches, filling 2 rooms of 80 calves. Calves were fed whey protein–based 26% CP, 20% fat milk replacer void of additives for 8 wks in a 2x/d strategy. Calves were randomly assigned at the onset of diarrhea (fecal score of 3 or 2 consecutive scores of 2) to one of 4 treatments (59.4% of the calves). Treatments included, (1) Colostrum (COL): 10 feedings of a 1 L mixture over 5 consecutive days of whole colostrum (Saskatoon Colostrum Company) mixed at 30 g/L; (2) porcine plasma (PP; NUTRAPRO P APC) fed the same as COL; (3) injection of 3 mL trimethoprim sulphadoxine (Borgal) intramuscularly 1x/d for 5 consecutive days with MR fed same as COL; or (4) control (CON; MR fed the same as COL). The mean days from arrival to enrollment were 4.2 ± 1.7 d, NSD (no significant differences; $P = 0.83$) between treatment groups. All diets were formulated to be isonutritious in protein and fat. Resolution of diarrhea was defined as 2 consecutive fecal scores of 0 or 1. Trimethoprim sulphadoxine was administered if fecal scores did not improve. Calves were individually weighed weekly through d 56, with fecal scores, respiratory scores (UC Davis system), and medical treatments recorded 2x/d. Blood was collected at arrival to determine serum total protein (STP). All statistical analyses were conducted in Stata 17 with linear regression and cox proportional hazard models. No differences in arrival STP or body weight were found between groups. Few differences were noted between treatment groups including no differences found in growth or quantity of antibiotics for diarrhea or respiratory disease; however, calves in the antimicrobial group had increased ADG over control during the 56 d experimental period.

Key Words: calf, diarrhea, colostrum

Table 1 (Abstr. 1729W)

Group	n	Mortality	Days to resolve diarrhea	% treated diarrhea (Borgal)	% treated respiratory 1×	28-d ADG (g)	56-d ADG (g)
CON	23	4	9	60.9	73.9	589.7	721.2^{x}
COL	24	5	11	75.9	54.2	571.5	766.6
PP	26	3	7	57.7	46.2	576	766.6
AB	22	5	8	100.0	59.1	585.1	816.5^{y}

^{x,y}Superscripts x or y different ($P = 0.01$).

1730W Evaluation of differing interventions at the onset of neonatal calf diarrhea. D. W. Wood*[1], A. J. Keunen[2], B. W. Keunen[2], R. M. Blome[1], L. C. Ribeiro[1], and D. R. Renaud[3], *[1]Animix, Juneau, WI, [2]Mapleview Agri, Palmerston, ON, Canada, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of the study was to investigate strategies to treat diarrhea. Farm and auction sourced male calves (n = 160; 155 Holstein, 5 Holstein x beef cross) arrived at a research facility in 2 batches filling 2 separate rooms of 80 calves. Calves were fed whey protein-based 26% CP, 20% fat milk replacer void of additives for 8 wks in a 2x/d strategy. Calves were randomly assigned at the onset of diarrhea (fecal score of 3 or 2 consecutive scores of 2; 67.5% of all calves) to one of 4 treatments. Treatments included, (1) Colostrum (COL): 10 feedings of a 1 L mixture over 5 consecutive days of whole colostrum (Saskatoon Colostrum Company) mixed at 30 g/L; (2) bovine plasma (BP; NUTRAPRO B APC) fed the same as COL; (3) injection of antibiotic (AB; 3 mL Borgal trimethoprim sulphadoxine; intramuscularly 1x/d for 5 consecutive days); or (4) control (CON; whey-based milk replacer) fed the same as COL. The mean days from arrival to enrollment was 5.1 ± 1.7 d, NSD (no significant difference; $P = 0.92$) between treatment groups. Resolution of diarrhea was defined as 2 consecutive fecal scores of 0 or 1. Calves were individually weighed weekly through d 56, with fecal scores, respiratory scores (UC Davis system), and medical treatments recorded 2x/d. Blood was collected at arrival to determine serum total protein (STP). All statistical analyses were conducted in Stata 17 with linear regression models and Cox proportional hazard models used. NSD in arrival STP or body weight was found between groups. Few differences were noted between treatment groups including no differences found in growth or the number of calves that received a

Table 1 (Abstr. 1730W). Health and growth measures comparing differing interventions

Group	n	Mortality	Days to resolve diarrhea	% treated diarrhea (Borgal)	% treated respiratory 1×	28-d ADG (g)	56-d ADG (g)
CON	25	3	4.5^x	84.0	64.0^x	580.6	793.8
COL	28	2	5.0	89.3	67.9	576.1	771.1
BP	27	2	4.0^y	81.5	63.0	535.2	789.3
AB	28	4	4.5	100.0	42.9^y	612.4	825.6

^{x,y}Superscripts x or y different $P < 0.10$.

follow-up antibiotic for diarrhea. Supplementing BP tended to resolve diarrhea faster and AB tended to reduce 1st treatment for respiratory compared with CON. A subsequent study should compare BP and COL in CMR formulas, so they precede onset of diarrhea.

Key Words: calf, diarrhea, colostrum

1731W Effects of time and colostrum composition on immunoglobulin G absorption in neonatal dairy-beef calves. J. M. V. Pereira*[1], G. Mazon[1], A. J. Geiger[2], and J. H. C. Costa[1], [1]*Dairy Science Program, University of Kentucky, Lexington, KY,* [2]*Zinpro Corporation, Eden Prairie, MN.*

The objective of this randomized controlled trial was to evaluate 3 types of colostrum on serum IgG concentration, serum total protein (STP), and apparent efficiency of absorption (AEA) dynamics in newborn dairy-beef calves. Holstein-Angus calves (n = 70; BW = 45.2 ± 5.5kg) were randomly assigned to one of 3 colostrum types at birth: maternal colostrum (MC; n = 23), colostrum replacer (CR; n = 23; 750g of CR; Premolac Plus IgG), or colostrum replacer supplemented with casein (CC; n = 24; Premolac Plus IgG; 95g/L of casein). Treatments had 300g total of IgG, and casein addition was calculated to match the maternal colostrum treatment. Calves were tube fed an equal volume of 3.75L of colostrum at 3h post-birth. To evaluate the dynamics of serum IgG concentration and STP levels, and AEA, 10 mL blood was collected at the time of colostrum feeding (0h), and at 24h, and 36h post colostrum feeding. We used mixed linear models to evaluate the effects of treatment, it contained treatment, calf weight, sex, dystocia score, calf age at colostrum feeding, and the interaction between treatment and hours related to colostrum feeding. There was a significant interaction between treatment by time interaction where CR calves had greater IgG concentrations at 24h (MC = 23.7 ± 1.1, CR = 25.5 ± 1.1, and CC = 20.8 ± 1.0mg/mL; $P < 0.01$) than CC calves. Furthermore, CR and MC calves had greater serum IgG than CC calves at 36h (MC = 24.6 ± 1.1, CR = 25.6 ± 1.1, and CC = 19.0 ± 1.0mg/mL; $P < 0.01$). Treatment also affected STP levels at 24h (MC = 6.2 ± 0.1, CR = 5.0 ± 0.1, and CC = 5.6 ± 0.1g/dL; $P < 0.01$) and 36h (MC = 5.9 ± 0.1, CR = 5.1 ± 0.1, and CC = 5.3 ± 0.1g/dL; $P < 0.01$) following colostrum feeding. Lastly, treatment affected AEA, where calves receiving CR treatment had an increased AEA (MC = 32.3 ± 0.0, CR = 37.4 ± 0.0, and CC = 28.7 ± 0.0%; $P < 0.01$). Our findings suggest that colostrum type may affect serum IgG concentration and STP dynamics, also affects AEA in dairy-beef calves. Future research should evaluate the effects of colostrum type on immune function and calf health throughout the calf rearing period.

Key Words: colostrum replacer, passive transfer, apparent efficiency of absorption

1732W Relationship between serum gamma-globulin concentration and morbidity in pre-weaned dairy calves. N. Kobayashi*[1], K. Murayama[1], N. Nishizawa[1], M. Oba[2,3], and T. Sugino[3], [1]*Dairy Technology Research Institute, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishi-shirakawa, Fukushima, Japan,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [3]*The Research Center for Animal Science, Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Japan.*

Our previous study showed a strong positive correlation between serum concentrations of immunoglobulin G (IgG) and gamma-globulin (γGLB). The aim of this study was to evaluate relationship between serum γGLB concentration and morbidity in pre-weaned dairy calves. Holstein heifer calves (n = 723) were transported from their origin dairies to a calf farm at 3 to 7 d of age, and blood was sampled immediately after arrival. Serum γGLB concentration was measured using cellulose acetate membrane electrophoresis. The calves were divided into 4 categories based on their serum γGLB concentration; ≥1.0 g/dL (Excellent), 0.7–0.9 g/dL (Good), 0.4–0.7 g/dL (Fair) and <0.4 g/dL (Poor). Morbidity, defined as veterinary intervention, for diarrhea or respiratory disease in pre-weaned dairy calves was determined for the first 28 and 56 d of age, and the 4 categories based on serum γGLB concentration were compared in the morbidity by chi-squared test using JMP 16 pro (SAS Institute Inc., Cary, NC). The proportions of calves in each category were 18.8% (Excellent), 15.9% (Good), 33.6% (Fair) and 31.7% (Poor). Calves that had diarrhea were 5.9, 9.6, 20.6 and 26.2% for 0 to 28 d of age, and 14.0, 18.3, 23.1 and 28.0% for 0 to 56 of age, respectively for Excellent, Good, Fair, and Poor categories. Calves with serum γGLB concentration higher than 0.7 g/dL (Good and Excellent) had less diarrhea during the first 28 d of age than those with lower serum γGLB concentration (Fair and Poor; $P < 0.01$). Calves that had respiratory disease were 14.7, 20.0, 22.6 and 22.7% for 0 to 28 d of age, and 31.9, 44.0, 51.5 and 53.2% for 0 to 56 of age. Calves with serum γGLB concentration higher than 1.0 g/dL (Excellent) had less respiratory diseases for the first 56 d of age than those with lower serum γGLB concentration (Good, Fair, and Poor; $P = 0.03$). These results suggested that serum γGLB concentrations in the first week of age are associated with morbidity before weaning in dairy calves, and that serum γGLB concentration may be used as a predictive indicator for calf health similar to serum IgG concentration.

Key Words: failure of passive transfer, gamma-globulin, immunoglobulin

1733W Evaluating the utility of genomically enhanced RFI as a selection criterion to improve feed efficiency in growing Holstein heifers. K. O'Reilly[1], G. E. Carstens[1], J. R. Johnson*[2], N. Deeb[2], and P. Ross[2], [1]*Texas A&M University, College Station, TX,* [2]*STgenetics, Navasota, TX.*

The objective of this study was to validate the utility of genomic EcoFeed scores as a selection criterion to improve feed efficiency and greenhouse gas emissions in Holstein replacement heifers. Genomic breeding values for residual feed intake (RFIg), implemented by STgenetics as EcoFeed scores, were based on RFI phenotypes of 6,563 growing Holstein heifers across 182 feeding trials. EcoFeed score is moderately heritable (0.24 ± 0.02) and uncorrelated with other traits selected for in Holstein females. To assess the utility of this breeding value, Holstein heifers (n = 55; initial BW = 351 ± 64 kg) with low RFIg (n = 29) or high RFIg (n = 26) were selected from a contemporary group of 453 heifers (RFIg = −0.023 ± 0.211 kg/d) who had an average reliability for RFIg of 42%. Selected low- and high-RFIg heifers were assigned to 1 of 2 pens, each equipped with electronic feedbunks (GrowSafe Systems) and a Greenfeed gaseous-exchange measurement system (GEM; C-Lock). Individual DMI was collected for 84 d and BW measured weekly. The RFIp was calculated as the residual from the regression of DMI on ADG and mid-test $BW^{0.75}$. Heifers had three 14-d gaseous exchange measurement periods. A time series analysis of gaseous exchange measurements, with a repeated measure of measurement period, fixed effect of RFIg class and random effect of pen was conducted. A mixed model with RFIg class as a fixed effect and pen as a random effect was used for analysis of other variables. There were no differences in BW and ADG for heifers with divergent RFIg; however, low RFIg heifers consumed 7.3% less ($P < 0.05$) feed per day. Consequently, low RFIg heifers exhibited a more favorable ($P < 0.05$) RFIp (−0.188 vs 0.211 kg/d, respectively). Low RFIg heifers had 7.7% lower ($P < 0.05$) methane emissions (g/d) and 6.1% lower ($P < 0.05$) carbon dioxide (g/d) production than their high RFIg counterparts. Results indicate that selection based on RFIg through EcoFeed is an effective strategy to select for cattle with favorable feed efficiency phenotypes to increase the economic and environmental sustainability of the industry.

Key Words: residual feed intake, EcoFeed, feed efficiency

1734W *Saccharomyces cerevisiae boulardii* CNCM I-1079 influence on gut permeability, intestinal microbiota, and host immune function in newborn dairy calves.

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Newborn calves' susceptibility to intestinal diseases is substantially increased due to unstable intestinal flora and increasing vulnerability to pathogenic microorganisms. It has been demonstrated that a live yeast probiotic: *Saccharomyces cerevisiae boulardii* CNCM I-1079 (SCB) supplemented to calves from birth to 1 week of age may play a key role modulating early microbial colonization. However, there is a lack of knowledge on if a live yeast probiotic has a direct effect on gut permeability, intestinal microbiota and intestinal mucosa immune function in calves. The objective of this study was to evaluate how SCB supplementation in calves at 1 week of age impacts gut permeability, intestinal microbiota, and mucosa immune function. At birth, 20 Holstein bull calves were randomly allocated to receive 1 of 2 dietary treatments: supplementation with live yeast (SCB, $10x10^9$ cfu/d, n = 10) fed at 5 g/d; or with a placebo for control (CON, n = 10), for the first 7 d after birth. Each animal received 2 meals of colostrum replacer at 2 and 12 h of life (expected total IgG fed = 300 g) before being fed milk replacer twice a day. Transcriptome and microbiota profiles of biopsied colon mucosa were obtained using RNA-sequencing and amplicon-sequencing on d 0 and 5. Gut permeability analysis was performed on d 2 and 6, and blood samples were taken at −1h (baseline), +2h, +4h, +6h, +8h, +10h and +12h. Gut permeability data were analyzed using PROC GLIMMIX on SAS to evaluate area under the curve for chromium, lactulose, and mannitol on d 2 and 6. Gut permeability results did not differ between treatments. There were no significant differences in term of α and β diversity and in differentially expressed genes between SCB and CON at d 5. However, functional analysis revealed that genes related to immune function were uniquely expressed on d 5 in SCB supplemented calves. These results suggest supplementation of SCB does not influence gut permeability during the first week of life in bull calves, however, it enhances colon mucosa immune function.

Key Words: probiotic, gut permeability, immunity

1735W The effect of aspirin on intestinal permeability of Holstein and Jersey heifers.

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Increased intestinal permeability has been implicated as a cause of metabolic transition diseases in dairy cows and aspirin has been demonstrated to increase intestinal permeability in humans. Thus, we evaluated the effect of aspirin on intestinal permeability, assessed from urinary excretion of Co from the nonabsorbable marker Co-EDTA, in heifers. Twelve heifers (6 Holsteins and 6 Jerseys) were randomly assigned to either 0 or 100 mg aspirin/kg BW/12 h for 21 d. Heifer age was 10.4 ± 0.7 mo and BW was either 362 ± 25 (Holsteins) or 269 ± 21 (Jerseys). Where appropriate, aspirin was administered orally as a slurry in 200 mL of water via a drench gun at approximately 0600 and 1800 h daily. On d 21, heifers were dosed orally with gelatin capsules containing 40 g of Co-EDTA. Urine was sampled at 0, 1, 3, 6, 8, 12, 18, 24, 30, and 36 h post-dosing. Samples were frozen for subsequent analyses of Co and creatinine. Urinary Co:creatinine ratios (g/g) were fit to the following function using a nonlinear mixed effects model: $f(t) = Ae^{-k_1 t}exp[-Be^{-k_2 t}]$, where t = time post-dosing (h). For the structural model, the following equation was obtained: Urinary Co:creatinine $= 0.374e^{-0.058t}exp[-2.84e^{-0.325t}]$. Evaluation of covariates revealed a significant ($P < 0.01$) increase in parameters A and k_2 for Jerseys vs. Holsteins and in parameter B due to aspirin. Residual plots, model root mean square error (0.028, corresponding to residual coefficient of variation of 7.5%) and the Shapiro-Wilks test of normality of residuals ($P = 0.3$) all indicated an excellent fit of the model. For each heifer, urinary Co excretion was determined as the product of the area under the Co:creatinine excretion curve and the daily creatinine excretion (29 mg/kg BW). These data were then analyzed by ANOVA where the model included the effects of breed, aspirin, and their interaction. For urinary Co excretion, a substantial increase ($P < 0.001$) for Jerseys vs. Holsteins (0.630 vs. 0.372 g/d) and a moderate increase ($P = 0.019$) due to aspirin (0.509 vs. 0.493 g/d) was obtained. These data suggest that intestinal permeability may be higher for Jerseys vs. Holsteins and may be increased by aspirin administration.

Key Words: dairy cattle, intestinal permeability

1736W Evaluation of serum concentrations of total protein and gamma-globulin as an indicator of serum immunoglobulin G concentration in dairy calves.

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Measurement of serum concentration of immunoglobulin G (IgG) is the standard method to assess passive transfer of immunity in newborn dairy calves, but its analysis is expensive and may not be cost-effective. The aim of this study was to evaluate precision of estimating serum IgG concentration from the total protein (TP) and the gamma-globulin (γGLB) as alternative approaches. Blood was sampled from 129 Holstein calves in the first week after birth, and serum concentrations of TP, γGLB, and IgG were measured by biuret test, cellulose acetate membrane electrophoresis, and single radial immunodiffusion, respectively. Mean, SD, and range of serum concentrations were 5.5, 0.64, and 4.1 to 7.2 g/dL for TP, 0.67, 0.390, and 0.07 to 1.9 g/dL for γGLB, and 16.8, 10.69, and 0.3 to 45.5 g/L for IgG, respectively. Spearman's correlation coefficients (r_s) were determined separately for calves fed maternal colostrum (MC; n = 74) and those fed colostrum replacer (CR; n = 55) on their first feeding after birth. The r_s between serum IgG and TP concentrations were 0.92 ($P < 0.01$) in MC calves and 0.77 ($P < 0.01$) in CR calves, but r_s between serum IgG and γGLB concentrations were 0.95 ($P < 0.01$) in MC calves and 0.96 ($P < 0.01$) in CR calves. Root mean squared error (RMSE) for the estimation of serum concentration of IgG from TP were 4.44 and 5.43 g/L in MC and CR calves, respectively, and there was a lack of fit in the simple linear regression model ($P < 0.01$ for MC; $P = 0.01$ for CR). However, RMSE for the estimation of serum concentration of IgG from γGLB were 3.33 and 2.05 g/L in MC and CR calves, respectively, and there was no lack of fit in the simple liner regression model ($P > 0.05$). These results suggest that serum IgG concentration can be estimated more precisely from concentration of γGLB than TP particularly for calves fed CR, and that passive transfer of immunity in dairy calves can be assessed effectively by serum γGLB concentration.

Key Words: passive transfer, gamma-globulin, immunoglobulin

1737W Further insights into the specificity of immunoglobulin G and protein absorption in the first 24 hours of life in newborn calves.

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On farm predictors of IgG transfer, such as serum total protein (STP), are used to determine if enough IgG from colostrum has been absorbed by the calf and passive transfer of immunity (PTI) is achieved. However, STP does not solely measure IgG and may be influenced by colostrum source (maternal vs. replacer). The objective of this study was to evaluate IgG and protein absorption in newborn calves fed diets with or without the IgG fraction. Ten, newborn, crossbred calves (n = 5/treatment) were fed one of 2 experimental diets: 1) A colostral whey isolate product derived from maternal bovine colostrum providing 150 g of IgG (PREM; Premolac Plus, Zinpro Corporation, Eden Prairie, MN), or 2) a whey protein concentrate fed to deliver the same protein intake as PREM without providing any IgG (WPC). Both treatments were fed within 2 h at the same rate (375 g of powder) to provide the same level of total protein intake with a portion of that protein being IgG in PREM. A serum sample was taken at birth and at 6-, 12-, 18-, 24-, and 30 h post-treatment feeding and analyzed for serum IgG, STP, and serum brix. Serum IgG values for calves fed PREM peaked at 12 h post-feeding (19.2 g/L). Serum IgG values were greater at all time points post-feeding for calves fed PREM compared with calves fed WPC ($P < 0.01$). However, STP and serum brix were not different between PREM and WPC at any of the time points ($P = 0.48$), and although all calves fed PREM achieved PTI, STP and serum brix for PREM calves was below traditionally-defined PTI cut points at every time point. Results from this study seem to indicate that calves absorb protein in a non-specific manner in the 24 h following birth regardless of protein type (IgG vs. whey). The potential may exist for other, non-IgG proteins to be absorbed in place of IgG during the first 24 h of life. However, more work with a more robust set of calves is needed to further explore this concept.

Key Words: immunoglobulin G, calf, whey

1738W Methods to determine immunoglobulin G content of the abomasal curd of calves fed different colostrum sources.

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Neonatal calves rely on the passive transfer of immunity via ingestion of maternal colostrum (MC) or a colostrum replacer (CR). Immunoglobulin G (IgG) absorption occurs at non-specific receptors in the small intestine in the calf. After 24–48 h of calf birth, downregulation of non-specific receptors occurs, reducing IgG uptake. In the pre-ruminant abomasum, casein protein from colostrum interacts with chymosin to coagulate colostrum and form curds. However, curd formation is an imperfect process. Curd formation may result in curd leakage into the small intestine, competing with IgG uptake. This research is an ongoing pilot study comparing curd formation in Angus x Holstein bull calves (n = 18) fed pooled MC or a whey-based CR (Zinpro Corporation, Eden Prairie, MN), fed equal amounts of IgG (150g) within 2h after calving. After 2–4h of colostrum ingestion, calves undergo ultrasonography to observe the presence of curd formation using a transabdominal convex ultrasound probe (eCLi4 3–7 MHz broadband array). Blood samples are collected via jugular venipuncture using red top tubes (BD Vacutainer) at h 0, 24, and 48. Serum is separated within 1–2h of collection and stored at −80C for further analysis of IgG using radio immunodiffusion (RID). The objectives of this abstract are to discuss methods to adequately determine abomasal curd formation in the neonatal calf and evaluate the IgG content of serum using RID. Curd formation was observed in calves fed MC compared with calves fed CR (images to be provided). This pilot study presents methods to adequately determine abomasal curd formation in the neonatal calf to better understand the apparent efficiency of absorption of IgG in the presence of curd formation.

Key Words: neonatal calf, curd formation, immunoglobulin G

1739W Stability evaluation of the direct-fed microbial *Enterococcus faecium* 669 in different feed matrixes.

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Probiotics are live microorganisms that confer a health benefit to the host when consumed in adequate amounts. Probiotics are administered to animals through the diet, milk replacer, or in premixes. Our objectives were to evaluate the stability of *Enterococcus faecium* 669 (Chr. Hansen A/S, Hørsholm, Denmark) during storage of i) milk replacer, ii) mineral-vitamin premix, and iii) on the preparation of milk replacer at different temperatures. In Exp. 1, the probiotic was included at 5.0E+06 cfu/g of milk replacer, corresponding to 2.5E+09 cfu/head daily. Samples were stored at 25°C for 12 mo and analyzed for cfu counting after 0, 1, 3, 6, and 12 mo. In Exp. 2, the same probiotic was included in a commercial premix (3.0E+07 cfu/g of premix), corresponding to 3.0E+09 cfu/head/day. Samples were stored at 25°C and enumerated after 0, 1, 3, 6, and 12 mo. In Exp. 3, the milk replacer was prepared by dissolving the powder in water at 37°C (cond. 1) or 50°C (cond. 2) and kept at 37°C and 50°C, respectively, for 1 h, or prepared at 50°C and incubated for 1 h at 25°C (cond. 3). The probiotic was added at 2.5E+06 cfu/g. In all conditions, samples were collected for enumeration of *E. faecium* after 0, 15, 30, 45, and 60 min of incubation. The enumeration of *E. faecium* was performed after incubation of Bile Esculine Agar plates at 37°C for 24 h. All data were analyzed using SAS (version 9.4) and comparisons were performed versus time 0 (T0). No effect on *E. faecium* viability (recovery, %) was observed at any time points in Exp. 1 ($P = 0.29$), Exp. 2 ($P = 0.36$), and Exp. 3 ($P \geq 0.14$). *Enterococcus faecium* was recovered on average at 108% in Exp. 1 ($P = 0.36$) and at 113% in Exp. 2 ($P = 0.29$) vs. T0. In Exp. 3, the cfu recovered after 60 min (compared with T0) did not differ in any of the experimental conditions ($P \geq 0.11$), and the setting did not impact the recovery ($P = 0.15$) (116% in cond. 1, 120% in cond. 2 and 108% in cond. 3). These experiments demonstrate that *E. faecium* 669 remained stable in the milk replacer and premix after 12 mo of storage and during the preparation of the milk replacer at 37°C and 50°C.

Key Words: *Enterococcus faecium*, milk replacer, stability

1740W Effects of *Enterococcus faecium* 669 on performance and health of preweaning dairy calves.

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In dairy calves, pre-weaning performance and health have a major effect on long-term productive performance and strategies that support health and performance during this critical developmental period are warranted. The objective of this study was to evaluate the effects of a direct-fed microbial (DFM) *Enterococcus faecium* 669 on performance and health of pre-weaning dairy calves. On d 0 of the study, 42 1-d Holstein calves [initial body weight (BW) 44 ± 4.5 kg] were assigned to either a control (n = 21) or a DFM-supplemented (n = 21; Chr. Hansen A/S, Hørsholm, Denmark) at a daily rate of 2.5×10^9 colony forming units/head. The DFM was mixed daily in whole milk, and a diet containing starter feed and alfalfa hay were offered *ad libitum* for a 63-d experimental period. Individual BW measurements were taken weekly, while diet intake and health observations were assessed daily. Individual fecal score was given to the animals, such as 1 = diarrhea and 5 = hard, pellet-forming feces. Rectal temperature was measured daily in the first 14 d of the trial and then once on d 21. Fecal pH was analyzed daily until d 21 and fecal samples were collected for parasitological and microbiological analysis. All data were analyzed with the MIXED procedure of SAS (v. 9.4, SAS Inst., NC). A treatment × day interaction was observed for BW ($P = 0.04$), as DFM-supplemented calves were heavier on d 56 and at weaning ($P < 0.01$). Similarly, a treatment × week interaction was observed for average daily gain and feed efficiency ($P < 0.01$), with improvements for DFM calves. All the diarrhea cases were observed in the first 2 weeks of the trial and there was a trend for a lower diarrhea occurrence in DFM calves (%; $P = 0.09$). Rectal temperature was lower for DFM-supplemented calves vs. CON ($P = 0.04$), but no treatment effects or interactions were observed for fecal pH, microbiological, and parasitological analysis ($P > 0.12$). In summary, daily supplementation of *Enterococcus faecium* 669 through the whole milk improved performance of pre-weaning dairy calves, while also showing promising results on diarrhea occurrence and rectal temperature.

Key Words: dairy calves, *Enterococcus faecium*, preweaning performance

Ruminant Nutrition: Carbohydrates and Lipids 3

1741W The relation and variation of odd and branched-chain fatty acids content in rumen fluid, blood, and milk from lactating dairy cows. Z. Luo[1,2], A. Evans[2], and D. Bu*[1], [1]*Institute of Animal Science, State Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*School of Agriculture & Food Science, University College Dublin, Belfield, Dublin 4, Ireland.*

Milk from ruminants has been found to have many beneficial components, such as odd and branched-chain fatty acids (OBCFA). It is important to determine how these components are produced and resynthesized in cows. The main purpose of this study was to investigate the correlations among rumen fermentation parameters, rumen fluid, blood and milk OBCFA, and to see the changing trend of their content in rumen fluid, blood and milk. Seven Holstein dairy cows with similar body weight and parity were fed a total mixed ratio (TMR) and rumen fluid, blood and milk samples were collected to detect their content of OBCFA, as well as rumen fermentation parameters. The correlation procedure of SAS (version 9.4) was used to test the level of significance of correlations among rumen fluid parameters, milk, blood and rumen fluid OBCFA. Another data was analyzed by repeated measures using the PROC MIXED procedures of SAS. Tukey's multiple range test was used to evaluate differences between fluids. The results showed that there was a significant correlation between rumen fermentation parameters (VFA) and rumen fluid OBCFA ($r = 0.45$ $P = 0.04$), blood OBCFA ($r = 0.50$, $P = 0.02$), but no correlation with milk OBCFA. There was a significant positive correlation between odd-chain fatty acids (OCFA) from blood and rumen fluid, especially C13:0 ($r = 0.43$, $P = 0.049$). The rumen fluid branched-chain fatty acids (BCFA) were negatively correlated with the content in blood, especially anteiso fatty acids ($r = -0.46$, $P = 0.04$). The iso fatty acids of blood were relatively abundant but the milk content of C15:0 ($P < 0.05$), C17:0 ($P < 0.05$), isoC17:0, and anteisoC17:0 were mostly higher than in rumen fluid and blood. The levels of medium-chain fatty acids, isoC13:0 ($P < 0.05$), anteisoC13:0 ($P < 0.05$), isoC14:0 ($P < 0.05$), iso C15:0 ($P > 0.05$), anteisoC15:0 ($P > 0.05$), isoC16:0 ($P > 0.05$), in milk were lower than those in the rumen fluid and blood. This study demonstrates the complex dynamic of OBCFA production in the rumen, its passage through the blood to the mammary gland and into the milk. It also highlights the likelihood of an important contribution of adipose tissue, and the de novo synthesis of milk fats in the mammary tissue and the need for further studies to determine their exact relative contributions to milk fat content.

Key Words: milk, rumen, fermentation parameters

1742W The effect of unsaturation degree of C18 fatty acids on the rumen fermentation and methane mitigation *in vitro*. X. Sun[1], Y. Li*[1], K. Giller[1], C. Kunz[1], X. Ma[1], R. Peng[1], M. Terranova[2], S. Yang[1], and M. Niu[1], [1]*Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland,* [2]*AgroVet-Strickhof, ETH Zürich, ETH Zürich, Lindau, Switzerland.*

The supplementation of unsaturated fatty acids (UFA) to ruminants has a significant effect on enteric methane (CH_4) mitigation and rumen fermentation. The objective of this study was to determine the effect of C18 fatty acids (FA) with different degree of unsaturation on in vitro gas production [CH_4, hydrogen (H_2)], pH, and ammonia (NH_3) and volatile fatty acids (VFA) concentrations. In vitro incubations were performed using the Hohenheim Gas Test. Individual rumen fluid were collected from 3 lactating rumen cannulated Original Brown-Swiss cows. We used Tween80 as the emulsifier to dissolve the FA (FA/Tween80, 50 mg/mL). The following treatments were included: 1) Control (Tween80), 2) stearic acid (SA; C18:0), 3) oleic acid (OA; C18:1), 4) linoleic acid (LA; C18:2), 5) α-linolenic acid (ALA; C18:3). The substrate [200 mg of TMR in dry matter (DM)-basis containing 40% grass silage, 40% maize silage, 15% hay and 5% concentrate] with FA (5% of DM) was incubated for 0, 2, 4, 8, 12, and 24 h in 6 replicates. Data were analyzed using a mixed model including fixed effect of treatment, and random effect of cow and syringe. There was a linear decrease ($P < 0.05$) in CH_4 production from incubation 8~24 h with increasing degree of unsaturation. For example, CH_4 production at 24-h decreased 1.9, 10.9, and 18.6% by OA, LA, and ALA, respectively, compared with SA. The decrease in CH_4 with increasing unsaturation degree may be due to competition of the H_2 for methanogenesis. Meanwhile, H_2 production increased linearly with increasing number of double bonds only at 12 h ($P < 0.01$). The addition of UFA compared with SA had little effect on other in vitro fermentation parameters, including pH, NH_3 and VFA concentrations and in vitro organic matter digestibility. In conclusion, the results suggest that the extent of CH_4 mitigation was positively associated with the number of double carbon bonds of 18 C UFA. Therefore, at identical FA concentration, a higher degree of unsaturation of 18 C FA within the diet of dairy cows may decrease CH_4 production without adverse effects on in vitro rumen fermentation.

Key Words: unsaturated fatty acids, in vitro, methane

1743W Effects of fatty acid supplements and lysophospholipids on nutrient digestibility in lactating cows. K. Park*[1], N. Porter[1], K. L. Clark[1], L. R. Rebelo[1], I. H. Kwon[2], and C. Lee[1], [1]*Department of Animal Sciences, The Ohio State University, Wooster, OH,* [2]*EASY BIO Inc., Seoul, Republic of Korea.*

The objective of the present study was to investigate changes in nutrient digestibility affected by saturated fatty acids (FA) sources and lysophospholipids (LPL), a natural emulsifier, supplementation. Total 48 mid-lactation cows blocked by parity and days in milk were used in a randomized complete block design. Cows within block were assigned randomly to the following treatments: Diet supplemented with 1) FA (1.8% of diet DM), primarily palmitic acid (PA); 2) FA, primarily stearic acid (SA); 3) PA and LPL (0.003% of diet DM); 4) SA and LPL. The experiment was conducted for 6 wk. Spot samples of feces were taken over 3 d (every 3 h within a 24-h cycle) during the last week of the experiment to assess total-tract digestibility of nutrients. The mixed model was used for statistical analysis with FA, LPL, and their interaction as fixed effects and block as a random effect. DMI was not affected by FA and LPL. The FA source did not affect the digestibility of DM, OM, and CP, and there was no interaction between FA and LPL. However, PA increased (41.4 vs. 38.8%; $P = 0.04$) NDF digestibility compared with SA without an interaction between FA and LPL. Supplementation of PA decreased the digestibility of 16-carbon FA (47.6vs. 59.1; $P < 0.01$) compared with SA, and SA decreased the digestibility of 18-carbon FA (65.3 vs. 77.0; $P < 0.01$) compared with PA. While LPL did not affect the digestibility of DM, OM, CP, and NDF, it increased FA digestibility (64.7 vs. 60.5%; $P = 0.02$) compared with no LPL. Furthermore, LPL increased (74.1 vs. 68.2%; $P < 0.01$) 18-carbon FA digestibility without an interaction with FA, but an interaction between FA and LPL was observed for 16-carbon FA digestibility, which occurred because LPL increased 16-carbon FA digestibility with SA but not with PA. In conclusion, we confirmed that fiber digestibility can be enhanced by PA but not by SA. Supplementation of LPL may be beneficial in increasing FA digestibility when used with a FA supplement containing primarily SA. Further research would be required to comprehend how LPL interacts with different FA sources.

Key Words: emulsifiers, palmitic acid, stearic acid

1744W Effects of medium-chain fatty acid supplementation on plasma metabolites of dairy cows in the transition period. G. C. Aguiar*[1], J. C. S. Lourenço[1], E. W. Carneiro[2], D. E. Rico[3], J. A. Negrão[4], and R. Almeida[1], *[1]Universidade Federal do Paraná, Curitiba, PR, Brazil, [2]Royal Agrifirm Group, Curitiba, PR, Brazil, [3]Deschambault Animal Science Research Centre (CRSAD), Deschambault, QC, Canada, [4]Universidade de São Paulo, Pirassununga, SP, Brazil.*

Dairy cows experience profound physiological and metabolic changes during the transition period. Medium-chain fatty acids (MCFA; 6:0, 8:0, 10:0, and 12:0) may have beneficial effects on the metabolism of lactating cows when supplemented at low doses (<1.0% of dietary DM). The objective of this study was to evaluate the effects of MCFA supplementation on plasma metabolites in periparturient dairy cows. One hundred and 68 primiparous (n = 66) and multiparous (n = 102) Holstein cows (695 ± 4.09 kg) were divided into 2 groups in a randomized complete block design for 35 d (21 d pre-calving and 14 d post-calving). The treatments used were: T1: Control (no FA supplementation) and T2: 45 g of MCFA (0.25–0.35% of dietary DM) based on coconut and palm oil (Aromabiotic Cattle, Royal Agrifirm Group, NL), which was supplemented individually as a *top-dress* once a day, mixed with corn meal. The Control animals received the same amount of corn meal. Blood samples were collected on different days for the analysis of plasma metabolites (−7, 0, 3, 7, and 14 d). Data were analyzed using the MIXED procedure of SAS, with the model containing the fixed effects of treatment, day, parity and their interactions, whereas block and animal within treatment were considered random effects. The treatment × parity interaction was tested based on other studies that indicate a metabolic difference between primiparous and multiparous cows, with differences in the activation of lipolysis for tissue mobilization. However, in this study, we did not obtain differences ($P > 0.10$) for the evaluated metabolites: GGT, AST, bilirubin, albumin, cholesterol, glucose, NEFA, BHB, and total Ca, related to treatment, and treatment × day and treatment × parity interactions. However, we detected a trend ($P = 0.09$) of higher GGT concentrations on Control cows compared with MCFA-supplemented cows; 27.89 vs. 26.92 U/L. In conclusion, MCFA supplementation at the dose used in this study had little impact on metabolism. Studies investigating the impact of higher doses during the transition period are needed to help us understand such metabolic responses.

Key Words: blood metabolites, coconut oil, medium-chain fatty acids supplementation

1745W Replacing ground corn with liquid molasses in diets containing red clover: Effects on production performance and enteric methane emissions. A. L. Konopka, D. C. Reyes, M. A. Rahman*, K. V. Almeida, and A. F. Brito, *Department of Agriculture, Nutrition, and Food System, University of New Hampshire, Durham, NH.*

We aimed to investigate the effect of partially replacing ground corn (GC) with sugarcane liquid molasses (LM) on DMI, milk yield and composition, and enteric CH_4 emissions in dairy cows. Eighteen multiparous and 2 primiparous Jersey cows averaging (mean ± SD) 90 ± 34.7 DIM, 27.8 ± 5.72 kg of milk, and 493 ± 61 kg of BW at the beginning of the study were blocked in pairs based on milk yield, DIM, or parity and, within pair, randomly assigned to treatments in a randomized complete block design. The diets contained (DM basis) 60% red clover baleage, 5.8% soybean meal, 2.5% roasted soybean meal, 1.5% fat, 2.5% minerals and vitamins premix, and: 1) 27.7% GC (COR) or 2) 22.7% GC and 5% LM (MOL). Diets averaged 16.5% CP and 33% NDF. The study lasted 9 weeks with a 2-wk covariate period. Data and sample collection were taken during the covariate period and wk 4 and 7 of the study. Measurements of gaseous emissions were obtained using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS with repeated measures over time. No significant treatment effect was observed for DMI and milk yield, which averaged 20.1 and 30.5 kg/d, respectively. Feeding COR vs. MOL also had no significant effect on concentration (mean = 5.0%) and yield (mean = 1.47 kg/d) of milk fat, as well as MUN concentration (mean = 8.65 mg/dL). Reductions in milk protein concentration (3.34 vs. 3.27%; $P < 0.01$) and yield (1.01 vs. 0.93 kg/d; $P = 0.01$) were observed in cows fed MOL compared with COR. Similarly, feed efficiency calculated as 4% FCM/DMI (1.83 vs. 1.72 kg/kg; $P < 0.01$) and ECM/DMI (1.93 vs. 1.81; $P < 0.01$) were lower with feeding MOL than COR. Diets did not affect enteric CH_4 production (mean = 398 g/d; $P = 0.33$) and CH_4 intensity (mean = 10.03 g/kg of ECM; $P = 0.44$), but CH_4 yield tended (20.4 vs. 19.5 g/kg of DMI; $P = 0.10$) and CO_2 production (11.1 and 10.8 kg/d; $P = 0.06$) both tended to decrease in cows fed MOL than COR. In summary, partially replacing GC with LM at a rate of 5% in the diet DM had no effect on enteric CH_4 emissions, but decreased milk protein synthesis and feed efficiency.

Key Words: forage, greenhouse gas, sucrose

1746W Rolling severity of reconstituted high moisture barley with variable kernel sizes and its effects on ensiling characteristics and in vitro ruminal fermentation. B. Lynch*, G. O. Ribeiro, T. Mutsvangwa, and G. B. Penner, *University of Saskatchewan, Saskatoon, SK, Canada.*

The objective was to evaluate how severity of rolling for reconstituted high moisture barley (RHB) with variable kernel size affects ensiling characteristics and in vitro ruminal fermentation. Three sources of light (605 g/L) and heavy (684 g/L) barley were blended to create variable kernel sized barley (n = 4; 646 g/L). Sub-samples were dry rolled (DRB) to a processing index (PI) of 75% or used to produce RHB (65% DM with 24 h of tempering). The RHB was rolled to achieve PI values of 65% (R65), 75% (R75), and 85% (R85). The RHB was packed into miniature silos (density 2.15 g/cm^3) and ensiled for 1 or 5 mo (n = 12). In vitro ruminal fermentation characteristics of the RHB ensiled for 1 mo and the DRB were evaluated (n = 4). The RHB had greater kernel width before rolling than DRB ($P = 0.02$), and greater kernel length, width, and thickness following rolling than DRB ($P < 0.01$). Decreasing the PI from 85 to 65% for RHB decreased kernel thickness ($P < 0.01$). The percentage of fine particles (<1.18 mm) was greater for DRB than all RHB treatments ($P < 0.01$). The severity of rolling for RHB did not affect post-ensiling pH, but lactate concentration and 7-h starch digestibility were greater after 5 than 1 mo ($P < 0.01$). The 7-h starch digestibility increased with decreasing PI ($P = 0.02$). Dry matter and organic matter disappearance were greatest for DRB and R65, intermediate for R75, and least for R85 ($P < 0.01$). Fermenter pH was least for DRB, greatest for R75 and R85, with R65 as the intermediate ($P = 0.04$). The DRB and R65 treatments had greater acetate, propionate, and butyrate production (mmol/d) than R85, with R75 being numerically intermediate but not different from the other treatments. Total microbial nitrogen production (mg/d) was greatest for DR, intermediate for R65 and R75, and least for R85 ($P < 0.01$). Relative to DRB, processing RHB results in fewer

Table 1 (Abstr. 1747W). Effects of rumen-protected fat supplementation on milk production, milk composition, BW and BCS of grazing dairy cows[1]

	Diet[2]				*P*-value[3]	
Item	CON	PFM	PFH	SEM	RPF	Type
Milk yield, kg d^{-1}	24.31	24.79	24.18	0.35	0.67	0.20
Fat, %	4.83	4.78	4.96	0.06	0.51	0.02
Protein, %	3.76	3.72	3.80	0.03	0.95	0.02
Fat, kg d^{-1}	1.15	1.17	1.18	0.02	0.30	0.70
Protein, kg d^{-1}	0.90	0.92	0.91	0.01	0.46	0.72
Milk solids, kg d^{-1}	2.05	2.08	2.07	0.03	0.39	0.85
BW, kg	478	476	474	4.60	0.58	0.70
BCS	3.02	3.04	3.04	0.02	0.20	0.85

[1] BW = body weight; BCS = body condition score; SEM = standard error of the mean.

[2] CON = P + 3.6 kg of DM of a concentrate with no RPF; PFM = P + 3.6 kg of DM of a concentrate containing 5%. RPF (C16:0 = 58%); PFH = P + 3.6 kg of DM of a concentrate containing 5% RPF (C16:0 = 97%).

[3] RPF = CON vs. (PFM+PFH)/2; Type = PFM vs. PFH.

fines and a slower rate and lesser extent of fermentation relative to DRB at the same PI. Aggressively processing RHB (R65) resulted in similar nutrient digestibility to DRB.

Key Words: barley grain, ensiling, high moisture

1747W Effect of rumen-protected fat supplementation on milk fat production in grazing dairy cows.

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The objective of this experiment was to investigate the effect of rumen-protected fat (RPF) supplementation on milk fat production in grazing cows during the early to mid-lactation period. One hundred and eighty cows, averaging 48 ± 18 DIM and 477 ± 78 kg of BW, were blocked and randomly assigned to 1 of 3 dietary treatments. The 3 dietary treatments were; pasture (P) supplemented with 3.6 kg of DM cow^{-1} day^{-1} of a concentrate with no RPF (CON); P supplemented with 3.6 kg of DM cow^{-1} day^{-1} of a concentrate containing 5% RPF with a medium concentration of palmitic acid (58%; PFM); and P supplemented with 3.6 kg of DM cow^{-1} day^{-1} of a concentrate containing 5% RPF with a high concentration of palmitic acid (97%; PFH). The experiment consisted of an initial 2-wk covariate period and an 11-wk period of data collection. Data were analyzed using a repeated measures mixed model including the random effect of cow and the fixed effects of parity, treatment and week. Preplanned contrasts were used: RPF supplementation [CON vs. (PFM+PFH)/2] and RPF type (PFM vs. PFH). Overall, RPF supplementation had no significant effect on milk fat production when compared with CON; however, PFH increased milk fat and milk protein concentration when compared with PFM (Table 1). In conclusion, higher levels of palmitic acid increased milk fat concentration, but did not influence milk fat yield.

Key Words: rumen-protected fat, milk fat, grazing

1748W Evaluating production effects of replacing calcium salts of fatty acids with stabilized liquid propionic acid in early lactation.

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Propionic acid and fatty acids have both been shown to increase milk yield in early lactation. Study objectives were to evaluate effects of replacing calcium salts of fatty acids (CSFA) with PropPeak (PP) a stabilized liquid propionic acid (Innovative Liquids, LLC.) on production and feed efficiency in early lactation. Cows (n = 48; 35 ± 4 DIM; 3.2 ± 1.3 parity; X ± SD) were enrolled in a 3x3 Latin square design with 35-d periods (14-d acclimation, 21-d experimental period) receiving 1 of 3 treatments (DM basis): 1) CON (0.23 kg/h/d CSFA, Nurisol, Global Agri Trade), 2) PP (0.23 kg/h/d) or 3) MIX (0.11 kg/h/d CSFA and 0.11 kg/h/d PP). Treatments were top-dressed to basal diets (% DM: 29.3 NDF, 16.8 CP, 26.9 Starch, 2.78 FA) with measured daily intakes (BioControl North America, Liberty, MO). Cows were milked 3x/d and yield was recorded. Samples for composition analysis were obtained once a week at 3 consecutive milkings. The statistical model included random effect of cow within sequence and fixed effects of treatment, period, sequence, and group analyzed using the MIXED procedure with Tukey adjustment of SAS version 9.1 (SAS Institute, Inc., Cary, NC). Overall, there were no treatment effects on DMI, feeding behavior, body weight, body condition score, or energy corrected milk (ECM) feed efficiency ($P \geq 0.26$). Milk yield did not differ between CON v MIX ($P = 0.16$) but decreased in PP v CON (51.6 v 53.4, kg/d; $P < 0.01$) and PP v MIX (51.6 v 52.7, kg/d; $P < 0.01$). Milk fat and protein yield did not differ between treatments ($P \geq 0.22$) but protein content increased in PP v CON (2.96 v 2.91, %; $P = 0.05$). ECM did not differ in CON v MIX or MIX v PP ($P \geq 0.45$) but tended to increase in CON v PP (57.6 v 55.8, kg/d; $P = 0.08$). De novo and mixed fatty acid yield did not differ among treatments ($P \geq 0.67$) while preformed fatty acid yield tended to increase in CON v PP (0.77 v 0.73, kg/d; $P = 0.08$). No production or component yield differences were determined feeding PP at 50% replacement of CSFA DM in early lactation. Replacing 100% of CSFA with PP decreased milk yield but had no effect on milk component yields.

Key Words: propionic acid, fatty acid, milk yield

1749W Effects of palmitic and oleic acid supplementation on milk production of dairy cows milked with an automatic milking system.

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The objective of our study was to evaluate the effects of supplementing calcium salts of palmitic (PA) and oleic (OA) acid on milk production in lactating dairy cows milked with an automatic milking system (AMS). Twenty-six Holstein cows (BW; 639 ± 10.1 kg, parity; 1.81 ± 0.12) were

blocked by days in milk (DIM) and assigned to 3 groups: early (0–60 DIM; n = 7), peak (61–120 DIM, n = 5), and middle and late (121- DIM; n = 14). This experiment was designed to compare milk production during a 30-d treatment period with those observed in a 14-d preliminary period preceding the treatment period. In the treatment period, all cows were fed a partial mixed ration (PMR) supplemented with 300 g of fatty-acid calcium salt (FA-Ca) per head per day; the FA-Ca was composed of 60% of PA and 30% of OA. We collected data (milk yield, milk composition, DMI) during the preliminary period and the treatment period (15 and 30 d). Preliminary milk yield (PMY) was determined during the last 3 d of the preliminary period. All data were analyzed using Proc Mixed procedure (fixed effects; group, treatment, interaction between group and treatment, PMY used as covariate, interaction between PMY and treatment, and random effect of cow). Linear effect for the interaction between PMY and treatment was added to evaluate responses to treatment by level of milk yield. Dry matter intake of PMR at 30 d of treatment period increased compared with that of 15 d in peak (21.1 vs 27.4 kg/d, $P < 0.05$), and middle and late (18.8 vs 24.0 kg/d, $P < 0.05$) groups, respectively. The de novo and preformed FA contents in milk at 30 d in the middle and late groups increased compared with that of preliminary period, respectively (de novo; 26.7 vs 23.5%, preformed; 37.7 vs 35.3%, $P < 0.05$). Interactions between treatment and PMY were detected for DMI of compound feed in AMS, 4% FCM (linear interaction); higher-producing cows (>35 kg/d milk yield) increased 4% FCM at 30 d ($P < 0.01$), and higher-producing cows (>41 kg/d) decreased DMI of compound feed at 30 d, compared with those at 15 d ($P < 0.05$). These results suggest that supplying calcium salt of PA and OA to PMR affected feed intake of lactating cows, and the long-term supplementation of these calcium salts may affect milk production of cows producing milk greater than 35 kg/d in AMS.

Key Words: palmitic acid, oleic acid, automatic milking system

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1750W Sensitivity analysis of INRAtionV5 for dairy cows: One-at-a-time method. S. Jeon*[1], S. Lemosquet[2], T. Senga-kiesse[3], A.-C. Toulemonde[1], and P. Nozière[1], *[1]UMR Herbivores, INRAE-VetAgro Sup, Saint-Genès-Champanelle, Auvergne-Rhône-Alpes, France, [2]UMR PEGASE, INRAE-Institut Agro Rennes Angers, Saint-Gilles, Bretagne, France, [3]INRAE-Institut Agro Rennes Angers, Rennes, Bretagne, France.*

The in sacco rumen degradability and intestinal true digestibility values are used to characterize the nutritional values of feedstuffs in the INRA feeding system for Ruminants (2018) and its rationing software (INRAtionV5). Nevertheless, as INRAE has decided not to retain fistulated ruminants from 2025, alternative in vitro methods are being developed. That raised the need to perform a sensitivity analysis (SA) for evaluating the effect of variations in nutritional input variables on the output variables of INRAtionV5. Five key variables were selected for the SA: organic matter digestibility (OMd), gross energy (GE) and crude protein (CP) contents, and variables obtained through in sacco experiments, i.e., effective ruminal degradability (ED6_N) and true intestinal digestibility (dr_N) of nitrogen. A one-at-a-time SA was performed on outputs of the INRAtionV5 module for dairy cows with 6 diets varying by the type of forages (corn silage, grass fresh/hay/silage) and concentrates (cereals and oil seed meals). These 6 pivot diets were formulated using INRAtionV5 to meet 95% of the potential milk production for a multiparous cow (wk 14 of lactation). Then, for each of the 6 diets considered as total mixed rations, the values of the 5 key input variables of each feedstuff were randomly sampled under the normal distribution (n = 50 test diets/input variable). The Normalized sensitivity coefficient (NSC, %), ratio of each input to output variables, was used as the SA index. Predicted dry matter intake was mainly affected by GE (NSC = −44 ± 34.0%). In addition, predicted milk protein yield (MPY), milk yield (MY), and milk protein contents were mostly affected by OMd (NSC = 98 ± 39.0%, 71 ± 29.0%, and 27 ± 10.0%, respectively). However, MPY and MY were mainly affected by ED6_N, especially in grass silage-based diet (NSC = −56% and −46%, respectively). The fecal N was most affected by ED6_N (NSC = −53 ± 14.7%) and urinary N was most affected by CP (NSC = 283 ± 34.5%). Results of SA showed that amplitude of responses depends on the diet, and that productive response with grass silage diet is particularly sensitive to in sacco ED6_N measurements.

Key Words: dairy cow, sensitivity analysis, INRAtion

1751W Effect of curing extent on ruminal in vitro gas production kinetics of red clover hay and silage across storage phases. D. Zamudio*, R. A. de Castro, A. P. Jimenez, M. Cardoso, J. Poblete, M. Killerby, and J. J. Romero, *University of Maine, Maine.*

This study evaluated the effects of insufficient (WET) or ample (CUR) curing on red clover silage (29.4 and 45.3% DM, respectively) and hay (65.1 and 89.1% DM, respectively) in vitro fermentation measures. Measurements were taken at the start of storage (STRT), after 14 d (MicA), and once storage processes had stabilized for hay and silage (50 and 78 d, respectively; LATE). The experimental red clover field was divided into 5 blocks and each block was divided into 2 subplots and then assigned randomly to silage and hay (storage methods). Hay was packed into mini-bales (~500 fresh g; ~232 kg DM/m^3) and silage into mini-silos (19.5 L; ~214 kg DM/m^3). Samples at each storage stage were analyzed for in vitro gas production (48 h). Gas kinetics were recorded using the Ankom RF Gas Production System. Data were analyzed as a randomized complete block design (5 blocks) with a 2 (curing extents) × 2 (storage methods) × 3 (storage phases) factorial. Differences were declared at $P \leq 0.05$. The data were analyzed using the GLIMMIX procedure of SAS 9.4. At STRT, the Asymptotic maximal gas production (M) and fermentation rate (k) were not different between CUR and WET hay ($\bar{x} = 240.5 \pm 7.33$ mL/g incubated DM and $\bar{x} = 13.2 \pm 0.56$% of gas production/h, respectively). However, at MicA, CUR hay had a higher M and k than WET hay (232.7 vs. 192.6 and 13.9 vs. 9.33, respectively). The same was observed at LATE (237.6 vs. 197.6 and 13 vs. 8.36, respectively). At STRT, the CUR and WET silage was not different for M and k ($\bar{x} = 233.4 \pm 7.33$ mL/g incubated DM and $\bar{x} = 14.06 \pm 0.56$% of gas production/h, respectively). A similar trend was observed at MicA ($\bar{x} = 220$ and 13.6, respectively) and LATE ($\bar{x} = 219.7$ and 14.7). In conclusion, ample curing of red clover hay preserves the fermentation kinetics relative to insufficient curing, especially after 14 d of storage. In the case of red clover silage, the fermentation kinetics was not affected by the curing extent across the storage period.

Key Words: legume, in vitro fermentation, wilting.

1752W Predicting dry matter intake of lactating Jersey cows using animal factors or diet composition. K. V. Almeida[1], M. Gindri[2], D. C. Reyes*[1], P. J. Kononoff[3], and A. F. Brito[1], *[1]Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH, [2]Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants, Paris, France, [3]Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE.*

We aimed to predict DMI of lactating Jersey cows with models using animal factors or diet composition. The final database included 26 individual studies (n = 366 cows, 1,218 observations) that were divided into 2 independent data sets for model development and evaluation. The first data set (n = 234 cows) consisted of 18 studies with 832 observations of individual animal factors (lactation number, DIM, BW, BCS, DMI, milk yield, ECM) and diet composition (CP, ADF, NDF, ADF/NDF,

Table 1 (Abstr. 1752W). Fit statistics and model evaluation using an independent data set

	Proposed models	
Item	Animal	Diet
Model goodness-of-fit		
Coefficient of determination (R^2)	0.95	0.74
CCC[1]	0.88	0.59
RMSE[2]	1.21	1.13
Model evaluation		
MSEP[3]	7.70	17.6
RMSEP[4]	2.77	4.20
Decomposition of MSEP, %		
Mean bias	0.0003	0.43
Slope bias	0.36	0.18
Random error	0.64	0.39

[1]CCC = concordance correlation coefficient.

[2]RMSE = root mean squared error.

[3]MSEP = mean squared error of prediction.

[4]RMSEP = RMSE of prediction.

starch, and EE). Cows averaged 170 ± 62 DIM, 460 ± 51 kg of BW, and 24 ± 6 kg of milk/d, and diet composition ranged from 14.8 to 19.1% CP, 21.4 to 43.0% NDF, 16.2 to 33.3% starch, and 0.60 to 0.72 for the ADF/NDF ratio. Mixed-effects models were fitted including DMI as a response variable and animal factors or diet composition as explanatory variables. All models included the random effect of cow within study. The natural logarithm of each candidate variable was tested to increase residual homoscedastic variance and the predictive ability of models. Akaike information criterion was used to rank the best models before final model selection. The second data set (n = 132 cows) consisting of 8 studies with 386 individual observations was used for model evaluation. Data are presented in Table 1. Our proposed animal-model was fitted as follows: DMI (kg/d) = –35.8 + (0.18 × lactation number) + (0.003 × DIM) + (0.27 × ECM) + $e^{(7.47 \times \text{BW})}$. The diet-model was fitted with the equation: DMI (kg/d) = 23.59 + (0.35 × CP%) – (0.08 × NDF%) – $e^{(2.44 \times \text{starch\%})}$. The animal-model had better goodness-of-fit than the diet-model for all fit statistics, indicating that it can be used to predict DMI for lactating Jersey cows.

Key Words: dairy cows, intake, prediction

1753W Effects of yeast probiotic on milk performance, digestibility, feed efficiency, and methane emission of high-yielding dairy cows.

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Since the limitation or even the prohibition of the use of certain antibiotics as growth promoters in animal feed, the need to find alternatives has accelerated to optimize feed valorization, feed efficiency and production. In this context, Probiotic yeasts are used widely in the diets of dairy cows. The aim of the study was to evaluate the effect of yeast probiotic Actisaf Sc 47 "*Saccharomyces cerevisiae* strain CNCM I-4407" on milk production, feed efficiency and methane emission in dairy cow during early lactation. In total, 50 cows were allocated in 2 groups: Yeast Probiotic at 10g/d/cow and Control based on parity, previous lactation, milk yield and calving month. Dry matter intake was recorded daily, milk yield (MY) was recorded at each milking, milk fat (MF) and protein (MP) were recorded once a week, digestibility was measured during wk 6 and 12 postpartum by using AIA as marker. Protein and NDF digestibility was determined from ratios of AIA and N and NDF in feed and feces. Methane emission was recorded at each milking by sniffer system. Data were analyzed using generalized linear mixed model with repeated measures. Fixed effects were Treatment, Calving Month, and Parity. Random effect was Cow. For MY and milk constituents, MY during the week preceding the trial was used as a covariate. Compared with control, cows fed Actisaf Sc 47 had higher MY (47.5 vs. 50.1 kg/d, $P = 0.033$), energy-corrected milk (47.7 vs. 50.5 kg/d, $P = 0.009$), fat corrected milk (46.3 vs. 49.2 kg/d, $P = 0.008$), milk fat (1823 vs. 1945 g/d, $P = 0.02$) and feed efficiency (1.96 vs. 2.1, $P = 0.04$). The use of Actisaf Sc 47 increased neutral detergent fiber (66.5 vs. 70.6%, $P = 0.02$) and protein (75.9 vs. 78.6%, $P = 0.05$) digestibility. For methane, no difference was observed.

Key Words: milk, digestibility, efficiency

1754W Effects of highly bioavailable rumen-protected choline on energy metabolism and lactation performance in dairy cows.

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Choline optimizes lipid metabolism in the transition period and supports adaptation to lactation in dairy cows. However, the success of this methyl donor in improving milk yield and components and metabolism in ruminants depends on its bioavailability which in turn is affected by the type of protection used in rumen-protected choline (RPC). The current study assessed a RPC with greater intestinally available choline. Our objective was to determine the DMI (21 d pre and postpartum), milk yield and composition from calving to 150 d in milk, and the energy-related metabolites (Glucose, NEFA, and BHBA) in cows fed with a highly bioavailable RPC. Holstein cows (n = 48) at 245 d of gestation were blocked by parity and assigned to Control or RPC group within each block. Cows enrolled in the RPC group received 15 g/d of CholiGEM (Kemin Industries Inc., Des Moines, Iowa) from 21 d prepartum and 30 g/d from calving to 21 d postpartum. There were no effects of RPC on DMI (kg/d) prepartum in primiparous (Control = 14.1 ± 0.5 vs. RPC = 13.4 ± 0.5, $P = 0.33$) and multiparous (Control = 22.6 ± 0.6 vs. RPC = 21.5 ± 0.7, $P = 0.54$). Postpartum DMI in primiparous (Control = 17.1 ± 0.5 vs. RPC = 16.1 ± 0.5, $P = 0.15$) and multiparous (Control = 27.2 ± 0.7 vs. RPC = 26.6 ± 0.7, $P = 0.33$) cows was also not affected. Cows receiving RPC increased fat-corrected milk up to 150 DIM in primiparous (Control = 37.7 ± 0.2 vs. RPC = 38.8 ± 0.2, $P < 0.001$) and multiparous cows (Control = 50.6 ± 0.2 vs. RPC = 52.9 ± 0.2, $P < 0.001$). Cows receiving RPC increased milk fat yield in multiparous (Control = 1.9 ± 0.01 vs. RPC = 2.0 ± 0.01, $P < 0.001$) but not in primiparous (Control = 1.4 ± 0.01 vs. RPC = 1.5 ± 0.01, $P > 0.05$) cows and positively impacted dairy cows' metabolism. In conclusion, highly bioavailable RPC enhanced lactation performance by increasing fat-corrected milk and milk fat yield and positively impacted dairy cows' metabolism.

Key Words: energy-related metabolites, milk yield, transition period

1755W Effects of oral calcium supplementation on serum calcium and health events in mature Jersey cows.

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Jersey cows (parity ≥3) from a commercial dairy were used to evaluate the effects of oral Ca supplementation on postpartum serum Ca concentrations, health parameters, and herd removal. Cows were enrolled at freshening into one of 2 treatments on alternating days, over a period of 55 d. Treatments consisted of either a control group (CON) or a group receiving 2 doses of 44 g of elemental Ca as a Ca oral supplement (CaOS) via bolus (No BS Cow Products, Grover Beach, CA) immediately postpartum and again between 12 and 24 h later. Before enrollment, all cows were group fed a common close-up diet containing a blend of anionic salts (prepartum urine pH was 5.4 ± 0.5, mean ± SD). Blood was collected immediately before administration of the 2nd bolus, after which cows were moved to the fresh pen. Blood serum was stored frozen for later Ca analysis. The final data set consisted of 119 cows of parity group = 3 (56 CON and 63 CaOS) and 82 cows for parity group >3 (49 CON and 33 CaOS). ANOVA revealed that serum Ca concentrations were higher ($P = 0.027$) for parity group 3 (2.02 mM) vs. parity group >3 (1.92 mM), tended to be higher ($P = 0.065$) for CaOS (2.00 mM) vs. CON (1.93 mM) and were unaffected ($P = 0.74$) by the interaction of parity group with treatment. For each cow, the sum over 30 DIM of all health events normally recorded (displaced abomasum, ketosis, lameness, mastitis, metritis, milk fever, and retained placenta) was computed. This value and the number of cows removed (sold or died) over 30 DIM were each modeled by logistic regression,

Table 1 (Abstr. 1756W). Effect of supplemental microbial additives on performance of Holstein cows

	Treatment				*P*-value	
Item	CON	G1	G2	SEM	MA	TMA
ECM, kg/d	37.9	39.3	39.9	0.6	<0.01	0.45
Fatty acids, g/d						
<16 C	285	296	303	8	0.10	0.55
16 C	463	478	495	11	0.06	0.26
>16 C	439	459	471	9	0.01	0.33
Digestibility 124 d, %						
OM	77.7	78.1	77.0	0.5	0.79	0.09
NDF	46.5	46.3	45.8	0.9	0.62	0.68
Starch	98.5	98.6	98.5	0.1	0.75	0.31
CP	70.4	72.2	70.1	0.8	0.42	0.05
EE	82.5	84.1	81.3	0.9	0.80	0.01
Total SCFA, m*M*	117	118	120	2	0.26	0.48
Rumination, min/d	575	587	579	6	0.30	0.33

where the independent variables consisted of parity group, treatment, and serum Ca (the interaction of parity group with treatment was not significant, $P = 0.47$). The area under the curve for the receiver operating characteristic curve from the final model for health events was 0.73, indicating good accuracy. The odds ratio for health events was 2.2 for parity >3 vs. parity = 3 ($P = 0.083$) and 3.07 for CON vs. CaOS ($P = 0.025$). The odds ratio for removal was 2.34 for parity >3 vs. parity = 3 ($P = 0.058$) and 1.48 for CON vs. CaOS ($P = 0.38$). Supplemental oral Ca marginally increased serum Ca postpartum and substantially reduced health events over 30 DIM in this study.

Key Words: hypocalcemia, supplemental oral calcium

1756W Effects of microbial additives supplementation on production, digestibility, and rumination in dairy cows.

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Objectives were to determine the effects of 2 rumen-native microbial additives (MA) supplemented in diets of dairy cows on production, total-tract (TT) digestibility, and rumination. One hundred seventeen cows were enrolled in a randomized block design. Cows were blocked by parity and energy-corrected milk (ECM) yield then assigned randomly to a diet that was top-dressed daily with either 100 g of corn meal containing no microbial additive (CON), 100 g of corn meal containing 4×10^7 cfu of *Clostridium beijerinckii* and 1×10^9 cfu *Pichia kudriavzevii* (G1; Native Microbials, San Diego, CA), or 100 g of corn meal containing 4×10^7 cfu of *C. beijerinckii*, 1×10^9 cfu *P. kudriavzevii*, 1×10^8 *Butyrivibrio fibrisolvens*, and 1×10^8 of *Ruminococcus bovis* (G2) for 140 d. Milk yield was measured daily, whereas milk samples (AM and PM) were evaluated twice weekly. Ruminal fluid, feces, and urine were sampled at (mean ± SD) 61 ± 1 and 124 ± 3 d post treatment. Rumination was measured during 53 d post treatment. Data were analyzed with mixed-effects models and orthogonal contrasts evaluated the effects of MA [CON vs. (1/2 G1 + 1/2 G2)] or type of microbial additive (TMA; G1 vs. G2). Supplementing MA increased yields of ECM and milk fatty acids with no effect on TT digestibility at 61 d post treatment. Treatment G1 tended to improve TT digestibility of organic matter (OM), and increased digestibility of crude protein (CP) and ether extracted (EE) at 124 d post treatment. Rumination time and short chain fatty acids (SCFA) were not affected by treatment. Supplementing MA present in treatment G1 enhanced production coupled with moderate improvements in digestibility.

Key Words: dairy cow, microbial additive, digestibility

1757W Effect of capsaicin supplementation on performance and health of dairy cows: A meta-analysis.

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A meta-analysis was performed to investigate the effects of capsaicin on lactational performance and health status of lactating dairy cows. The database was comprised of 14 peer-reviewed studies published between 2011 and 2022. Supplement type [control = CON; capsaicin only = CAP; capsaicin plus other additives (i.e., essential oils and sweeteners) = CAP+] as well as health challenges (e.g., ketosis, glucose tolerance, lipopolysaccharide) were considered modifier variables in the analysis. Lactation performance response variables included: body weight, dry matter intake, milk yield (MY), energy corrected milk (ECM) yield, feed efficiency, and milk components. Health response variables included: red and white blood cell counts and circulating blood β-hydroxybutyrate (BHB), fatty acid, insulin and glucose concentrations. Data were analyzed using the comprehensive meta-analysis (version 3.3) software. Means, standard deviations and number of observations were used to compute standardized mean differences (raw mean difference of treatment and control means divided by the pooled standard deviation of the means) and the corresponding 95% confidence interval. Across both supplement types, MY was increased ($P = 0.01$) 0.29 ± 0.120 kg/d (n = 13) when compared with CON with the effect due to CAP+ treatments (n = 4), which increased ($P = 0.007$) MY 0.45 ± 0.168 kg/d. In studies which included a health challenge (n = 2), CAP/CAP+ increased ($P < 0.001$) MY 1.52 ± 0.342 kg/d compared with CON. Yield of ECM was not affected by CAP but was decreased ($P < 0.001$) 0.23 ± 0.023 kg/d by CAP+ compared with CON. No other treatment effects in CAP, CAP+, or challenge studies on lactational performance were observed. A trend for increased BHB ($P = 0.10$) levels was observed for CAP (n = 8) compared with CON. Neither CAP or CAP+ had other effects on health response variables. In conclusion, analysis at the study level, without consideration of supplement dose, suggests CAP+ had a positive effect on MY, and both CAP and CAP+ alleviated MY loss associated with health challenges.

Key Words: capsicum, phytonutrients, performance

1758W Culled fruit waste fed to dairy cows. L. D. Baker*, J. S. Bender, D. W. Pitta, and Z. Dou, *University of Pennsylvania Veterinary School, Kennett Square, PA.*

Large amounts of fruit waste occur at all stages of the supply chain. Opportunities for reuse and upcycling wasted fruit through ruminant diets is a viable option. In this study, we incorporated citrus fruit waste from a processing-repacking center into a dairy cow feeding experiment for 24 d. Culled citrus fruit arrived daily at the University of Pennsylvania Marshak Dairy for incorporation into the TMR. Cows are housed in 4 different production groups of 40 cows each, varying in parity, DIM and milk production, and within each group, cows were randomly assigned to control or fruit treatment diets. Both treatment and control diets were formulated for 24.9 kg DM/d and offered ad libitum. The treatment diet contained 1.2 kg DM citrus fruit (7.1 kg AF), reducing other feed ingredients proportionally to maintain the formulated 24.9 kg DM/d. Milk production was recorded daily for each cow, and DMI measured for each group of cows. Using a multi-level mixed effect regression model, the results indicated there was no significant difference in milk production between control and treatment cows ($P > .05$), and no difference in DMI between control and treatment within each group of cows. Milk fat and protein yields did not differ by treatment group. At 24.9 kg intake, the treatment diet was $0.36 less expensive than the control diet. Using actual DMI per group, the feed cost of the cows fed culled citrus fruit was $16.37/d less expensive than the cost of the control cow diet, supporting that feeding culled citrus fruit can be profitable for dairy farms. Feeding fruit waste avoids landfill, reducing methane emissions and offsetting enteric emissions. This study demonstrates that feeding culled citrus fruit to dairy cows did not affect milk production or component yield, with a reduction in GHG emissions.

Key Words: profitability, environment, food-waste

1759W Developing an algorithm to predict intake of concentrates in dairy cows fed a partial mixed ration. L. F. Martins*, S. F. Cueva, N. Stepanchenko, D. E. Wasson, and A. N. Hristov, *The Pennsylvania State University, University Park, PA.*

Objective was to develop an algorithm based on individual animal parameters to predict the intake of concentrate premixes (CMix) in dairy cows fed a partial mixed ration (PMR) and CMix delivered using an automatic feeding system (AFS). This study was part of a randomized complete block design experiment where cows within blocks were randomly assigned to 1 of 2 treatments: TMR fed ad libitum (n = 16 cows) or PMR fed ad libitum + CMix delivered using 2 AFS (DeLaval, Alpro 6.0/Feeding; n = 32 cows). Concentrate feeds used in the TMR were combined into 2 different CMix to be offered in the AFS as energy (13% crude protein and 45% starch) and protein (30% crude protein and 23% starch) CMix at a ratio of 1:2.4, respectively. Individual CMix allowance was calculated based on the weekly averaged dry matter intake and a 60:40 forage to concentrate ratio diet. Production data were used to estimate net energy (NE_L) and metabolizable protein (MP) balances, NE_L and MP allowable milk, and microbial protein synthesis using NRC (2001) and NASEM (2021) equations. The estimated values and actual production data (PROD) were then included as independent variables in multiple linear regression models to predict intake of energy and protein CMix for the PMR cows. Stepwise backward selection was used for the exclusion of variables when $P > 0.05$. Model performance was assessed using root mean squared error (RMSE) and Lin's concordance correlation coefficient (CCC) calculated from predicted and observed data collected from PMR cows. Using NRC and NASEM outputs to predict CMix intake resulted in a lower RMSE (10 and 11 vs. 19%) and a greater CCC (0.93 and 0.91 vs. 0.73), compared with PROD model, respectively. Precision and accuracy of the NRC and NASEM models were high, but both overestimated energy and protein CMix intakes by 0.28 and 0.68 kg/cow/d, respectively. Increasing the number of observations in the data set would enhance algorithm performance and could potentially improve precision feeding of dairy cows; however, effects on nutrient use efficiency remain to be determined.

Key Words: nutritional model, precision feeding

1760W Effects of increasing doses of a rumen modifier based on plant extracts on production performance of lactating dairy cows. P. Piantoni*[1], K. Dieho[2], J. de Jong[2], and G. Schroeder[1], [1]*Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN,* [2]*Cargill Animal Nutrition and Health, Global Innovation Center, Velddriel, the Netherlands.*

The objective of this experiment was to determine the effect of increasing doses of a rumen modifier based on condensed tannins and spice extracts on production performance of lactating dairy cows. Eight rumen-cannulated Holstein Friesian dairy cows (140 ± 86 DIM; 39.0 ± 5 kg/d milk yield; mean ± SD), were used in a Latin Square design experiment with 4-wk periods. Treatment sequences were balanced for carryover effects, and cows were randomly assigned to treatment sequence within square. Treatments were: 0, 10, 20 and 30 g/d Valido PRO (Cargill Inc.; 0VP, 10VP, 20VP, and 30VP, respectively). Treatments were top-dressed and thoroughly mixed with the feed offered once a day immediately after feeding. The formulated grass and corn silage-based diet was 54.2% forage, 15.7% CP, 18.1% starch, and 39.0% NDF. Data from the last week of each period were analyzed using the Lme4 package in R with a model containing the fixed effect of treatment and the random effect of square, period, day, and cow. Orthogonal contrasts were used to evaluate the linear and quadratic effect of increasing the dose of Valido PRO. Results follow the order: 0VP, 10VP, 20VP, and 30VP. Increasing doses of Valido PRO quadratically increased dry matter intake (26.6, 27.1, 27.3, and 26.3 kg/d; $P_{quad} = 0.05$) and milk yield (36.8, 38.2, 37.6, and 36.1 kg/d; $P_{quad} = 0.03$), tended to increase fat-and-protein-corrected milk (37.1, 37.9, 37.4, and 36.3 kg/d; $P_{quad} = 0.08$), and did not affect feed efficiency (1.38 ± 0.2). Treatments did not affect milk urea concentration (17.8 ± 1.7 mg/dL) or milk crude protein (3.39 ± 0.2%) or fat (4.06 ± 0.2%) content. Treatments did not affect milk fat yield (1.48 ± 0.2 kg/d) but increasing doses of Valido PRO quadraticallyincreased milk protein yield (1.22, 1.27, 1.26, and 1.20 kg/d; $P_{quad} = 0.02$). Quadratic responses in milk and fat-and-protein-corrected milk yield indicate that the more appropriate dose of Valido PRO was around 10 g/d. This experiment demonstrates the importance of dose response experiments with phytogenic products to determine recommended dosage for improved performance.

Key Words: dose response, phytogenic, rumen modifier

1761W The induction of nutritional ketosis in lactating dairy cows using calcium butyrate: Effects on lactation performance and health. M. A. Barrientos-Blanco*, A. Celemin-Sarmiento, M. da Silva, N. Bagheri, C. Mercado, N. Galdos, and J. E. Rico, *University of Maryland, College Park, MD.*

In addition to their roles as energy substrates, research in non-ruminants suggests that ketones of dietary origin may exert health-promoting effects. We have recently used intra-ruminal calcium butyrate for inducing hyperketonemia in dairy cows and observed no adverse effects on health. Herein, we aimed to induce nutritional ketosis using calcium butyrate (CaBu) as an exogenous ruminal ketogenic precursor. Six multiparous (2.8 ± 0.9 parity) Holstein lactating dairy cows (128 ± 52 DIM), fed ad libitum, were enrolled in a study with a 2x2 Latin Square design. Cows were randomly allocated to CaBu diet (CB; 4% of dietary DM) or without CaBu control (CON) over 2 72h periods. CB boluses were fed daily as a top-dress in 2 doses at 0h (CB1) and 8h (CB2) (0800h and 1600h). Changes in blood β-hydroxybutyrate (BHB) were monitored using a hand-held meter, and blood samples were collected 0, 2, 4, 8, 10, 12, 16, 24, and 48h relative to the first bolus. Milk yield (MY), milk components, and dry matter intake (DMI) were recorded at d −1, 0, 1, and 2 of each experimental period. Rectal temperature (RT), respiration rates (RR), and pain scores (PS) were measured twice at 0830h and 1600h on d 0, 1, and 2. Continuous and categorical variables were analyzed using mixed models in SAS, with the random effects of cow and period, and the fixed effects of time, treatment, and their interactions. Average blood BHB concentrations were 31% higher in CB relative to CON (0.76 mM vs. 0.58 mM, respectively; $P < 0.05$). Plasma BHB peaked at h4 post-CB1 (~1.1 mM) and h4 post-CB2 (~0.9 mM). Although CB exhibited an 11% reduction in DMI, MY did not differ, and feed efficiency was significantly improved in CB relative to CON (11.7% and 14.5% for MY/DMI and FCM/DMI respectively; $P < 0.05$). The ketogenic diet did not affect milk fat, protein, lactose, SCC, or MUN ($P > 0.15$). We did not detect differences in RR and RT, nor signs of pain. Our data indicate that a ketogenic diet using CaBu can induce nutritional ketosis, improving feed efficiency, with no negative impacts on milk quality or health status of dairy cows.

Key Words: nutritional ketosis, calcium butyrate, dairy cow

1762W The effectiveness of a negative DCAD diet on fresh cows through urine pH analyses. E. Prybylski*, E. O'Meara, and F. Cardoso, *University of Illinois Urbana-Champaign, Champaign, IL.*

This study aimed to determine whether the environment (ENV, laboratory vs. farm) would affect measurement of urine pH levels of cows fed a negative DCAD (dietary cation-anion difference) diet prepartum. The secondary aim was to analyze within day effects of negative DCAD in urine pH. Fourteen multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, and body condition score (BCS) during the far-off dry period were assigned to 1 of 3 dietary treatments (TRT). Cows were assigned either a controlled-energy diet (straw-based diet, 1.45 NEL, Mcal/kg of DM; −181.98 mEq/kg DCAD) with RPL (Kemin Industries Inc., Des Moines, IA) and RPM (Kemin Industries Inc., Des Moines, IA) [CEAA; 0.15% RPL and 0.09% RPM of dietary dry matter intake (DMI)], controlled-energy diet without RPL and RPM (control; CENAA), or high-energy diet (corn silage based diet, 1.71 NEL, Mcal/kg of DM; −186.79 mEq/kg DCAD) with RPL and RPM (HEAA; RPL 0.22% and RPM 0.12% of dietary DMI). Starting at hour 0 (9 a.m.) once a week on Sundays, which was one hour after daily feed push-up and return from morning milking, dry cows had urine collected and pH analyzed, followed by hours 6 (3 p.m.) and 24 (9 a.m. the following day). Urine was collected in polypropylene containers, and pH was determined using pH strips. This process was repeated weekly (n = 9) until the experiment's completion. Statistical analyses were performed using the MIXED procedure of SAS. The model included the fixed effects of ENV and TRT. Cow was the experimental unit. Hour was included as a repeated measure. Tukey's adjustment was used for comparison of means. There were no urine pH differences ($P = 0.83$) for TRT. Additionally, there were no differences among treatments ($P = 0.85$) evaluated at 0, 6, or 24 h among ENV. Laboratory measures resulted in a higher urine pH ($P = 0.04$). There were also greater differences between the ENV and TRT days on urine pH levels ($P = 0.04$). In conclusion, higher urine pH in the laboratory than farm was likely due to improper storage and higher temperatures resulting in alkaline pH levels; thus, on farm measurement resulted in more accurate scores.

Key Words: pH, dietary cation-anion difference, environment

1763W Summarizing the effects of organic versus inorganic zinc in lactating dairy cows. M. J. Oconitrillo and J. A. D. R. N. Appuhamy*, *Department of Animal Science, Iowa State University, Ames, IA.*

Despite the higher bioavailability compared with their inorganic counterparts, the effects of dietary organic zinc supplements in dairy cows are mixed in published literature. The study objective was to combine the literature data and summarize the effect sizes (ES) of organic zinc (Zn-o) vs. inorganic zinc (Zn-i) added to dairy cow diets. A literature search using the journal of dairy science and the science direct databases resulted in 9 articles offering 12 comparisons of diets with only Zn-i (CTL) vs. the same diets where the Zn-i was completely replaced with Zn-o (TRT). The Zn-i in CTL were ZnO (n = 5) or $ZnSO_4$ (n = 7), and the Zn-o in TRT were Zn-amino acid chelates containing only Zn (n = 5) or Zn plus a few other trace minerals (n = 7). The added Zn concentration of the diet (Zn-con), days in milk (DIM), and trial duration ranged between 41 and 540, 1 and 166, and 7 and 125 with the means of 124 mg/kg of DM, 77, and 49 d, respectively. The mean DMI, milk yield (MY), milk protein (MP%) and fat (MF%) concentrations, milk protein (MPY) and fat (MFY) yields, and somatic cell counts (SCC) of CTL were 24.4 kg/d, 38.0 kg/d, 3.04%, 3.57%, 1155 g/d, 1356 g/d, and 97 × 1000 cells/mL, respectively. The ES (ES = TRT mean – CTL mean) were summarized across the studies by using multilevel mixed-effect meta-analyses conducted with the *metafor* package of the R software. The summarized ES of DMI, MY, MP%, MPY, MF%, MFY, and SCC were 0.42 kg/d ($P < 0.01$), 0.48 kg/d ($P = 0.10$), 0.04% ($P = 0.02$), 17.4 g/d ($P = 0.03$), 0.07% ($P = 0.02$), 24.1 g/d ($P = 0.07$), and 16.0 × 1000 cells/mL ($P < 0.01$), respectively. The DMI increment decreased ($P < 0.01$), whereas the SCC reduction increased ($P < 0.01$) as Zn-con increased. The Zn-con explained 72 to 97% of the heterogeneity of those ES. The ES of MY decreased ($P = 0.02$), whereas the ES of MF% increased ($P < 0.01$) as DIM or the trial duration increased. The Zn-i in CTL or the Zn-o in TRT did not affect any of the ES. Overall, positive effects of Zn-o compared with Zn-i were revealed when the literature data were combined and statistically summarized.

Key Words: dairy cows, inorganic zinc, organic zinc

1764W Feeding direct-fed *Bacillus subtilis* and *Clostridium beijerinckii* affected plasma metabolites in Holstein cows. F. F. Cardoso*[1], L. Garcia[1], J. S. Thompson[2], M. N. de Jesus[2], A. H. Smith[2], T. G. Rehberger[2], and F. C. Cardoso[1], *[1]Department of Animal Sciences, University of Illinois, Urbana, IL, [2]Arm & Hammer Animal and Food Production, Waukesha, WI.*

This experiment was conducted to determine the effects of feeding 2 strains of *Bacillus subtilis* (2.0×10^9 cfu/daily); 6 strains of environmental *Clostridium beijerinckii* (4×10^6 cfu/daily), or both from −21 d relative to expected calving until 70 d in milk on animal health focusing on blood biomarkers. Seventy-one multiparous Holstein cows, blocked by parity, previous 305-d milk production, and expected calving date, were assigned to 1 of 4 dietary treatments in a randomized, complete block design. Treatments consisted of TMR top-dressed with *B. subtilis* (CER; Certillus, Arm & Hammer and Food Production, Waukesha, WI; 20g/cow/day), *C. beijerinckii* (BJE, 0.001% of dietary DM), both (CBJ), and neither *C. beijerinckii* or *B. subtilis* (CON). Blood samples (Triglycerides, BUN, GGT, Cholesterol, Total protein and Globulin) were taken from coccygeal artery or vein on d −25 ± 5 (covariate); −8 ± 3; 7 ± 2; 28 ± 2; and 70 ± 2 d relative to calving. Statistical analysis was performed using the MIXED procedure of SAS. Prepartum and postpartum data were analyzed separately. In the prepartum period, cows in CER tended ($P = 0.06$) to have greater concentrations of plasma triglycerides than cows in CON (19.7 and 15.3 mg/dL, respectively). Cows in CBJ tended ($P = 0.09$) to have greater concentrations of plasma BUN than cows in CER (14.9 and 13.6 mg/dL, respectively). Cows in CER had lower concentrations of plasma GGT ($P < 0.01$) than cows in CBJ (16.4 and 19.8 U/L, respectively). During the postpartum period, cows in CER had greater ($P = 0.02$) concentrations of plasma cholesterol than cows in CON (154 and 130 mg/dL, respectively). Cows in BJE had greater ($P \leq 0.01$) concentrations of plasma total protein compared with cows in CON (7.43 and 6.97 g/dL, respectively) and had greater concentrations of plasma globulin compared with cows in CON (4.03 and 3.41 g/dL, respectively). Supplementation with CER increased plasma total cholesterol postpartum, which can be correlated to a better liver function, perhaps related to higher plasma TG, associated with better transport of lipids. Our data suggested that cows in CER and BJE may have decreased inflammation due to the lower plasma GGT concentration.

Key Words: GGT, inflammation, transition period

1765W Partitioning variance in nutrient concentrations of dry cow total mixed rations.

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Dry cow diet nutrient composition can vary from targets across dairies. However, both sampling on- farm and subsampling methods in a commercial laboratory are also contributors to variation in feed analyses. Therefore, the objective of this study was to quantify the contribution associated with these variance sources in crude protein (CP), starch, neutral detergent fiber (NDF), ash, and fat concentrations. To achieve this, dry cow TMR samples were collected in duplicate from 14 dairies across the US by dairy professionals. These samples were submitted to a commercial laboratory (Rock River Laboratory, Watertown, WI; RRL) for analysis. At RRL each sample was divided into subsamples using a mixing and quartering procedure. Subsamples were dried, ground, and divided into 3 subsamples. Each of these subsamples (n = 167) were analyzed for nutrient concentrations by near infrared spectroscopy, in addition to mineral composition which was analyzed using inductively coupled plasma-optical emission spectrometry. Data were analyzed using a Mixed Model in SAS JMP version 17.0.0. Farm was included as a fixed effect while farm level sampling within farm and lab subsample within farm sample were included as random effects. Variance components for farm level sampling and laboratory subsampling were estimated using restricted maximum likelihood covariance estimates. Total variation was partitioned between the sampling at the farm level and subsampling at the laboratory level. The fixed effect of farm was significant for all nutrients analyzed ($P < 0.0001$). The variance associated with laboratory subsampling was less than 5% of total for all nutrients analyzed. Farm sampling variance accounted for 14.9%, 31.8%, 17.7%, 13.2% and 16.8% of total for CP, starch, NDF, ash, and fat concentrations, respectively. Coefficient of variation (CV) was 11.3% for CP, 35.5% for starch, 17.1% for NDF, 9.0% for ash, and 17.0% for fat. The significance of farm in the model and large CV among nutrients showcases the large variability of nutrient concentrations within dry cow TMR at the farm level. Additionally, on farm sampling accounted for most of the variance in the TMR analysis.

Key Words: dry cow, total mixed ration, sampling

1766W Effects of supplementing a high protein corn coproduct on energy and nitrogen utilization in lactating Jersey cows fed different proportions of corn silage and alfalfa haylage.

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The corn-ethanol industry has advanced dry-milling processes to include the mechanical separation of fiber particles from a portion of the plant and yeast-based nitrogenous particles. These portions can be added back to result in the production of a high protein corn coproduct (HPCoP), which contains a higher concentration of CP and NDF (52.4 and 36.2%, respectively) than traditional distillers grains (31.0% CP, 30.8% NDF). The objective of this study was to compare the effects of feeding diets differing in the proportion of corn silage (CS) or alfalfa haylage (AH) and in the supplementation of either non-enzymatically browned soybean meal (NBSM) or HPCoP on N and whole-animal energy utilization using indirect calorimetry in lactating dairy cows. Twelve multiparous Jersey cows (mean ± SD; 417 ± 6 kg BW; 97 ± 4 d in milk) were used in a 4 × 4 Latin square with 4 periods of 28 d. Cows were randomly assigned to 4 different mixed diets with the following factorial arrangement: high CS and HPCoP (19.0% CP), high CS and NBSM (18.3% CP), high AH and HPCoP (18.2% CP), and high AH and NBSM (17.5% CP). Diets high in CS contained 39% DM CS and 18% AH; these proportions were reversed in diets high in AH. Diets were supplemented with either 10.0% DM HPCoP or 7.78% DM NBSM. Treatment did not affect ($P \geq 0.55$) DMI, averaging 19.7 ± 0.44 kg/d across treatments. Additionally, no difference ($P \geq 0.23$) was observed in diet concentration of ME Mcal/kg of DM (averaging 2.56 ± 0.05 Mcal/kg of DM). Cows consuming high CS diets had a greater ($P = 0.01$) N intake than those consuming AH diets (592 vs 563 ± 14.2 g/d). Cows consuming HPCoP supplemented diets also had a greater ($P = 0.02$) N intake (590 vs 565 ± 14.2 g/d) than those consuming NBSM supplemented diets. Neither aNDFom nor CP apparent total-tract digestibility differed ($P \geq 0.22$) across diets and averaged 43.2 ± 1.57 and 65.3 ± 0.88%, respectively). Lastly, no difference ($P \geq 0.31$) was observed in total fatty acid digestibility, averaging 61.5 ± 3.29% across treatments. Results of this study suggest that a high-protein corn milling coproduct can be added to diets high in either corn silage or alfalfa haylage without impacting energy utilization.

Key Words: corn fermented protein, corn silage, alfalfa haylage

1767W Internal temperature and THI tolerance level of cows supplemented with *Acacia mearnsii* tannins.

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Based on theories of protein mobilization during heat stress in dairy cows, this study evaluated whether supplementation of 150 g/cow/day of *Acacia mearnsii* could alter internal temperature and THI tolerance. Cows with an average of 63 ± 10 d in lactation (DEL) and average production of 25 ± 3 kg/day, were homogeneously distributed in tannin group (GT; n = 10) supplemented on top condensed tannin from *Acacia mearnsii* (Tanac, Rio Grande do Sul, Brazil) and Control Cow Group (GC; n = 10), both kept in the same barn. After 10 d of adaptation, the cows received an intravaginal device for 72 h/week containing a data logger thermometer (Thermochron ibutton, KY, USA) to record vaginal temperature every 30 min (n = 144/cow/week) for 2 weeks. The ambient temperature and humidity were obtained from a meteorological station and used to calculate the temperature and humidity index (THI). For statistical analysis, IT data were evaluated using the Wilcoxon-Mann-Whitney test, rejecting the null hypothesis $P < 0.05$. The milk yield data were analyzed by repeated measures in the Proc Mixed statistical package, in the SAS software (SAS Studio Institute Inc., Cary, USA, 2019). In addition, a Receiver Operating Characteristic Curve (ROC) analysis was used to assess the THI required to raise the TI above 39.2°C. The groups were also compared by Chi Squared Test for total hours above this temperature. The minimum and maximum THI during the experimental period was 63.04 and 78.38, respectively. The milk yield was not different between groups, but the IT were lower in TG (39.4°C ± 0.72, vs. CG 39.6°C ± 0.73 and null hypothesis rejected ($P >$ 0.05). The number of hours with temperature above 39.2°C was lower for TG ($P < 0.05$). The TG showed a temperature above 39.2°C when the THI reached 64.06, while overheating in the CG occurred when the THI reached 63.66 ($P < 0.001$). These data demonstrate that tannin supplementation can change the tolerance to high THI and modulate the internal temperature of cows without changing the milk yield.

Key Words: additives, heat stress, thermotolerance

1768W Effects of feeding *Bacillus subtilis* and *Clostridium beijerinckii* on performance of Holstein cows during the transition period and early lactation.

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Feeding *Bacillus subtilis* has shown to improve cow's performance. However, there is scarce evidence on the impact of the non-toxigenic clostridia *Clostridium beijerinckii* on animal performance. This experiment was conducted to determine the effects of feeding 2 strains of *B. subtilis* (2.0×10^9 cfu/daily); 6 strains of environmental *C. beijerinckii* (4×10^6 cfu/daily), or both from −21 d relative to expected calving until 70 d in milk on cow's performance. Seventy-one multiparous Holstein cows, blocked by parity, previous 305-d milk production, and expected calving date, were assigned to 1 of 4 dietary treatments in a randomized, complete block design. Treatments consisted of TMR top-dressed with *B. subtilis* (CER; Certillus, Arm & Hammer and Food Production, Waukesha, WI; 20 g/cow/day), *C. beijerinckii* (BJE, 0.001% of dietary DM), both (CBJ), and neither (CON). Cows were milked 2 × per d and milk samples were taken on 7, 14, 28, 42, 56, and 70 d relative to calving. Milk yield and dry matter intake (DMI) were obtained daily. Statistical analyses were performed using the MIXED procedure of SAS. There was no difference for DMI and milk yield. There was a tendency ($P =$ 0.08) for a treatment × wk interaction, where cows in CER had greater milk fat content (5.32%) compared with cows in BJE (4.60%) in wk 1; cows in CER had a greater (4.52%) milk fat concentration compared with cows in CON (3.73%), BJE (3.83%), and CBJ (3.83%) in wk 4. There was a treatment × wk interaction, in which cows in BJE had a greater milk protein ($P < 0.01$) and casein concentrations ($P = 0.03$) (4.42, 4.28%) compared with cows in CER (3.92, 3.17%) and CBJ (3.74, 3.28%) at wk 1, respectively. In conclusion, feeding *B. subtilis* and/or *C. beijerinckii* did not improve or hinder DMI and milk yield but had a modest impact in milk components in early lactation.

Key Words: direct-fed microbials, *Bacillus subtilis*

1806W Effect of removing Rumensin from diet on milk production efficiency.

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Studies comparing diets with and without Rumensin often include a period before the study where all cows receive a common diet, including Rumensin, as a covariate before the study period. This acclimation time likely is an issue both when Rumensin is introduced to diets as well as when it is removed from diets. If rumen adaptation exists when Rumensin is removed from a diet, the milk production efficiency (MPE) of control treatments may be biased at the beginning of trials. The objective of this study was to examine how MPE is impacted when Rumensin is removed from diets in post-peak lactating dairy cows. Data from 4 previous studies were pooled for this analysis where these studies were chosen due to them having similar designs for Rumensin versus Control treatments and how cows were treated at the onset of the studies (covariate period). Data analyzed were pen-level weekly averages for milk, energy-corrected milk (ECM), solids-corrected milk (SCM), and dry matter intake (DMI). In the 2-week covariate period (weeks −1 and 0), all cows received Rumensin and then for the study period (wk 1–9) cows were in either a control group (no Rumensin) or a Rumensin group. Least squares models with Milk/DMI, ECM/DMI, and SCM/DMI as the dependent variable were estimated with week, treatment, week × treatment interaction, production efficiency in the covariate period (2-week average) as fixed effects, and study as a random effect. Total observations used in model estimation were 504 (9 weeks × 56 pens). Overall fit (R^2) of the 3 models ranged from 0.59 to 0.72. Differences in LS means by treatment were compared for each week with significance denoted as * ($P = 0.05$) and † ($P = 0.10$). Percent advantage (PA) in Milk/DMI for Rumensin from wk 1 to 9 were 0.30, −1.35, 3.47*, 2.15, 4.07*, 0.12, 4.67*, 3.63*, and 5.00*. PA for ECM/DMI were −0.24, −2.37†, 2.87*, 1.30, 3.58*, −0.90, 3.70*, 2.41†, 4.23* for wk 1–9, respectively, and PA for SCM/DMI were −0.09, −2.25†, 3.08*, 1.48, 3.81*, −0.63, 4.07*, 2.64†, 4.41* for wk 1–9, respectively. This analysis suggests it takes up to 3 weeks for the effect of Rumensin to dissipate.

Key Words: monensin, milk production efficiency

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion 3

1769W Evaluation of blanched *Sargassum horneri* as feed source for greenhouse gas mitigation in the rumen. M. J. Seo*[1], S. K. Kim[2], I. K. Hwang[2], H. C. Kim[3], S. S. Lee[4], Y. H. Joo[1], S. M. Jeong[1], J. Y. Kim[1], and S. C. Kim[1], [1]*Division of Applied Life Science (BK21Four, Insti. of Agric. & Life Sci.), Gyeongsang National University, Jinju, Gyeongsangnam-do, Republic of Korea,* [2]*Aquaculture Research Division, National Institute of Fisheries Science, Busan, Republic of Korea,* [3]*Research and Development Planning Division, National Institute of Fisheries Science, Busan, Republic of Korea,* [4]*Animal Nutrition and Physiology Division, National Institute of Animal Science, Wanju, Jeollabuk-do, Republic of Korea.*

This study was conducted to evaluate the feed values and greenhouse gas emissions of *Sargassum horneri* (SH) for the ruminant. In Experiment 1, a 4 (blanching temperatures; 21, 60, 70, and 80°C) × 3 (durations; 1, 2, and 3 min) factorial design was used to determine the sodium reduction method for SH. In experiment 2, the SH blanched at 21°C (CON) and 70°C (LSH) for 3 min were used as the supplements, and a 2 (supplements; CON vs. LSH) × 3 (supplementation levels; 0, 1, 3, and 5%) factorial design used to determine the greenhouse gas mitigation in the rumen. All data were analyzed using PROC MIXED of SAS version 9.4(2013) to test the effects of factor only or their interactions. Polynomial contrasts (linear and quadratic effects) were used to evaluate the effects of blanching temperatures (Experiment 1) and supplementation levels (Experiment 2). Rumen fluid was collected from 2 cannulated Hanwoo heifers, and mixed with *Van Soest* medium at a 1:2 ratio for experiment 2. As the results in experiment 1, crude ash content decreased ($P < 0.01$) linearly with increasing the blanching temperature, while crude protein increased ($P < 0.01$) linearly. The contents of Na, K, and As decreased ($P < 0.01$) linearly with increasing the blanching temperature, especially lowest at 70°C for 3 min of blanching. As the results in experiment 2, in vitro digestibilities of dry matter (IVDMD) and organic matter (IVOMD) and total volatile fatty acid decreased ($P < 0.05$) linearly with increasing the supplementation levels. However, the supplementation levels did not affect the carbon dioxide and methane emissions, except for the tendency of decreases only in LSH ($P < 0.10$). By the sodium reduction methods, IVDMD and IVOMD in LSH were higher ($P < 0.05$) than in SH. However, total gas, carbon dioxide, and methane emissions in LSH were lower ($P < 0.05$) than those in CON. In conclusion, the blanching SH at 70? for 3 min could be recommended as the sodium reduction method. And LSH supplementation may have potential benefits for greenhouse gas mitigation in the rumen, but needed further investigations to clarify it.

Key Words: greenhouse gases, *Sargassum horneri*, blanching

1770W A first assessment of safety and metabolism of iodoform when used as a methane mitigating feed additive to dairy cattle. M. Rønn*, S. Purup, N. P. Nørskov, and M. O. Nielsen, *Aarhus University, Foulum, DK-Tjele, Denmark.*

Iodoform (IF) has proven to be a potent inhibitor of methane emission from dairy cattle. As a first attempt to evaluate its use as a feed additive, cell lines of bovine mammary epithelial cells (MAC-T) and fetal human epithelial cells (FHs-74 Int; FHI) were exposed to blood serum and milk whey samples originating from dairy cows exposed to IF in an intensive dose response experiment. The experiment was conducted with 4 Danish Holstein dairy cattle given 4 doses of IF (x0, x1, x2, x2.5) directly into the rumen twice daily through a rumen cannula. Cell viability was tested using a resazurin metabolism assay with cells exposed to 1%, 5%, and 10% of serum or whey in triplicate wells and plates. To test the effect of IF itself, a cell viability trial with MAC-T cells exposed to increasing doses of 1–1000 ng/mL was additionally performed. Furthermore, rumen fluid, duodenal fluid, blood serum and milk samples from the cow trial were quantified for the content of IF using liquid-liquid extraction and GC-MS/MS. The kinetic metabolism of IF in rumen fluid was tested in vitro using the ANKOM fermentation system, where 35 µg IF dissolved in ethanol was added to 90mL buffered rumen fluid in 100 mL bottles, whereafter samples of liquid were collected from time 0–24h of fermentation for IF quantitation. Differences in cell viability were analyzed using a linear mixed effects model, while kinetic studies were analyzed using linear mixed effect models with quadratic or eksponententical effects of time or IF concentration. There were no significant effects of serum or whey from IF dosed cows on FHI or MAC-T cell viability, except for a reduced viability of MAC-T cells, when media contained 10% serum from cows exposed to dose x2.5 of IF compared with serum from control cows. This was, however, not caused by IF itself, as cell viability did not decrease when MAC-T was exposed to high doses of pure IF, and IF could not be detected in any of the biological fluids obtained from cows. The in vitro kinetic study showed that IF disappears from rumen fluid within 30 min of rumen fermentation, which explains why IF could not be detected in any biological samples collected from cows, irrespective of the dose of IF they had been exposed to.

Key Words: methane mitigation, safety assessment, metabolism

1771W Effects of direct fed microbials on in vitro ruminal fermentation, methane production, and ruminal microbial communities. E. Sarmikasoglou[1], P. Sumadong[2], G. Dagaew[2], M. L. Johnson*[1], J. R. Vinyard[1], G. K. Salas-Solis[1], M. U. Siregar[1], W. Rottman[3], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*Khon Kaen University, Khon Kaen, Thailand,* [3]*Locus Agricultural Solutions, Solon, OH.*

The objective of this study was to examine the effects of Amplio, a direct fed microbial product on in vitro ruminal fermentation and methane production utilizing batch culture serum bottles. One hundred 68 batch culture 200 mL bottles were used in a complete randomized block design where treatment was analyzed as a fixed effect and run was a random effect. Treatments were arranged in 3x3 factorial to evaluate the effects of inoculum, diet and their respective interaction. Among the 168, 114 bottles were used for total gas, CH_4, pH, NH_3, VFA, and lactate measurements at 3, 6, 24, and 48 h after inoculation. Each treatment had 3 batch culture bottles (3 replicates per treatment) and 3 blank bottles (rumen fluid only) without a diet to correct for background gas production. Three inoculums (microcrystalline cellulose, CON; 10 billion Amplio, A1; and 60 billion Amplio, A2) were supplemented with a dry cow diet (D, 18% starch), mid-lactation diet (M, 25% starch), or early lactation diet (E, 30% starch), resulting in total of 9 treatments. There were 3-experimental runs total, where run was used as a blocking factor. Total gas was measured at 3, 6, 24, and 48 h after inoculation, there were no dose effects within the same diet but there was an increase in the total gas production from D to E diets, due to greater fermentable matter from D to E diets ($P < 0.01$). At 6 h after inoculation, we observed reduced CH_4 production in M/A2 compared with M/C ($P < 0.01$), while the E/A2 had greater CH_4 production compared with E/C ($P < 0.01$). At 24 h after inoculation, both M/A1 and M/A2 reduced CH_4 production compared with M/C ($P < 0.01$); and E/A2 had greater CH_4 production compared with both E/C and E/A1($P < 0.01$). At 48 h after inoculation, the M/A1 and M/A2 reduced CH_4 production compared with M/C, while the M/A2 had lower CH_4 production compared with M/A1 ($P < 0.01$). Furthermore, the E/C had lower CH_4 production compared with E/A1 and E/A2, while E/A2 had greater CH_4 production compared with E/A1 ($P < 0.01$). Our results demonstrate that Amplio affects CH_4 production and its effects depend on diet type. Notably, it reduces CH_4 production when included in mid-lactation dairy diets, containing 25% starch.

Key Words: pH, batch culture, methane

1772W Characterization of the diurnal pattern of exhaled volatile fatty acids and enteric methane emissions of dairy cows.

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The methods used for evaluation of rumen fermentation are invasive. Volatile organic compounds (VOC) in a ruminant exhalome originate from lungs and rumen, and contain VOC, such as ruminal methane (CH_4) and exhaled volatile fatty acids (eVFA). In this study, we aimed to use a non-invasive metabolomics approach using secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) platform to assess rumen fermentation parameters in dairy cows. Enteric CH_4 emission from 7 cows was measured 8 times over 2 d using GreenFeed. Simultaneously, exhalome samples were collected and analyzed using SESI-HRMS platform. Data were analyzed using a mixed model in R with fixed effect of time as repeated measures and random effect of cow. In total, 1,298 features were detected, among them 3 targeted eVFA (i.e., acetate, propionate, butyrate), which were annotated using their exact mass-to-charge ratio. Exhaled acetate had the greatest concentration among the eVFA, averaging (±SD) 21.3 ± 13.94 count-per-second (CPS) followed by propionate at 11.5 ± 3.10 CPS, and butyrate at 2.67 ± 2.495 CPS. Further, exhaled acetate was the most abundant at around 60% of the total eVFA captured, followed by 32% propionate and 8% butyrate, which correspond well with the previously reported ruminal VFA proportions. The diurnal patterns of CH_4 emission and individual eVFA were characterized using a linear mixed model with cosine function fit, and it demonstrated a daily pattern ($P < 0.05$) for ruminal CH_4 and H_2 emissions and eVFA concentrations over a 24-h period. Based on the model, the phase of total eVFA, and exhaled acetate, propionate, and butyrate was at 1354, 1348, 1448, and 1312 h (i.e., 4–6 h post-feeding), respectively, whereas the peak of CH_4 occurred at 1436 h and that of H_2 at 1600 h. This corresponds well with the expected relationship between rumen VFA and CH_4 formation. Results from the present study revealed a great potential to assess rumen fermentation and the daily pattern using exhaled metabolites as a non-invasive approach. Further validation and application of the proposed method are required.

Key Words: exhalomics, exhaled VFA, CH_4 emission

1773W A proposed in vitro method for measuring methane production in ruminal batch culture.

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As interest in understanding methane (CH_4) production by ruminants grows, there is a need to develop novel ways to measure CH_4 production from in vitro ruminal batch culture. We devised a method to collect fermentation gases using syringes and a series of valves. Batch culture fermentations were incubated in a 38.6°C shaking water bath for 24 h. Fifty mL round bottom tubes (Thermo Scientific 31100500) with rubber stoppers (size 5.5, Fisherbrand 14–135H) were used as the fermentation vessels. Fermentation gases were collected in a 60 mL syringe that was connected to the stopper using a male Luer to hose barb adapter (Masterflex 45504–19) and 2 Luer on/off valves in series (female x female, male x female: McMaster-Carr 7033T26, 7033T24). Gas production was recorded hourly by checking syringe plunger displacement. When gas production approached 60 mL (and at final time points), both valves were closed and disconnected from each other. A Luer-lock needle hub was connected to the valve adapter of the filled syringe and the needle cannula was inserted into a 6 mL vacuum tube (BD Vacutainer 366408). The valve was opened and about 10 mL of fermentation gas was injected into the vacuum tube. CH_4 concentrations were measured by manually injecting 250 µL of the stored sample from the vacuum tube into a GC within 24 h of collection. CH_4 production was calculated by multiplying the known gas volume by the measured CH_4 concentration at each sampling point. The system was validated to test if our method could detect differences in CH_4 from 30 mL batch cultures utilizing a 2 × 2 factorial using 90:10 (HF) and 10:90 (LF) ground timothy hay to fine ground corn (0.5 g total) with and without monensin (0.002 g Rumensin, Elanco Animal Health). Compared with LF, HF increased total CH_4 yield from 7 mL to 9 mL ($P < 0.05$) but did not significantly impact CH_4 concentration ($P = 0.2$). Monensin treatment decreased CH_4 concentration from 17% to 15% and total CH_4 yield from 9 mL to 7 mL ($P < 0.05$) which is consistent with Capelari and Powers (2017), validating it as an effective method to measure CH_4 production in ruminal batch culture.

Key Words: methane, in vitro, batch culture

1774W Withdrawn.

1775W *Pinus koraiensis* cone essential oil mitigates rumen methane emission by altering the rumen microbial compositions and functions in goats.

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The objectives of the current study were to investigate *Pinus koraiensis* cone essential oil (PEO) as a methane (CH_4) inhibitor and determine its impact on ruminal bacterial community and functional characteristics in goats. In the present study, 10 growing Korean native goats (29.9 ± 1.58 kg, male) were assigned to different dietary treatments; control (CON; basal diet without additive) and PEO (basal diet + 1 g/d of PEO) by a 2 × 2 crossover design. The data obtained from in vivo experiment was analyzed using PROC GLIMMIX procedure. Methane concentration were measured every 4 consecutive days for 17–20 d. Rumen fluid and feces were sampled at the end of the experimental period to determine the biological effects and dry matter (DM) digestibility of PEO supplementation. Rumen microbiota was analyzed using 16S rRNA gene amplicon sequencing. The PEO supplementation resulted in reduced CH_4 emission (eructation CH_4/body weight$^{0.75}$, $P = 0.079$) without affecting DM intake; however, it lowered the total volatile fatty acids ($P < 0.05$), molar proportion of propionate ($P = 0.075$), and ammonia nitrogen (P

= 0.087) in the rumen. The absolute fungal abundance ($P < 0.01$) was reduced by PEO supplementation, whereas ciliate protozoa, total bacteria, and methanogen abundance were not affected. The composition of rumen prokaryotic microbiota was altered by PEO supplementation with lower evenness ($P = 0.054$) observed for the PEO group than the CON group. The PICRUSt2 analysis indicated that the metabolic pathways of prokaryotic bacteria, such as pyruvate metabolism, was enriched in the PEO group. The entire co-occurrence networks showed that more nodes and edges were detected in the PEO group. Overall, our findings provide an understanding of how PEO supplementation affects CH_4 emission and rumen prokaryotic microbiota composition and function. This study may help develop potential manipulation strategies to find new essential oils to mitigate enteric CH_4 emissions from ruminants.

Key Words: feed additive, metataxonomic, methane

1776W An in vitro gas production system produces more repeatable estimates of starch digestibility in ensiled corn ingredients than in vitro starch digestibility. J. R. Knapp*[1], N. Schlau[2], K. Taysom[2], and D. M. Taysom[2], *[1]Fox Hollow Consulting LLC, South Vienna, OH, [2]Dairyland Laboratories Inc., Arcadia, WI.*

The objective was to compare starch digestibility predicted from in vitro gas production (IVGP) incubations to in vitro starch digestibility (IVSD) for high-moisture corn (HMC), corn earlage (EAR), and corn silage (SIL). Three data sets were assembled with a total of 207 samples. For the SIL, blended ruminal fluid from 3 donor animals fed a 30% starch diet was used to measure IVSD at 8h in triplicate and IVGP from 0 to 48 or 72h in duplicate using an Ankom RF system (Macedon, NY) over 2 in vitro runs. Three- and 4-carbohydrate pool models were statistically fitted to the gas production data using exponential growth functions and nonlinear mixed models. For the HMC and EAR, IVGP was as described for SIL and IVSD was determined by near-infrared spectroscopy (NIR). The ranges for starch digestibility estimates were similar for IVGP, IVSD, and NIR. The IVGP variation between duplicates within a run was substantially less than that of IVSD among triplicates, while IVGP had more variation among runs than IVSD. One advantage of IVGP is it is a direct measure of carbohydrate fermentation in contrast to IVSD which measures starch disappearance by difference. A second advantage is IVGP is measured continuously with 240 time points in an 8h incubation vs. 2 time points in IVSD, providing precise estimates of fermentation lag times vs. assumed lag times in IVSD. More precise estimates of lag times contribute to more precise estimates of degradation rates. Overall, with coefficients of variation < 3% in starch digestibility, IVGP provides more repeatable estimates of starch digestibility in ensiled corn feed samples than IVSD.

Key Words: starch digestibility, corn silage, in vitro gas production

Table 1 (Abstr. 1776W). Comparison of 8-h starch digestibility in ensiled corn ingredients determined by IVGP vs. IVSD

Data set	2017 SIL	2021 SIL	2020 HMC+EAR
Number of independent samples	42	18	137
IVSD method	In vitro	In vitro	NIR
IVSD mean	54.2	60.9	66.7
IVSD among-run SD	11.45	8.92	–
IVSD within-run SD	4.61	3.28	–
IVGP mean	51.2	53.9	54.5
IVGP among-run SD	13.19	13.1	16.47
IVGP within-run SD	3.79	1.14	1.77

1777W Effect of chemical and enzymatic treatment of corn silage on lactation performance in dairy cattle. P. A. LaPierre*[1], D. Stucker[2], D. M. Barbano[1], and M. E. Van Amburgh[1], *[1]Cornell University, Ithaca, NY, [2]Impetus, LLC, Clay, NY.*

The objective of this study was to identify potential differences in productive performance in dairy cattle fed a chemically and enzymatically treated corn silage compared with a non-treated silage. Forty (n = 40) non-cannulated Holstein cattle (DIM = 36 ± 10 d) were randomly enrolled to one of 2 diets, which either included corn silage that was sequentially treated with 50 g of sodium hydroxide per kilogram of forage DM and an enzymatic treatment at a rate of 1.67 mL/kg of forage DM or the same silage treated with water only to maintain similar DM content. Cattle were adapted to their respective diets for 21 d until sampled longitudinally for 9 weeks. Fluid milk and component yield, DMI, daily rumination, BW, body condition score (BCS), and feed chemistry were measured weekly. Diets were formulated to include 63.0% forage DM, 30.5% aNDFom, and targeted 95% and 103% of ME and MP requirements, respectively, using a 730 kg cow producing 50 kg of 4.20 and 3.15% fat and protein percentages, respectively. Data were analyzed using a mixed model, where dietary treatment and time were considered fixed effects and cow within treatment was made a random effect. Repeated measures of time were stated with cow within treatment defined as the subject. Cattle fed the treated corn silage consumed more DMI than cattle fed the control silage (29.7 vs. 28.4 kg DMI; $P < 0.01$) but daily rumination was lower (565 vs. 573 min; $P = 0.01$). Milk yield (52.2 kg; $P = 0.96$), milk fat yield (2.10 vs. 2.04 kg; $P = 0.34$), and milk protein yield (1.57 vs. 1.54 kg; $P = 0.42$) were similar for both groups of cattle. Relative de novo milk fatty acid (FA) production was not different between treated and control fed cattle (28.4 vs. 28.3 g/100g FA; $P = 0.63$); however, both mixed (40.1 vs. 39.7 g/100g FA; $P = 0.14$) and preformed (31.3 vs. 32.0 g/100g FA; $P = 0.15$) FA production approached statistical tendencies and were inversely related with each other. Cattle fed the treated diet tended to gain more weight over the study than control cattle (750 kg vs. 740 kg; $P = 0.08$). Results from this study suggest that chemical and enzymatic treatment of corn silage improves DMI, resulting in productive responses relating more toward weight gain than lactation differences.

Key Words: forage, enzyme, digestibility

1778W Effects of zinc and manganese source on 48-hour in vitro fermentation. C. Peterson*[1], G. Boerboom[2], M. McCarthy[1], J. Heldt[1], K. Griswold[1], and J. Johnston[3], *[1]Selko USA, Indianapolis, IN, [2]Selko Feed Additives, Amersfoort, Utrecht, the Netherlands, [3]Fermentrics Technologies Inc., Arnprior, ON, Canada.*

Two trials were completed to determine the effect of zinc (Zn) and manganese (Mn) source on 48 h in vitro rumen fermentation characteristics. Targeted supplemental trace minerals (TM) were 750 mg/d and 500 mg/d for Zn and Mn, respectively, for a dairy cow consuming 25 kg DM with 120 L rumen volume. Targets were scaled to fit a 250 mL fermentation vessel containing a mix of KSU buffer (80%) and rumen fluid (20%) with 400 mg of ground dairy TMR (6 mm) inside 5 × 10 cm bags. Trial 1 evaluated 7 Mn treatments (6 reps per treatment) simulating 20 ppm supplemental Mn: Control (CM, no TM), Mn oxide (OM, 2.60 mg), Mn sulfate (SM, 4.96 mg), IntelliBond M (IBM, 3.55 mg), Vistore Mn (VM, 3.55 mg), Availa Mn (AM, 19.53 mg), and Mintrex Mn (MM, 12.02 mg). Trial 2 evaluated 7 Zn treatments (6 reps per treatment) simulating 30 ppm supplemental Zn: Control (CZ, no TM), Zn oxide (OZ, 2.17 mg), Zn sulfate (SZ, 4.40 mg), IntelliBond Z (IBZ, 2.84 mg), Vistore Zn (VZ, 2.84 mg), Availa Zn (AZ, 13.02 mg), and Mintrex Zn (MZ, 9.77 mg). Vessels were incubated for 48-h in a 39.5°C insulated water bath. Data were analyzed as a complete randomized design with PROC MIXED in SAS using 6 replications/treatment (SAS Institute Inc., Cary, NC). Significance equaled $P \leq 0.05$. For Trial 1, IBM resulted in significantly greater ($P \leq 0.03$) apparent microbial biomass production (aMBP) than MM, SM, and AM (192.6 vs. 174.9, 172.3, 170.8), tended to be greater ($P = 0.08$) than OM (178.8) and was not different ($P \geq 0.25$) from CM and VM (193.3 and 183.8). For Trial 2, IBZ resulted in significantly greater ($P \leq 0.04$) aMBP than CZ, MZ, VZ, and SZ (212.0 vs. 200.6, 197.6, 190.2, 202.3), tended to be greater ($P = 0.07$) than AZ (203.1) and was not different ($P = 0.14$) from OZ (205.1). The IBZ treatment also resulted in significantly greater ($P \leq 0.02$) apparent organic matter disappearance (aOMD) than all other treatments (58.7% IB, 55.6% CZ, 54.6% OZ, 55.7% SZ, 55.4% VZ, 54.7% AZ, and 54.0% MZ). Overall, IntelliBond is an improved source of trace minerals that increased in vitro fermentation compared with other trace mineral sources.

Key Words: hydroxy trace minerals, apparent microbial biomass, fermentation

1779W Effects of different slow-release urea compounds on ruminal fermentation and nutrient utilization in a dual-flow continuous culture system.

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Slow-release urea (SRU) compounds are feed additives that gradually release ammonia-N (NH_3-N) in the rumen, which could improve N uptake by ruminal microorganisms and improve overall N utilization. The objective of this study was to evaluate the effects of different sources of SRU on ruminal fermentation, nutrient degradation, and N utilization in a dual-flow continuous culture system. Eight fermentors were used in a replicated 4 × 4 Latin square design with 4 treatments and 4 experimental periods. Treatments were defined by the source of non-protein N, as follows: 1) Control with 0.62% (diet DM) as regular urea (CON); 2) 0.51% as SRU compound 1, (SRU1); 3) 0.51% as SRU compound 2, (SRU2); 4) 0.51% as SRU compound 3, (SRU3). Diets were formulated to be isonitrogenous across treatments and fed 2x/d. The last 3 d of each 10-d period were used for sample collection. Samples for pH, lactate, volatile fatty acid (VFA), and NH_3-N kinetics were collected from the fermentors at 0, 1, 2, 4, 6, and 8 h after morning feeding. Daily composite samples for lactate, VFA, NH_3-N, and nutrient degradability were collected at 3, 6, 9, and 24 h after morning feeding. Data were analyzed using the MIXED procedure of SAS. Model included fixed effect of trt. and random effects of per., sq., ferm.(sq.) and day. There were no treatment effects on pH or NH_3-N kinetics for samples collected from the fermentors, nor was there an interaction between treatment and time. For lactate kinetics, there was an interaction between treatment and time ($P = 0.03$). For VFA kinetics, there were treatment effects ($P < 0.05$) on acet:prop ratio (A:P), acetate, valerate, and BCVFA proportion. Compared with CON, SRU1 had lower A:P ratio and acetate proportion, which could represent a favorable fermentation partner compared with CON. SRU1 had lower BCVFA proportion than SRU2 and SRU3, which indicates less protein degradation. In conclusion, there was no effect of the inclusion of SRU compounds on NH_3-N or pH. There was an interaction on lactate kinetics and time. Preliminary results show little differences among SRU compounds.

Key Words: slow-release urea, volatile fatty acids, N efficiency

1780W Microbiota changes in calves with *Cryptosporidium parvum*-associated diarrhea.

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The objective of this study was to describe and compare the fecal microbiota of healthy calves and calves with *Cryptosporidium parvum* associated diarrhea and to investigate the fecal microbiota of calves positive for *C. parvum* with and without diarrhea. Fecal samples from dairy calves housed in a farm experiencing an outbreak of *C. parvum* were collected. A total of 26 neonate calves aged 10 to 24 d old were enrolled in the study. In total, 12 calves remained healthy through the study, with 5 calves testing positive for *C. parvum* (H-Cp) and 7 healthy calves testing negative for *C. parvum* (Healthy). A total of 14 calves had *C. parvum*-associated diarrhea (CPAD). Diarrheic calves were defined as calves with loose or watery feces. *Cryptosporidium parvum* infection was determined using ELISA. Microbiota composition was characterized by 16s RNA gene amplicon sequencing. Alpha diversity was assessed using the Chao1 (Richness) and Inverse Simpson (Diversity) indices, whereas β-diversity was assessed using Bray-Curtis distances. Linear discriminant analysis effect size (LEfSe) analysis was used to determine enriched taxa in each group. Richness and diversity were not different between healthy calves, H-Cp and CPAD ($P > 0.05$ for all comparisons). Similarly, Bray-Curtis distances showed no difference in bacterial structure among the 3 groups (AMOVA >0.05, for all comparisons). However, in CPAD calves had an enrichment of *Fusobacteria* spp. and *Akkermansia* spp. (LDA >3, $P < 0.05$) while in H-Cp calves there was an enrichment of *Flavinofractor* spp. (LDA >3, $P < 0.05$). The increase in *Fusobacteria* identified in calves with *Cryptosporidium* parvum associated diarrhea is similar to that reported in a previous study suggesting that blooming of *Fusobacteria* spp. may play a key role in the onset on of *C. parvum* infection. Alternatively, it is also possible that intestinal inflammation and dysbiosis caused by *C. parvum* infection favors the proliferation of *Fusobacterium*. Further investigation into the interaction between these microorganisms is warranted.

1781W Microbial diversity of the gastrointestinal tract of healthy and diarrheic calves before, during and at recovery from diarrhea.

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The objective of this study was to describe the α diversity of the gastrointestinal microbiota of healthy and diarrheic calves before, during, and at recovery from diarrhea. Fecal samples from dairy calves on a farm experiencing an outbreak of diarrhea were collected longitudinally on d 5, 10, 14 and 21 after birth. A total of 24 Holstein calves were included in the study. The calves were divided into 8 healthy, 8 developing diarrhea on d 10, and 8 developing diarrhea on d 14. Diarrheic calves were defined as calves with loose or watery feces. Microbiota composition was characterized by amplifying the V4 region of the 16s rRNA gene, and sequence analysis was performed using Illumina MiSeq. Alpha-diversity indices Chao-1 (richness), Shannon Evenness (evenness), and Inverse Simpsons (diversity) were calculated using the software Mothur (1.48.0). Data normality was assessed using normal probability Q-Q plots, and data were analyzed accordingly. Statistical analysis was conducted using the software JMP 17 (SAS). The differences in α-diversity indices within and between groups were assessed using a 2-way ANOVA, and Tukey's honestly significant difference test was used to adjust for multiple comparisons. Diversity, richness, and evenness increased over time in healthy and diarrheic calves. These indices were not different between groups at any time before diarrhea; however, on the day of diarrhea, a lower diversity (Median: 8.62; Range: 2.11 to 20.43) and evenness (2.67; 1.01 to 3.41) was observed in diarrheic calves compared with healthy animals (diversity, 7.80; 3.62 to 16.86; evenness: 2.48; 1.85 to 3.14) ($P < 0.001$). Recovery from diarrhea coincided with a normalization of the evenness and diversity in calves that experienced diarrhea as there were no differences in α-diversity indices between groups on d 21 ($P > 0.05$). These results indicate that intestinal inflammation causes dysbiosis and that re-establishing the "normal" bacterial communities may play an essential role in resolving diarrhea. Further investigation into therapies aiming to modulate microbial diversity in diarrheic calves, including probiotics or fecal matter transplantation, is warranted.

1782W Investigating the bacterial profiles of cows differing in rumination and eating times. A. Castaneda*[1,2], N. Indugu[1], K. Narayan[1], S. Rassler[1], J. Bender[1], T. Webb[1], B. Vecchiarelli[1], L. Baker[1], and D. Pitta[1], *[1]School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA, [2]Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

Methane emissions pose energetic losses to the host and environmental costs. To develop a mitigation strategy, it is vital to understand the differences between animals differing in rumination and eating times. Multiple studies have reported that CH_4 emissions between "high rumination" (HR) and "low rumination" (LR) cows are different; however, it is unknown whether the bacterial profiles of these groups vary. Thus, this study aimed to investigate the bacterial profiles of LR and HR cows. We used the Dynamic Time Warping algorithm to select cows differing in rumination and eating times. The algorithm was developed using hourly data collected from dairy cows. We calculated the distances between cows differing in rumination and eating times and depicted them in a plot using principal components. From 50 cows in early lactation (30–100 DIM), 20 were selected to represent the clusters LR and HR. The cows were enrolled in a 5-week trial: a 2-week training phase, a 2-week adaptation phase, and one week for sampling. The CH_4 emissions were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD). Rumen, bolus, and fecal samples were collected from each cow. Fecal samples were taken to compare their microbial profiles with those of rumen and bolus samples and obtain a representative sample of the microbial profile of the cows' gastrointestinal tract. Genomic DNA was extracted, the 16S rRNA gene of bacteria was amplified using barcoded primers and sequenced using the Illumina MiSeq platform to analyze bacterial diversity. Using a GLM model, the daily average CH_4 mass flow was higher ($P < 0.05$) in the LR group (430 ± 6.27) than in the HR group (404 ± 6.04). When the bacterial communities were compared, a difference ($P < 0.05$) among sample types was observed, indicating that the amplicon sequence variants of bacteria differ. The Unweighted UniFrac analysis revealed that the bacterial populations vary ($P < 0.05$) by group and sample type. Specifically, using an LMER model, the bacterial populations in fecal and bolus samples differed in the HR and LR groups. In summary, the bacterial profiles of the HR and LR groups are different. However, future work should explore how the bacterial profiles of HR and LR groups vary over a 24-h cycle.

Key Words: rumination, eating, rumen microbe

1783W Evaluation of cobalt from a glycinate premix on rumen function and vitamin B_{12} synthesis. E. Ramos-Morales[1], A. Belanche[1,2], P. Romero[1], E. Jimenez[1], I. Martin-Garcia[1], H. Khelil-Arfa[2], M. V. Zoom[2], A. Blanchard[2], D. R. Yanez-Ruiz[1], J. W. Hickman[3], and G. Acetoze*[3], *[1]CSIC, Granada, Spain, [2]Universidad Zaragoza, Zaragoza, Spain, [3]ADM International Sàrl, Rolle, Switzerland, [4]ADM Animal Nutrition Technology Center, Decatur, IL.*

Vitamin B_{12} or cobalamin is synthesized by prokaryotes in the rumen, and this has implications in human nutrition since humans rely mainly on the consumption of ruminant derived products for vitamin B_{12} acquisition. In addition, vitamin B_{12} is an important growth factor for some ruminal microorganisms, and it is utilized by others in pathways that produce propionate. Cobalt, as a trace element in the diet, is essential for the formation of vitamin B_{12} in the rumen. The objective of this experiment was to evaluate the effect of a premix based on metal amino acid complexes including cobalt (AAC) on rumen fermentation, feed degradability and vitamin B_{12} synthesis, using an in vitro continuous culture system. Treatments consisted of a 50:50 hay: concentrate diet alone (control) or with the AAC premix providing an equivalent of 12 mg of Co per animal and day. The trial consisted of 2 incubation periods (2 replicates per period) of 18 d each, with the first 5 d for adaptation of the rumen microorganisms to the in vitro conditions before the administration of the treatments. On d 15 and 16 of the incubation, samples of the fermentors were collected for VFA, ammonia, lactate, Cobalt and vitamin B_{12} determination. On d 17 and 18, the content of the effluent of each fermenter was collected to calculate DM disappearance and the residue was then analyzed for organic matter (OM), nitrogen (N) and neutral-detergent fiber (NDF) to determine nutrient disappearance. The effect of applying the different treatment was analyzed using one-way ANOVA and Period as blocking factor. For those parameters analyzed in different days a repeated measures analysis was used. The addition of AAC premix did not have any significant effect on the fermentation pattern or the disappearance of DM, OM and N ($P > 0.05$). A significant increase in the concentration of Vit B_{12} in the effluent ($P < 0.001$) was observed in vessels fed the ACC premix as compared with those of the control treatment, which resulted in a 14-fold increase of the daily flow of Vit B_{12}. Results showed that the cobalt from AAC was efficiently used in the synthesis of vitamin B_{12} by rumen microorganisms. Further studies with a range of doses and diets representing different ruminant production systems are required to optimize a recommended dose of AAC premix.

Key Words: cobalt, rumen, vitamin B_{12}

1784W Effects of thiamine supplementation on rumen fermentation and odd- and branched-chain fatty acids content in vitro. X. Guo[1,2], T. Zhan[1], D. Bu[1], and L. Ma*[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Life Science, Ningxia University, Yinchuan, China.*

Thiamine promotes branched-chain α-keto acid dehydrogenase in odd- and branched-chain fatty acids (OBCFA) synthesis pathways. This study aimed to investigate the effects of thiamine supplementation in total mixed ration on rumen fermentation characteristics and the rumen OBCFA content in vitro. Treatments were set as dose response with 0, 15, 30, 60, 120 and 240 mg/kg DM thiamine. Each incubated with 75 mL of buffered rumen fluid and 0.5 g of fermentation substrate for 24h at 39°C. Five replicate parallel fermentation flasks were prepared by each level of supplemented thiamine, and incubation was repeated twice. Filtered fluid with Nylon bags were collected from each fermentation flask. The contents of volatile fatty acids (VFA) and OBCFA were determined using an Agilent 6890N GC equipped with a flame ionization detector. All data were analyzed using the linear mixed model of nlme Package in R (version 4.2.1). As the dose of thiamine increased, the concentrations of total volatile acid, acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid and isovaleric acid changed quadratically with lowest at 30 and 60 mg/kg dose of thiamine. Compared with the control group, the concentration of total volatile acid at 30 and 60 mg/kg thiamine decreased by 12.31% and 3.96%, respectively. The OBCFA content changed quadratically with highest at 60 mg/kg dose of thiamine ($P < 0.01$). Compared with the control group, the contents of C13:0, C14:0, C15:0, C16:0, iso-C14:0, iso-C15:0, iso-C17:0, anteiso-C13:0, anteiso-C15:0, iso, anteiso and BCFA at 60 mg/kg thiamine significantly increased ($P < 0.05$). The contents of total iso, anteiso and BCFA increased by 31.48%, 38.02% and 33.66%, respectively, compared with the control. In summary, the results of this study indicated that 60 mg/kg thiamine had beneficial effects on increasing OBCFA content than other groups.

Key Words: thiamine, odd- and branched-chain fatty acids, rumen

1785W Effects of dietary betaine supplementation and partial rumen content transplantation on rumen volatile fatty acid profile in Holstein dairy cows.

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Heat stress alters the rumen fermentation in dairy cows, which may be modified by dietary betaine supplementation. Twelve rumen-cannulated multiparous Holstein cows (39 ± 6.4 kg milk/d; 82 ± 27 d in milk [DIM]) were used in a split-plot design testing the effects of betaine and partial ruminal content transplantation (PRCT) on cow performance during heat stress. The main plot was the level of dietary betaine supplementation (CON: unsupplemented; or BET: 100 g/d intra-ruminal betaine hydrochloride 95%; AB Vista, Canada). Within each plot, cows were randomly assigned to 1) heat stress (HS), 2) thermoneutral pair-feeding (TNPF), or 3) HS with PRCT (HSPRCT; 25% replacement of rumen contents from 4 donor cows fed ad libitum under thermoneutrality; d 8–14) in a replicated 3 × 3 Latin square design with 14-d periods. A mock transplantation was performed in HS and TNPF cows as a handling control. Rumen samples were collected pre- (h 0, relative to feeding) and post-prandial (h 4, relative to feeding) on d 0, 7 and 13. The statistical model included the random effects of cow and period, and the fixed effects of plot, treatment, day, and their interactions. No plot effect or interaction was observed with the supplementation of betaine. Pre- and post-prandial molar total VFA concentration were decreased by 19% and 21% in TNPF relative to HS ($P < 0.01$), respectively, while no effect of transplantation was detected. Postprandial molar proportions of lactate and acetate increased by 23% and 7%, respectively on d 7 and 13 ($P < 0.05$), while propionate decreased by 9% ($P < 0.05$) in TNPF cows relative to HS. Similarly, postprandial molar ratios of acetic to propionate was increased in TNPF by 14% relative to HS ($P = 0.01$). On d 13 molar proportion of preprandial formic acid was decreased 40% ($P < 0.05$) and postprandial isovaleric acid was increased by 28% ($P < 0.05$) in HSPRCT compared with HS. These results suggest that dietary betaine supplementation and PRCT during heat stress of dairy cows have a limited ability to modify rumen VFA profile. Supported by FFAR.

Key Words: betaine, heat stress, ruminal microbiota

1807W *In-situ* ruminal dry matter degradability of three hydroponic fodders and corn silage in lactating Holstein cows.

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Climate change may have an adverse effect on feed production. Hydroponic fodders produced in controlled environments are alternative feeds that may be used for dairy cattle. The objective of this study was to evaluate in-situ dry matter ruminal degradability of hydroponic fodders and corn silage. Two rumen-canulated lactating Holstein cows were used in a complete randomized block design with four treatments and 9 times (0, 2, 4, 8, 12, 24, 48, 72, and 240 h). Treatments were corn silage (CS), hydroponic sprouts of barley, wheat, and triticale. Quadruplicate samples were placed in Dacron bags (10 x 20 cm, 50 μm porosity) and incubated in the rumen. After incubation, samples were washed and dried in an air-forced oven set at 60°C for 48 h and DM recovery was weighed. Ingredients' nutrient compositions were: CS (32.3% NDF, 8.4% CP, and 41.6% starch, 1.3% WSC; % of DM), barley (34.3% NDF, 17.1% CP, and 4.2% starch, 32.9% WSC; % of DM), wheat (23.7% NDF, 23.3% CP, and 8.5% starch, 32.7% WSC; % of DM), triticale (20.0% NDF, 16.2% CP, and 21.7% starch, 33.2% WSC; % of DM). Model included fixed effects of treatment and random effects of cow and replicate(cow). Data was analyzed using PROC MIXED of SAS. Degradation fractions were calculated based on DM disappearance. There were treatment effects ($P < 0.01$) on fraction A (degradable), fraction B (potentially degradable), fraction C (undegradable), effective ruminal degradability (ERD), and rate of degradation (k_d: %/h). Fraction A was greater for triticale (74.1%) compared to wheat (70.2%), CS (63.1%), and barley (55.0%). Fraction B was greater for barley (28.5%) compared to CS (23.2%), wheat (19.1%), and triticale (13.9%). Fraction C was lower for wheat (10.6%) compared to triticale (12.0%), CS (13.7%), and barley (16.4%). The ERD was greater for wheat (90.8%) compared to triticale (89.0%), CS (88.0%), and barley (85.6%). The k_d for barley (2.4%) was lower, compared to triticale (4.9%). The k_d for CS (1.8%) was lower, compared to triticale (4.9%). Overall, triticale had the greatest fraction A, barley had the greatest fraction B and wheat had the lowest fraction C and the greatest ERD and k_d.

Key Words: alternative feeds, fodder, fractional degradability

Ruminant Nutrition: Protein and Amino Acids 3

1786W Effects of feeding controlled-energy and high-energy diets with rumen-protected lysine and methionine prepartum on colostrum quality of Holstein cows. E. O'Meara*[1], D. del Olmo[2], J. Aguado[2], F. Valdez[2], J. Drackley[1], and F. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Kemin Industries Inc., Des Moines, IA.*

The aim of this study was to determine cows' colostrum quality when rumen-protected methionine (RPM) and lysine (RPL) were fed in the same amino acid to metabolizable energy (ME) ratio in prepartum diets (1.21g of digestible methionine/Mcal of dietary ME and 3.21g of lysine digestible /Mcal of dietary ME) with different net energy of lactation (NE_L) concentrations. Sixty-two multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, and body condition score (BCS) during the far-off dry period were assigned to 1 of 3 dietary treatments. Prepartum (–21 d to expected calving), animals were fed a controlled-energy diet (straw-based diet, 1.45 NE_L, Mcal/kg of DM) with RPL (Kemin Industries Inc., Des Moines, IA) and RPM (Kemin Industries Inc., Des Moines, IA) [CEAA; 0.15% RPL and 0.09% RPM of dietary dry matter intake (DMI)], controlled-energy diet without RPL and RPM (control; CENAA), or high-energy diet (corn silage based diet, 1.71 NE_L, Mcal/kg of DM) with RPL and RPM (HEAA; RPL 0.22% and RPM 0.12% of dietary DMI). Colostrum was collected at the first milking after calving and was weighed and tested for IgG (Colostrometer, Biogenics, Florence, OR) by correlating density to IgG concentration. Colostrum samples were chemically analyzed for content of fat (ether extraction) and true protein (Kjeldahl). Statistical analyses were performed using the MIXED procedure of SAS. A contrast CONT1 (average of CEAA and HEAA vs. CENAA) along with the treatment effects were compared. Tukey's adjustment was used for comparison of means. There was no treatment effect ($P > 0.23$) for colostrum weight (CENAA = 5.9 ± 1.1; CEAA = 6.57 ± 1.0; and HEAA = 7.98 ± 0.1 kg); IgG (CENAA = 69.0 ± 6.7; CEAA = 74.7 ± 5.1; and HEAA = 71.14 ± 5.3 mg/dL); fat percentage (CENAA = 3.5 ± 0.4; CEAA = 2.6 ± 0.4; and HEAA = 3.25 ± 0.4%); and crude protein percentage (CENAA = 15.98 ± 0.8; CEAA = 15.46 ± 0.8; and HEAA = 14.38 ± 0.8%). In conclusion, providing RPL and RPM prepartum did not improve or hinder colostrum quality.

Key Words: colostrum, amino acids, immunoglobulin G

1787W Effects of feeding controlled-energy and high-energy diets with rumen-protected lysine and methionine prepartum on muscle and adipose tissue depth of Holstein cows. E. O'Meara*[1], D. del Olmo[2], J. Aguado[2], F. Valdez[2], J. Drackley[1], and F. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Kemin Industries Inc., Des Moines, IA.*

The aim of this study was to determine cows' muscle and adipose tissue depth when rumen-protected methionine (RPM) and lysine (RPL) were fed in the same amino acid to metabolizable energy (ME) ratio in prepartum diets (1.21g of digestible methionine/Mcal of dietary ME and 3.21g of lysine digestible /Mcal of dietary ME) with different net energy of lactation (NE_L) concentrations. Sixty-two multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, and body condition score (BCS) during the far-off dry period were assigned to 1 of 3 dietary treatments. Prepartum (–21 d to expected calving), animals were fed a controlled-energy diet (straw-based diet, 1.45 NE_L, Mcal/kg of DM) with RPL and RPM (Kemin Industries Inc., Des Moines, IA) [CEAA; 0.15% RPL and 0.09% RPM of dietary dry matter intake (DMI)], controlled-energy diet without RPL and RPM (control; CENAA), or high-energy diet (corn silage based diet, 1.71 NE_L, Mcal/kg of DM) with RPL and RPM (HEAA; RPL 0.22% and RPM 0.12% of dietary DMI). Tissue depth was determined by ultrasound (Ibex Pro, E.I. Medical Imaging). Measurements were taken on days −28 (as a covariate), −14, −7, and at calving. Statistical analyses were performed using the MIXED procedure of SAS. A contrast (CONT1; average of CEAA and HEAA vs. CENAA) along with the treatment effects were compared. Tukey's adjustment was used for comparison of means. There was no treatment effects for adipose tissue ($P > 0.35$; CENAA = 0.64; CEAA = 0.73; and HEAA = 0.71 cm; SEM = 0.73) and muscle tissue depth ($P > 0.52$; CENAA = 5.3; CEAA = 5.5; and HEAA = 5.5 cm; SEM = 0.18). Cows in CENAA had greater (−0.09 cm; CONT1; $P = 0.05$) adipose tissue depth change than cows in CEAA (0.02 cm) and HEAA (0.04 cm; SEM = 0.05). Cows in CENAA tended to have greater (−0.63 cm; CONT1; $P = 0.07$) muscle tissue depth change (−28 to 0 d) than cows in CEAA (0.05 cm) and HEAA (0.06 cm; SEM = 0.3). In conclusion, feeding RPM and RPL prepartum appeared to reduce muscle and adipose tissue loss prepartum independently from dietary energy content.

Key Words: muscle depth, back fat, amino acids

1788W Effect of rumen-protected methionine supplementation in a low starch diet with or without supplemental sugar on the productive performance of dairy cows. J. H. Carneiro*[1], L. S. Nogueira[1], F. Lopes[2], J. S. Osorio[3], and R. Almeida[1], *[1]Universidade Federal do Paraná, Curitiba, PR, Brazil, [2]Adisseo South America, São Paulo, SP, Brazil, [3]Virginia Tech, Blacksburg, VA.*

The objective of this study was to evaluate the effect of rumen-protected methionine (RPM) supplementation in a low-starch diet, with or without an extra source of sugar, on the productive performance of mid-lactation cows, fed isonitrogenous diets based on corn and grass silage, dry ground corn and soybean meal. Eighty multiparous Holstein cows with 2.4 ± 0.6 lactations, 36.5 ± 4.5 kg/d, and 160 ± 68 DIM, were randomly distributed in a 2 × 2 factorial arrangement. Cows were blocked by parity, milk yield, and DIM, and allocated in 4 treatments: CON (control diet with 23% starch (%DM) without sugar supplementation or RPM); MET (control diet with RPM supplementation; 14 g of Smartamine M, Adisseo); SUG (control diet with 1.7% DM of crystal sugar); and MET+SUG (the inclusion of both treatments). The total experimental period was 28 d, with the first 3 d used for data collection as covariates. A TMR was provided, and treatments were top-dressed once a day. Milk yield and composition were recorded weekly and in the last 9 milkings. Data were analyzed using the MIXED procedure of SAS containing the fixed effects of MET, SUG, parity, time, and their interactions, and the random effects of block and cow. Treatments were compared with Tukey multiple comparison adjustment for the difference of LSM when a $P < 0.05$ was detected. The inclusion of both sugar treatments (SUG and MET+SUG) increased milk yield and the inclusion of both RPM treatments (MET and MET+SUG) increased milk protein content (Table 1).

Key Words: amino acid, energy, sugar

1789W Effects of iso-acids and dietary protein concentration on milk production, feed intake, and blood metabolites in dairy cows. M. Suazo*[1], M. T. Socha[2], D. H. Kleinschmit[2], and I. J. Salfer[1], *[1]Department of Animal Science, University of Minnesota, Saint Paul, MN, [2]Zinpro Corporation, Eden Prairie, MN.*

Table 1 (Abstr. 1788W). Effects of methionine and sugar supplementation on productive performance

Variable	CON	MET	SUG	MET+SUG	SEM	MET	SUG	MET×SUG
Met (%MP)	2.10	2.40	2.10	2.40				
Lys:Met	3.06	2.68	3.06	2.70				
Met:ME	0.91	1.04	0.91	1.04				
Milk yield, kg/d	34.20^{b}	32.70^{c}	35.40^{a}	35.10^{a}	0.28	0.33	<0.01	0.03
ECM, kg/d	35.00	33.90	36.20	36.00	0.29	0.69	<0.01	0.06
Fat, %	3.55	3.89	3.76	3.77	0.04	0.03	0.03	1.00
Total protein, %	3.35^{b}	3.46^{a}	3.39^{b}	3.43^{a}	0.02	<0.01	0.68	0.02
Lactose, %	4.56	4.51	4.65	4.51	0.02	<0.01	0.22	0.49
Fat, kg/d	1.23	1.25	1.32	1.31	0.01	0.57	0.08	0.34
Total protein, kg/d	1.16	1.12	1.19	1.19	0.01	0.83	0.01	0.06
Lactose, kg/d	1.56	1.48	1.65	1.59	0.02	0.03	<0.01	0.15
MUN, mg/dL	22.55	23.56	23.99	22.09	0.29	0.55	0.48	0.78

Protein is the most expensive nutrient in dairy cattle diets and can cause environmental ammonia emissions when overfed. Due to these factors, there is an increased focus on feeding low CP diets and improving rumen nitrogen use efficiency. Iso-acids (IA) may improve nitrogen use efficiency by providing carbon skeletons for rumen cellulolytic bacteria. The objective of this study was to examine the effects of feeding IA in low and high CP diets on milk yield, DMI and circulating metabolites. Twelve cannulated late-lactation multiparous Holstein cows (210 ± 40.3; DIM ± SD) were enrolled in a 4x4 Latin-square design (n = 12 cows/treatment). Treatments included a diet formulated for 15% CP diet without IA (LO-CON), or with IA (LO-IA; 40 g IsoFerm, Zinpro Corp., Eden Prairie, MN), a diet formulated for 16.5% CP diet without IA (MOD-CON), or with IA (MOD-IA). Periods consisted of 4 wk of adaptation and 1 wk of sampling. Milk and DMI were determined daily through the experiment. Milk was analyzed for fat and protein concentration the final 3 d of each period. Plasma was sampled on the final 3 d of each period and analyzed for BUN and BHB concentration. Data were analyzed as a 2x2 factorial arrangement using a mixed-effects model and multiple comparison test in SAS 9.4 with main effects of CP and IA inclusion and random effects of period and day nested within period. Milk and ECM yields were affected by the interaction of CP x IA ($P < 0.05$, Table 1). Milk fat concentration was not affected by treatment ($P > 0.20$; SE = 0.12). Milk protein concentration was increased in MOD vs LO CP ($P = 0.007$; SE = 0.08). Dry matter intake was not affected by IA ($P = 0.15$; SE = 1.98). The BHB tended to be affected by CP x IA interaction ($P = 0.07$; SE = 0.06). The BUN was lower in LO compared with HI ($P < 0.01$; SE = 1.13). Overall, IA effects on milk production were dependent on CP of the diet.

Key Words: iso-acids, protein, dairy cows

1790W Development and validation of an efficient method for hydrolysis and analysis of amino acids in ruminant feeds, tissue, and milk using isotope dilution ratio Z-HILIC coupled with LC-MS/MS triple quadrupole. A. F. Ortega*[1], H. Zhao[2], and M. E. Van Amburgh[1], [1]*Cornell University, Ithaca, NY,* [2]*Agilent Technologies Inc., Wilmington, DE.*

Current analytical methods for amino acid (AA) analysis are time-consuming and expensive. This study aimed to develop a method for AA analysis that is faster, more efficient, rugged, and accessible. Four matrixes were selected for this study: one milk sample, one head/hide/feet/tail tissue sample, a corn silage (CS), and a NIST soy flour standard reference. Three methods were used to hydrolyze 18 AA for 24 h 1) Acid hydrolysis (0.1% phenol + 6 *M* HCl) for 15 AA, since Asn and Gln are recovered as Asp and Glu, respectively; 2) Performic acid oxidation before HCl hydrolysis for analysis of the 2 sulfur AA; 3) Alkaline hydrolysis to analyze Trp. Separation of underivatized AA was performed using a Z-HILIC column in an 18 min run and analyzed with a LC/TQ system using isotope dilution ratio for all AA except sulfur AA. LC/TQ was in positive and negative electrospray ionization for identification and quantitation. Z-HILIC allows for better determination of smaller polar compounds and coupling with LC/TQ improves selectivity. Linearity was achieved for all methods with all linear regression coefficients being above 0.99. The limit of detection and quantification were below measured values for all AA. Intraday and inter-day precision, expressed as relative standard deviation (RSD, %), were all below 15%. Accuracy, expressed as recovery %, was determined in blank samples and with matrixes of interest and was between 75% and 118.0%, except for Arg in the CS matrix where recovery was 65%. The AA values for milk and tissue were compared with values used by the CNCPS and NASEM, and the soy flour values were compared with the NIST data. Finally, the 3 methods were applied to analyze the AA profile of 8 additional feeds

Table 1 (Abstr. 1789W). Effect of IA and protein level on milk production, milk composition and blood metabolites

Variable	LO-CON	LO-IA	MOD-CON	MOD-IA	SEM	*P*-value
Milk yield, kg	35.9^{b}	34.7^{b}	35.4^{ab}	36.1^{a}	2.35	0.05
ECM, kg	37.4^{a}	35.6^{b}	37.1^{a}	37.6^{a}	2.42	0.05
Milk fat, %	3.69	3.67	3.74	3.73	0.12	0.33
Milk protein, %	3.14^{b}	3.17^{ab}	3.23^{a}	3.19^{a}	0.083	0.007
BHB, mmol/L	0.53^{xy}	0.57^{x}	0.61^{x}	0.50^{y}	0.058	0.07
BUN, mmol/L	9.14^{b}	8.79^{b}	11.34^{a}	10.87^{a}	1.13	0.0001

^{a,b}Variables within a row with different superscripts are significantly different ($P \leq 0.05$).

^{x,y}Variables within a row with different superscripts are significantly different ($P \leq 0.05$).

with an N content of 0.9% to 14.5%. The analysis of these feeds and the CS values were compared with values in the CNCPS feed library. Many of the measured AA were different from library values regardless of matrix ($P < 0.05$) particularly the polar AA Arg, His, Lys. This study demonstrates a promising approach to routine AA analysis in ruminant feeds, milk, and tissue.

Key Words: amino acids, LCMS, feed chemistry

1791W Determination of bovine plasma amino acids and metabolites using Z-HILIC coupled with LC-MS/MS triple quadrupole.

A. F. Ortega* and M. E. Van Amburgh, *Cornell University, Ithaca, NY.*

Circulating free plasma AA and derivatives could be used as potential biomarkers for determining metabolic and health status of animals. The objective of this study was to validate a new analytical technique to measure AA and metabolites in bovine plasma at 2 deproteinization times. The use of Zwitterion-hydrophilic interaction liquid chromatography (Z-HILIC) columns for analysis of underivatized analytes have allowed simpler sample preparation of bovine plasma for sensitive and selective analysis, when coupled with mass spectrometry (MS). A robust method using Z-HILIC coupled to a triple quadrupole LC/MS (LC/TQ) was validated to quantitatively analyze 19 AA and qualitatively 8 metabolites using isotope dilution ratio in bovine deproteinized plasma. The timing of deproteinization was investigated to determine if plasma should be deproteinized upon collection (on-site) or immediately before analysis (in-lab). Analytes were separated using a HILIC column in a 21 min run and analyzed with a LC/TQ in positive electrospray ionization for identification and quantitation. The method was validated for standard curve linearity, limits of detection (LODs) and quantitation (LOQs), intra- and inter-day precision (RSD, %), recovery (%), and freeze-thaw stability (RSD, %) after one month. Coefficients of determination (R^2) were over 0.993, and LODs and LOQs were below measured values for all AA. Intraday and interday precision for on-site deproteinization were on average 8.65% and 12.08%, respectively, and 10.33% and 11.31% in-lab, respectively. Blank and sample recoveries in-lab were 92.4% and 98.5%, respectively, while on-site they were 90.9% and 96.2%, respectively. Stability RSD was below 20% for all AA and metabolites regardless of timing except for OH-Pro (28.34%). Furthermore, this method was applied to samples collected for an unrelated experiment and deproteinized both on-site and in-lab. Timing differences were analyzed using paired *t*-test in R. Only the concentration of Cys-Cys was affected by timing of deproteination ($P < 0.001$), and Glu and OH-Pro were different between timings ($P < 0.01$) but within the standard error.

Key Words: amino acids, liquid chromatography-mass spectrometry, plasma

1792W Effects of supplementing rumen-protected arginine (RPA) on health and survival of dairy cows.

B. Souza Simoes*[1], T. Adeoti[1], M. Nehme Marinho[1], M. C. Perdomo[1], F. T. Saputra[1], U. Arshad[1], A. Husnain[1], R. Malhotra[1], Z. Sarwar[1], Y. Sugimoto[2], C. D. Nelson[1], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*Ajinomoto Co. Inc., Tokyo, Japan.*

The objectives were to study the effects of supplying 30 g/d of metabolizable arginine (Arg) as RPA from 250 d of gestation to 21 DIM on health and survival. Forty-two nulliparous and 60 parous cows were blocked by parity and predicted transmitting ability of energy-corrected milk (ECM) in nulliparous or 305-d ECM yield in parous cows. Within block, cows were assigned to control (CON), 30 g/d of metabolizable protein (MP) from heat-treated soybean meal, or RPA, 30 g/d of metabolizable Arg (Ajinomoto Co., Inc., Japan), which increased the dietary Arg from 5.7 to 7.5% of the MP (NASEM Dairy, 2021). Pre- and postpartum diets contained, respectively, 74 and 51% forage, 14.3 and 16.9% CP, 10 and 11% MP, and 38 and 25% NDF. Vaginal discharge (VDS, 1 to 5) and rectal temperature were evaluated at 4, 8, 11, and 15 DIM. Fever was considered when temperature > 39.5°C. Endometrial cytology was collected at 21 and 36 DIM and cows were classified with subclinical endometritis when polymorphonuclear leukocytes (PMN) > 10%. Morbidity included milk fever, retained placenta, metritis, displaced abomasum (DA), mastitis, and lameness by 84 DIM. Data were analyzed by generalized mixed-effects models fitting a binomial or Poison distribution with the GLIMMIX procedure of SAS (ver. 9.4). Survival by 300 DIM was analyzed by the Cox's proportional hazard regression. Three cows developed DA, 2 CON and 1 RPA. The risk of leaving the herd (CON = 17.4 vs. RPA = 16.0%; $P = 0.88$) or the rate of leaving the herd by 300 DIM did not differ between treatments (adjusted hazard ratio = 1.08; 95% CI = 0.41–2.87). Supplementing RPA during the transition period did not benefit health or survival of dairy cows (Table 1).

Key Words: arginine, health, transition

Table 1 (Abstr. 1792W). Effects of supplemental rumen-protected arginine (RPA) on health in the first 84 DIM in Holstein cows

Item (% as LSM)	CON (n = 50)	RPA (n = 52)	SEM	*P*-value
Milk fever	1.9	1.7	1.4	0.93
Retained placenta	2.0	2.1	2.5	0.96
Metritis (VDS = 5)	23.5	14.3	8.9	0.40
Clinical endometritis				
32 DIM	42.0	11.8	8.8	0.01
39 DIM	10.5	8.0	10.3	0.65
Subclinical endometritis	58.7	51.6	10.9	0.51
Proportion of PMN in cytology				
21 DIM	26.8	21.4	12.1	0.34
36 DIM	8.2	7.3	4.9	0.73
Mastitis	3.8	1.8	2.3	0.56
Morbidity	36.0	31.4	11.7	0.71
Multiple diseases	10.4	10.2	6.0	0.97

1793W Replacing soybean meal in dairy rations with urea or rumen-protected urea.

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Replacing soybean meal (SBM) with human-inedible N sources in ruminant rations can improve food resource efficiency, specifically human-edible protein efficiency. We investigated the replacement of a portion of dietary SBM with urea or rumen-protected (RP) urea using 42 Holstein-Friesian dairy cows (139 ± 39 DIM). Cows were blocked by DIM, parity, and fat- and protein-corrected milk yield (6 per block) and assigned to 1 of 6 treatment sequences according to a balanced replicated 3 × 3 Latin square design. Treatments were isonitrogenous supplementation of the total-mixed ration with SBM (3.4% of dry matter; DM), urea (0.5% of DM; UR), or RP-urea (0.6% of DM; RPUR; prototype consisting of 84% urea and 16% hydrogenated fat coating, ~80% rumen-undegradable; Trouw Nutrition R&D). Treatment supplements increased the crude protein content of the basal ration from 15.0 to 16.3% on a DM basis. Dry matter and net energy differences between SBM, urea, and RP-urea were compensated with ground corn. Dry matter intake, lactation performance, digestibility, and constituents in ruminal fluid and plasma were measured in the last 7 d of each 28-d period. Statistical models included period and treatment as fixed effects and block as a random effect. Dry matter intake, milk yield and content of protein, fat, and lactose, milk N efficiency, and digestibility were not affected by treatment ($P > 0.10$). Concentration of urea in arterial plasma and milk did not differ between SBM and RPUR ($P \geq 0.44$), increased with UR over SBM ($P < 0.01$), and increased with UR compared with RPUR ($P \leq 0.07$). Higher concentrations of urea in plasma and milk with UR compared with SBM and RPUR suggest that N excretion via urine may have also been greater with UR. Ruminal NH_3 concentration did not differ between UR and RPUR ($P = 0.64$) and increased with both over SBM ($P \leq 0.04$), suggesting that urea released in the post-ruminal digestive tract was recycled to the rumen. In conclusion, dietary CP content can be increased from 15.0 to 16.3% by supplementing SBM or urea at an isonitrogenous urea-equivalent inclusion rate of 0.5% of DM with no negative effects on DM intake, milk production, or digestibility.

Key Words: nonprotein nitrogen

1794W Assessing bacterial protein metabolism response to supplemental branched-chain volatile fatty in dual-flow cultures varying in forage and corn oil concentrations.

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Some cellulolytic rumen bacteria cannot transport or synthesize enough branched-chain AA (BCAA) and branched-chain lipids without branched-chain volatile fatty acid (BCVFA) precursors. Thus, they depend on cross-feeding for BCVFA, which can be limited with decreasing RDP concentration. Our objective was to assess the response in bacterial protein metabolism to supplemental BCVFA in continuous cultures under varying dietary conditions. The study was an incomplete block design with 8 dual flow fermenters used in 4 periods with 8 treatments (n = 4) arranged as a 2 × 2 × 2 factorial. The factors were: high forage (HF) or low forage (LF, 67 or 33% forage), without or with supplemental corn oil (CO, 3% DM), and without or with 2.15 mmol/d each of isovalerate, isobutyrate, and 2-methylbutyrate. The diets were isonitrogenous and the 33:67 alfalfa:orchard grass pellet was replaced by ground corn and soybean meal with LF. In a previous report, BCVFA increased ($P = 0.07$, Diet × CO × BCVFA) bacterial total AA flow when supplemented in HF–CO (16.6%) and in LF+CO (12.4%) diets. Herein, we report another 3-way interaction ($P < 0.06$, Diet × CO × BCVFA) in which BCVFA increased nonbacterial total AA flow when supplemented with HF+CO (35.6%) and LF–CO (34.2%) diets. Due to both increased bacterial and nonbacterial AA flows, supplemental BCVFA increased ($P < 0.01$) total AA flow from 11.6 g/d without BCVFA to 13.1 g/d with BCVFA. Most of the flows of individual AA were greater ($P \leq 0.01$) when BCVFA were supplemented, but the greatest relative ratios (with BCVFA/without BCVFA) were Ala (1.21) and Pro (1.17). The effluent flow of total AA tended to be greater ($P = 0.07$) with HF than LF diets (12.6 vs. 12.0 g/d), but supplemented CO did not have any main effects ($P > 0.35$) on total AA flow. Both Ala and Pro serve as preferred hydrolysis sites for several dipeptidyl peptidases, so increased flows of Ala and Pro could indicate decreased peptidolysis with supplemental BCVFA, which could result in increased ruminal outflow of nonbacterial or lysed bacterial AA.

Key Words: branched-chain volatile fatty acids, branched-chain amino acids, protein metabolism

1795W Effects of adding field peas to the diet of lactating dairy cows on feed intake, milk production, and rumen ammonia-nitrogen.

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Field peas have potential as dairy cow feeds, but the rate of dietary inclusion and performance outcomes require further study. Our objectives were, therefore, to evaluate the effects of including field peas at 2 different inclusion levels in the diet of lactating dairy cows on dry matter intake (DMI), yield and composition of milk, and rumen ammonia-nitrogen. Twelve lactating, non-pregnant Holstein cows were included in a repeated 3 × 3 Latin square design with 3, 3-week experimental periods. Samples were collected during the third week of each period. Cows were fed a basal TMR (Control), or partial mixed diet to which 3.9% DM (LP), or 7.8% DM field peas (HP) were added. The basal TMR contained 17.4% DM crude protein (CP), 4.5% DM crude fat, 28.0% DM starch, and an estimated 1.67 Mcal net energy for lactation (NEl)/kg DM. The field peas contained 22.5% DM CP, 1.67% DM crude fat, 44.7% DM starch, and an estimated 1.81 Mcal of net NEl/kg DM. Peas were coarsely ground and provided as a top dress. Rumen fluid digesta was collected by rumenocentesis once per period at 6 h after feed delivery. Adding field peas to the diet did not affect the milk yield, milk fat yield, nor milk protein yield or content. However, addition of field peas increased DMI, milk urea nitrogen (MUN), and rumen

Table 1 (Abstr. 1795W). DMI, production and composition of milk, and ruminal of lactating dairy cows fed experimental diets (no peas, Control; 3.9% DM peas, LP; and 7.8% DM peas, HP)

Item	Control	LP	HP	SEM	*P*-value
DMI, kg/d	23.7^{b}	24.8^{a}	24.4^{a}	0.33	<0.01
Milk yield, kg/d	36.8	36.6	36.1	1.13	0.78
Fat, %	4.16^{y}	4.21^{xy}	4.38^{x}	0.09	0.10
Fat, kg/d	1.49	1.53	1.56	0.04	0.28
Protein, %	3.41	3.40	3.38	0.02	0.54
Protein, kg/d	1.21	1.24	1.20	0.03	0.56
MUN, mg/dL	11.4^{b}	11.9^{b}	12.7^{a}	0.44	<0.01
Rumen NH_3-N, mg/dL	6.16^{b}	6.52^{b}	8.53^{a}	1.02	0.05

[a,b]Means with different superscripts differ ($P < 0.05$).

[x,y]Means with different superscripts tend to differ ($P < 0.10$).

ammonia-nitrogen, and tended to increase milk fat content. These effects on MUN and rumen ammonia-nitrogen may be due to the high rumen degradability of the crude protein in field peas. Our results demonstrate that diets containing field peas at up to 7.8% DM, may be fed lactating dairy cows, however content and yield of milk protein did not increase compared with Control due to increased CP degradation in the rumen.

Key Words: field peas, rumen, protein

Small Ruminants 1

1796W Performance of ewe lambs fed stearic acid (C18:0). C. G. Padilha, T. R. Wiggers, R. Horstmann, R. Larsen*, and D. E. Oliveira, *Universidade do Estado de Santa Catarina, Lages, Santa Catarina, Brazil.*

Lipid supplements have been used both to supply energy needs when these are compromised and in periods of positive energy balance. However, fatty acids differ in their metabolic functions and partition, in this sense, stearic acid (C18:0) has been the subject of studies that evaluate animal performance and development. The objective of this study was to evaluate C18:0 on the performance of growing ewe lambs. Eight ewe lambs (Lacaune x Texel), weighing 35 ± 2.8 kg in a completely randomized design, were assigned the following treatments: 1) Control and 2) 1.5% of the dry matter intake (DMI) of C18:0 (87% of C18:0). The experiment lasted 63 d and the basal diet was formulated according to the SRNS (2010), consisting of corn silage and concentrate. The concentrate was fed individually and the corn silage per group, twice a day, in the proportion of 110% of voluntary DMI. The DMI evaluation was performed daily and adjusted every 21 d according to the development of the animals. The diets were isoenergetic and isoproteic. Body condition score (BCS) and body weight (BW) assessment were performed on d 0, 21, 42 and 63. The average daily gain (ADG) was obtained individually according to development during the 63 d. Data were analyzed by SAS University (2017) using PROC MIXED, with treatment as a fixed effect, animals as a random effect and data normality was tested. Animals supplemented with C18:0 showed higher BCS and a reduction of 15.8% and 8.6% in DMI of concentrate and silage, respectively, compared with the control treatment (Table 1). The ADG and BW did not differ between treatments. Overall, feeding C18:0 improves BCS and reduces DMI without affecting ADG and BW of ewe lambs.

Key Words: fatty acid, lipid, intake

1797W Litter male ratio in multifetal pregnancies affects placental gene expression in ewes. T. Alon*[1,2], M. Ross[1], A. Rozov[1], L. Lifshitz[1], J. Shpirer[1,2], G. Kra[1], and U. Moallem[1], [1]*Department of Ruminants Science, Agriculture Research Organization, Volcani Institute, Rishon-LeZion, Israel,* [2]*Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel.*

Late pregnancy partial litter loss (PLL) occurs in 28% of lambings in the Volcani experimental sheep flock, by unknown mechanism. In human, the male fetus is an independent risk factor for adverse pregnancy outcomes, such as perinatal death. In a previous study, we found that the male ratio (MR) influenced the ewes' metabolic and physiologic status, and pregnancy outcome. In the current study, we followed 26 multifetal ewes during the last trimester of pregnancy, and examined the association between the MR, PLL, dam metabolic status, and placenta gene expression. Litter size (LS) was determined at ~60 d in pregnancy (DIP), blood samples were collected at 80, 138, and 143 DIP for analysis of glucose, triglycerides (TG), and lactate. Placenta cotyledons were collected from 12 ewes at lambing. Lambs' BW, crown to rump length, and vitality were recorded at lambing. Placenta cotyledons were analyzed for the relative mRNA abundance of genes related to steroids, glucocorticoids and metabolite transport – AR, CYP19, SLC2A1, IGF2, NR2C1, and CRHR1. Lambings were categorized into high male ratio (HMR; > 50% males) or low male ratio (LMR; ≤ 50% males). Data were analyzed with PROC MIXED of SAS, and the model included the effects of pregnancy duration and BCS. The average LS was 3.3 ± 0.7 (range 2–4), and 50% of lambings experienced PLL. The MR did not affect glucose, TG, and lactate plasma concentrations. Relative gene expression of SLC2A1, which encodes the glucose membrane transporter, was higher in HMR compared with LMR ($P = 0.02$), and the gene expression of the aromatase CYP19 that converts testosterone to estrogen tended to be higher in HMR ($P = 0.07$). The gene expression of the growth factor IGF2 that influences fetus growth was higher in LMR than HMR ($P = 0.02$). In conclusion, expression of several genes in placenta that are associated with fetus growth and sex were influenced by MR, which may be related to the PLL in multifetal pregnancies in ewes. However, further research is required to establish these findings, and to discover the underlying mechanism for PLL in ewes, and the effect of MR.

Key Words: sheep, partial litter loss, male ratio

1798W Evaluation of the effect of milk recording intervals on the accuracy of an empirical model fitted to dairy sheep lactations. L. Guevara[1], L. S. Glória[1], E. E. Corea*[2], M. Ramírez-Zamora[3], J. A. Salinas-Martinez[3], and J. C. Angeles-Hernandez[3], [1]*Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Rio de Janeiro, Brazil,* [2]*Universidad Nacional Autnoma de México, Ciudad de México, CDMX, Mexico,* [3]*Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, Mexico.*

Mathematical models are useful for identifying the characteristics of sheep lactation curves to develop and implement improved strategies. However, the accuracy of these models is influenced by factors such as the recording regimen, mainly the intervals between test day records (TDR). The current study aimed to evaluate the effect of different TDR intervals on the goodness of fit of the Wood model (WM) applied to dairy sheep lactations. A total of 4,494 weekly TDRs from 156 lactations of

Table 1 (Abstr. 1796W). Effect of stearic acid (C18:0) on dry matter intake and performance of lambs

	Treatment			
Item	C18:0	Control	MSE[1]	P value*
DMI concentrate (kg/d)	0.53	0.61	0.004	<0.0001
DMI silage (kg/d)	0.69	0.75	0.032	<0.0001
BW[2] (kg)	43.2	44.1	1.100	0.36
ADG (kg)	0.26	0.26	0.013	0.96
BCS[2] (1–5)	3.8	3.6	0.130	0.004

[1] Mean standard error.

[2] BW and BCS obtained at the end of the experiment; at the beginning of the experiment there was no difference between treatments.

* Significance level ($P < 0.05$).

dairy crossbred sheep were analyzed. Three new databases were generated from the original weekly TDR data (7D), comprising intervals of 14 (14D), 21 (21D), and 28 (28D) days. The parameters of WM were estimated using the “minpack.lm” package in the R software. The shape of the lactation curve (typical and atypical) was defined based on the WM parameters. The goodness of fit was evaluated using the mean square of prediction error (MSPE), Root of MSPE (RMSPE), Akaike’s Information Criterion (AIC), Bayesian’s Information Criterion (BIC), and the coefficient of correlation (r) between the actual and estimated total milk yield (TMY). WM showed an adequate estimate of TMY regardless of the TDR interval ($P = 0.21$) and shape of the lactation curve ($P = 0.42$). However, we found higher values of r for typical curves compared with atypical curves (0.9 vs. 0.74), with the highest values for the 28D interval ($r = 0.95$). In the same way, we observed an overestimated peak yield (0.92 vs. 6.6 l) and underestimated time of peak yield (21.5 vs. 1.46) in atypical curves. The best values of RMSPE were observed for the 28D interval in both lactation curve shapes. The significant lowest values of AIC ($P = 0.001$) and BIC ($P = 0.001$) were shown by the 7D interval for typical and atypical curves. These results represent the first approach to define the adequate interval to record the regimen of dairy sheep in Latin America and showed a better fitting for the Wood model using a 7D interval. However, it is possible to obtain good estimates of TMY using a 28D interval, which reduces the sampling frequency and would save additional costs to dairy sheep producers.

Key Words: gamma incomplete, curves, ewes

1799W Novel quality feed from a wasted resource: Measuring the nutritional value of low-glycoalkaloids potato haulm in sheep.

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Objectives aimed to evaluate the potential of low-glycoalkaloid potato haulm (LGPH) as a high-quality feed for ruminants. With 200M tons of potato haulm being discarded annually worldwide, LGPH, developed by Rumafeed, Israel, could serve as an untapped resource for animal feed. A metabolic experiment designed in a 4x4 Latin square arrangement was conducted to investigate the nutritional value of LGPH. Four female lambs (4 m of age at the begining of the study) were assigned to metabolic cages and fed a control maintenance diet consisting of 73% roughage, with LGPH added at 10%, 15%, and 20% (on DM basis) to form the experimental diets. Each period lasted 21 d, with 14 d of adaptation and 7 d of collection and sampling. Refusals and feces were collected for 6 d followed by 24 h urine collection. On the 6th d, one h after morning feeding, blood was withdrawn and rumen liquid was collected using a vacuum facilated stomach tube. Data were analyzed using ANOVA on JMP Pro software utilizing Latin square design where diet and period as fixed effects and sheep as random. Orthogonal contrasts were used to detect linear and quadratic effects of LGPH in the diet. Linear or polynomial equations were produced to extract nutrients digestibility of LGPH. Sheep were healthy during the trial, and no toxicological clinical signs were observed. Results showed that LGPH did not affect DMI (1.3 kg/d). However, the apparent digestibility of DM and CP increased ($P = 0.01$) linearly as the proportion of LGPH in the diet increased (61.3 to 64.7%, and 63.2 to 70.6%). The inclusion of LGPH had a quadratic effect on NDF digestibility ($P = 0.027$), with the peak at 13.34% on DM basis. The N retention was higher in the 20% LGPH diet than in the control ($P = 0.032$; 19.1 vs. 10.2 g/d), indicating enhanced nitrogen utilization. The computed digestibility of DM, OM, CP, and NDF for LGPH were 78.4, 80.7, 93.3, and 53.6%, respectively, and ME was 2.11 kcal/kg DM. In light of the ever-growing global shortage in feed supply, the results show that LGPH can successfully be used as high quality roughage feed in ruminant diets, thus adding a new feed source with zero increase in land and water resources.

Key Words: low-glycoalkaloid, potato haulm, digestibility

1800W Assessment of rumen fermentation in sheep fed babassu oil associated with sunflower oil.

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Babassu (*Orbignya phalerata* Mart.) is a palm tree available in Northeast of Brazil. Because its great medium chain fatty acid content in coconut oil, mainly lauric acid, the use on lamb’s diet is limited to avoid the rumen defaunation. Therefore, it is interesting to associate babassu oil (BAO) with other source of vegetable oil to maintain high dietary energy density. Then, 35 lambs (BW 28 ± 3.9 kg) were used in a complete randomized block design to determine the effects of association babassu oil (BAO) and sunflower oil (SO) on rumen fermentation. Lambs were penned individually during 21 d (15 d for diet adaptation and 1 d for ruminal content collection at 0, 3, 6, and 9 h after feeding. Control diet (CONT, without oil) containing 70% concentrate and 30% Tifton 85 hay. In the remaining 4 treatments, SO replaced BAO oil (4.5% on DM basis) at 0, 33, 50, and 66%. A representative sample of ruminal content from each animal was collected via esophageal tube. The first portion of rumen fluid was discarded, and the second portion, after being squeezed through 2 layers of cheesecloth, was used for determining pH, ammonia nitrogen, and short-chain fatty acids (SCFA). Data were analyzed by ANOVA as repeated measures over time and when significant diet effect ($P < 0.05$), the previously described contrasts (CONT vs 0SO and CONT vs. diets containing SO blend) were performed. The effects of SO blend content (0SO, 33SO, 50SO, or 66SO) added in the diets as the replacement for BAO were evaluated using linear and quadratic orthogonal contrasts ($P < 0.05$). There was observed a quadratic effect ($P < 0.05$) for pH in rumen content (6.36, 6.13, 6.30, 6.49, and 6.36), and proportion of propionate (30.19, 38.81, 36.77, 28.06, 35.44%), butyrate (10.78, 4.48, 6.46, 13.58, and 7.84%), isovalerate (2.11, 1.48, 2.19, 2.43, 1.90%), and acetate:propionate ratio (1.89, 1.43, 1.59, 2.03, and 1.55). The N-NH_3 content (9.46, 7.16, 6.69, 8.45 and 11.32 mg/dL) increased linealy when SO was added in diets. However, experimental diet did not affect ($P > 0.05$) acetate proportion (54.74, 53.72, 52.94, 54.41, and 53.17%) and total short chain fatty acids content (52.65, 52.18, 47.23, 38.46, 43.37 mmol/L). Sunflower oil can replace up to 66% of the BAO in diet of lambs without negative effects in ruminal fermentation.

Key Words: lipids, rumen pH, short-chain fatty acid

1801W Ingestive behavior of goat kids fed by diets with non-forage diets.

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High concentrate diets are normally used in feedlot system to reduce the age at slaughter. Additionally, the use of non-forage diets in ruminant production has increased. In these diets, the whole corn grain (WCG) is used as a fiber source, to stimulate the chewing. But it is an expensive ingredient of, because the use in human and non-ruminant nutrition. Then, the objective of this study was to evaluate the effects of replace the WCG by whole millet grain (WMG) in non-forage diets on ingestive behavior of goat kids. Twenty-one crossbred Anglo Nubiano goat kids (21.6 ± 2.91 kg BW) were used in a randomized complete block design. Animals were individually confined and fed a control diet (CON; 16% of crude protein) containing 90% concentrate and 10% Tifton hay (DM basis) for 58 d, with 12 d for diet adaptation and 46 d for experimental period. The remaining diets were based on 20% protein pellet and 80% of whole corn grain (WCG) or 80% of whole millet grain (WMG) as a fiber source. Data collection occurred on d 32 of the experimental period. Animals were monitored every 5 min during 24 h, according to the activities: eating, rumination, idle and other activities. The feed and rumination efficiencies, expressed as g/hour were obtained by dividing the average daily intake by the total time spent eating and/or ruminating in 24 h, respectively. Data were analyzed by ANOVA and when a significant effect of diet (fixed effect) was found, means were compared using Tukey test. The blocks were considered as random effect. Effects were declared significant at $P \leq 0.05$. The experimental diets did not affect ($P \geq 0.05$) the others activities (30.7, 22.8 and 28.5 min/day for CONT, WCG and WMG), feeding efficiency (167.8, 152.1 and 224.9 g/min for CONT, WCG and WMG) and rumination efficiency (429.1, 661.1 and 600.0 g/min). CONT increased the time spent in feeding (267.8, 236.4 and 175.4 min/day for COM, WCG and WMG) and time spent in rumination (127.1, 65.7 and 61.4 min/day), but decreased the time spent in idle (1029.3, 1130.0 and 1190 for CONT, WCG and WMG) compared with WMG. The whole millet grain can replace WCG in non-forage diets without modifying the ingestive behavior.

Key Words: corn grain, millet, rumination

1802W Use of coffee by-product in the diet of dairy goats. S. Carta[1], A. Nudda*[1], F. Correddu[1], G. Battacone[1], M. Lunesu[1], E. Tsiplakou[2], and G. Pulina[1], [1]*Dipartimento di Agraria, University of Sassari, Sassari, Italy,* [2]*Department of Nutritional Physiology and Feeding Agricultural University of Athens, Athens, Greece.*

Spent ground coffee (SGC), is a by-product obtained after coffee beverage preparation. It has been estimated that about 6 million tons per year of SGC are globally produced. The SGC could be recycled in animal feeding as a potential source of carbohydrate, mostly cellulose and hemicellulose, protein, and lipids. In addition, SGC is a good source of polyphenols that could exert a potential antioxidant effect. In this experiment, the SCG composition was characterized and the effects of its inclusion in the diet of dairy goats on milk yield and composition, and antioxidant status of milk have been evaluated. Twenty-four Saanen goats were divided into 3 groups balanced for milk yield, body weight and DIM. The animals were fed a TMR supplemented with increasing doses of SGC: D0 (Control group; 0 g/d), D50 (50 g/d), and D100 (100 g/d). Each treatment had 8 animals. Data were analyzed by a linear mixed model with diet, sampling, and their interaction as fixed effects and animal as random effect. The differences among the levels of the effects were tested using orthogonal polynomial contrasts (linear and quadratic). The SGC contained 55.5% of NDF, 15.0% of CP. Ferulic acid was the most abundant polyphenolic compound present in this by-product (1.2 g/kg of SGC) followed by caffeine (0.9 g/kg of SGC). Dry matter intake, body weight and milk yield were not affected by SGC supplementation. The SGC supplementation did not change milk protein, fat and lactose contents. The somatic cell counts were reduced only by the low dose. The fatty acid profile of milk showed that SGC linearly decreased the short chain fatty acids and increased the branched chain fatty acids. The SGC supplementation did not modify the milk antioxidant status. The results show that SGC up to 100 g/d did not exert negative effect on milk yield and main components. However, quadratic effects on some parameters suggest that further investigations are necessary, especially about the optimization of the supplement dose. Funded by NextGenerationEU; PNRR M4C2, CN00000022 AGRITECH.

Key Words: spent ground coffee, goat milk, by-product

Teaching/Undergraduate and Graduate Education 1

1803W Evaluating the impact of paper color on exam grades; an observational study. P. Hartoonian, S. E. Omale, and J. A. D. R. N. Appuhamy*, *Department of Animal Science, Iowa State University, Ames, IA.*

The objective of this observational study was to determine if exam paper color affects grades, both overall and by question type. Scores were derived from 132 (102 female and 30 male), and 171 (140 female and 31 male) students in an introductory animal nutrition class taught at Iowa State University during the spring semesters of 2022 and 2023, respectively. Data were from 3 exams (2 midterm and the final) in 2022, and one midterm exam in 2023. The midterm exams were printed on blue or yellow papers, and the final exams were printed on green or white papers in 2022. The exams were printed on all 4 color papers in 2023. The exams consisted of true or false (TF), multiple choice (MC), and written response (WR) questions. The paper color effects were statistically analyzed while accounting for the effects of sex, sex × color interaction, and the covariate effects of previous exam or assignment scores by using the MIXED procedure of the SAS software. The previous scores represented the baseline aptitude of students. When adjusted for the covariate effect of the baseline aptitude ($P < 0.01$), printing exams on blue vs. yellow papers did not affect the total score or scores by question type ($P > 0.05$) in 2022. The TF scores were higher for exams printed on green papers than those printed on white papers ($P = 0.04$), and that difference tended to be more prominent in male than female students ($P = 0.07$). The green vs. white paper color did not affect the score of the other questions or the total score ($P \geq 0.29$). When all 4 colors were evaluated simultaneously in 2023, the WR and total scores of exams printed on blue papers were lower than those printed on white and green papers ($P < 0.05$). Male students had lower WR and total scores ($P < 0.05$) and tended to have lower MC scores ($P = 0.07$) than female students for exams printed on blue papers. Consistent with 2022 observations, the scores were similar between exams printed on yellow and blue papers ($P > 0.10$). In conclusion, exam paper color can affect students' grades, and those effects can be different between question types and sexes.

Key Words: exam scores, paper color, sex

1804W Student perception of hands-on activities in an introductory dairy science course do not differ by experience or background. W. Brown*, G. Hock, and B. Disberger, *Kansas State University, Manhattan, KS.*

Hands-on activities are critical to enhancing student perception of learning outcomes, interest in subject material, and resulted in higher scores. Several studies have assessed their value to improve student learning outcomes in dairy science classes, but those studies have focused on a single strategy of laboratory instruction over a semester, such as a computerized simulation program or students operating a university farm. Our objective was to assess student perception (n = 89) of the value of specific laboratory activities in a limited 7-wk introductory dairy science course the during fall 2022 semester. Using a Likert scale (1 = poor; 2 = fair; 3 = good; 4 = excellent) students (n = 37; 41% response rate) rated laboratory activities for their value in helping them learn about dairy science. Students were asked to rank all activities in order of their perceived value (1 = best; 8 = worst). Further, one-way ANOVA was conducted (SPSS) to analyze differences in perceived value of activities across student hometown demographics (urban, rural non-farm, rural farm) and previous large animal experience (none, some, moderate, extensive). Student rankings of the value of lab activities were the following (overall ranking with composite score): 1) farm tour (2.6); 2) rumen cannulated cow (3.3); 3) tracking the production of an assigned university cow (3.8); 4) total mixed ration particle size separator (4.5); 5) feed dry matter determination (4.9); 6) colostrum quality determination (5.0); 7) blood-based pregnancy diagnosis (5.8); 8) somatic cell count quantification (6.1). The perceived value of observation of rumen function using a cannulated cow differed by students' previous large animal experience category ($P = 0.05$; none = 3.29; some = 3.88; moderate = 3.50; extensive = 2.78) but means would not separate using Tukey's HSD. There was no evidence of a difference in perceived value of the other laboratory activities across demographic or large animal experience classifications. Overall, students perceived more value in activities that allowed observation on a macro level.

Key Words: undergraduate education, learning, survey

1805W Can Excel improve the learning of ration balancing for animal science students? M. Marcondes*, K. Stadler, N. Hossain, G. Leite, and I. Carrari, *Washington State University, Pullman, WA.*

It was pointed out the need to increase the use of Solver (Microsoft Excel) to teach ration balancing during the release of the system of nutrient requirements of dairy cattle in 2021. This study aimed to evaluate the student acceptance of balancing rations using Solver compared with by-hand calculation. An optional 9-question survey was provided to 109 students in a 300-level class (Feeds and Feeding) at Washington State University. Google Docs coded all students' names to avoid bias and conflicts of interest. The survey was opened in the third week of class, after 2 classes of by-hand calculation and no Excel class. Then, the same survey was provided at the end of the semester after grades were submitted. Data were summarized in an Excel file, and percentages of each question were calculated. Seventy-seven students participated in the survey at the beginning of the semester, and 54 students at the end of the semester. Of the 77 students who completed the survey at the beginning of the semester, 38 answered the post-class survey. Thus, 2 pre-post descriptive statistical analyses were performed: A1) surveys were evaluated with all respondents, and A2) responses were evaluated only with respondents that answered both surveys. Overall, students thought the class was hard or very hard (A1 49%, A2 46%). For that reason, students were not engaged early in the semester (A1 43%, A2 50%), but this changed in the post-evaluation (A1 69%, A2 63%). Most students thought by-hand ration balancing was hard or very hard in the third week of class (A1 48%, A2 40%), but that dropped to 26% (A1) or 32% (A2) in the second survey. Regardless of the moment, most students (64%) preferred Excel to balance ration, even though in the early semester, most students considered themselves with either no or basic experience with Excel (A1 74%, A2 68%). Interestingly, those numbers dropped to only 22% (A1) or 27% (A2) in the second survey. For that reason, their confidence in Solver increased from 12.5% to 66.5%, and most students (91%) considered that Excel could simplify balancing rations. Producing videos for Excel and by-hand calculation was helpful for 86% of students. We concluded that implementing Excel to balance rations for cattle was successful and appreciated by the students.

Key Words: diet, formulate, nutrients

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