ABSTRACTS
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2024 ISRP ABSTRACTS

Session 1: Insights from Precision Technology and Data Science and their Application to Ruminant Physiology and Management

1 **Big data and artificial intelligence assisted prediction of physiological outcomes.** J. R. R. Dorea*, A. Negreiro, E. Casella, L. Hernandez, and G. J. M. Rosa, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

One of the constraints on advancing livestock research is the lack of large-scale quantification of physiological traits, a process often constrained by financial, labor-intensive, and scalability limitations, in addition to being traditionally performed through invasive methods. However, in the era of big data, the collection of large, extensive, and heterogeneous data sets via digital technologies coupled with artificial intelligence (AI) techniques have catalyzed significant progress across various research domains, including precision medicine, education, finance, and environmental and socioeconomic studies. This presentation will highlight our research efforts in employing computer vision systems for the longitudinal phenotyping of body growth and development in dairy cattle, from mammary gland tissue to comprehensive body morphology changes. The discussion will include the methodology for integrating physiological traits and phenotypes derived from these assessments and their association with dairy cow performance indicators. Our proposed approach offers a novel perspective on animal growth development, contributing to the optimization of livestock management and opening new avenues to study animal physiology through AI.

2 **What has precision technology taught us about grazing behavior?** M. Trotter*[1], D. Bailey[2,1], R. Reuter[3,1], J. Kinder[4,1], A. Garcia-Guerra[4], S. Quigley[1], K. McCosker[5], C. Tobin[6], C. Knight[7], C. Wade[8], D. Costa[1], J. Manning[1], A. Chang[1], C. Wilson[1], and T. Williams[1], *[1]CQ University Australia, Rockhampton, Queensland, Australia, [2]Deep Well Ranch, Prescott, AZ, [3]Oklahoma State University, Stillwater, OK, [4]The Ohio State University, Columbus, OH, [5]The University of Queensland, Brisbane, Queensland, Australia, [6]North Dakota State University, Carrington, ND, [7]University of Maine, Orono, ME, [8]University of Alberta, Edmonton, Alberta, Canada.*

Sensor technologies are being touted as the next big revolution in grazing animal management. They have been applied in a research context for many years and are now emerging as everyday tools for use by producers. But what insights will these sensor technologies provide researchers and animal managers beyond the traditional options for monitoring grazing animal behavior, and how will these technologies impact the sustainability and profitability of livestock enterprises? Sensors such as GPS tracking and accelerometer-based motion monitoring in leg and ear tags, collars, and rumen bolus forms provide data that can be interpreted to provide measures of key behaviors. These include bite rate, time spent grazing and resting, location of grazing activity, rumination duration and bouts, and distance traveled. In turn, the quantification of these behaviors can be used to explore broader issues such as landscape utilization, energetics, nutritional deficiencies and disease state, animal welfare, and reproductive efficiency, and can be used to generate data for inclusion in nutritional, economic and systems models. There are several ways in which producers anticipate using this information. From simply understanding where their animals are grazing, to optimizing fencing and water infrastructure, to more complex applications such as targeting paddock rotations, supplementation decisions based on animal behavior, and developing measures of feed intake from pasture. Although there are several challenges to overcome before we see widespread application of precision technologies in the grazing industries, the potential impacts are so significant that their application may one day be as ubiquitous as smartphones within the human population.

Key Words: precision livestock management, grazing, sensors

Session 2: Gastrointestinal Microbial Ecology, the Microbiome, and Gut Physiology Spanning from Microbial–Host Interactions to an Update on Methane Production and Mineral Interactions

3 Overall perspective on rumen microbial ecology to improve fiber digestibility. J. L. Firkins*[1] and P. B. Pope[2,3], [1]*The Ohio State University, Columbus, OH,* [2]*Queensland University of Technology, Woolloongabba, Queensland, Australia,* [3]*Norwegian University of Life Sciences,* Ås*, Norway.*

Optimizing fiber digestibility remains a key research priority to improve feed efficiency and environmental sustainability. Further research is needed for a complete comprehension of the divergence of other fiber components and their depolymerization. Hundreds of variants of glycosyl hydrolases are configured in multi-domain carbohydrate-active enzymes (CAZymes) and operons, which are attributed to taxa that detected via 16S rRNA gene profiling techniques, whereas future research must uncover deeper complexity in the rumen microbiome through omic and next-generation cultivation approaches. Network analyses (and potential markers within networks) of microbial consortia should help to associate efficient rumen fiber digestibility among animals fed differing dietary conditions through integration with chemical analyses of feed and digesta for improved ration evaluation. The particulate phase harboring most of the fibrolytic microbes and the spatial (including epimural) and temporal differences of ruminal digesta need better characterization to improve core fibrolytic microbes' competitiveness in varied diets. So far, potential manipulation of rumen microbial communities has yielded inconsistent benefit, and further microbiome resolution is needed to better inform the timing and duration of approaches. Our overall objective will be to integrate nutritional, microbial, and computational approaches to help guide future research to improve fiber digestibility in ruminants.

Key Words: rumen fiber digestibility, rumen microbiome, CAZymes

4 The giants and the dwarfs within the rumen ecosystem—Rumen ciliates and viruses. Z. Yu*, M. Yan, and S. Somasundaram, *The Ohio State University, Columbus, OH.*

The rumen ecosystem is home to diverse microbes including multiple kingdoms. Unlike rumen bacteria and archaea, ciliates and viruses have not been extensively studied due to the difficulty in analyzing these predators. Sitting at the top of the food chain in the rumen, ciliates affect feed digestion, fermentation, methane emissions, and recycling of microbial protein, the major source of amino acids for cattle. Recent studies have provided new insights into the genome architecture, taxonomy, metabolism, and physiology of rumen ciliates. Some of the ciliate genes and enzymes are being explored as targets to specifically control rumen ciliates and reduce their negative impact on nitrogen utilization and methane emissions. Rumen viruses are extremely diverse and abundant, but they have been largely overlooked due to the limitations of bioinformatics tools to identify them reliably. Recent advancements in bioinformatics tailored to virome analyses have led to the development of a new rumen virome database, providing a key resource for investigating the rumen virome comprehensively. Recent studies have shown that rumen viruses can infect the core rumen microbiome, including bacteria, archaea, and ciliates. Lytic viruses can contribute to nutrient recycling, including the intraruminal recycling of microbial protein. Lysogenic viruses can augment or modify the metabolism, ecology, and physiology of their hosts by providing auxiliary metabolic genes. By affecting the rumen microbiome in top-down and bottom-up fashions, through their lytic and lysogenic life cycles, respectively, the rumen virome can substantially regulate the rumen microbiome in terms of composition, interactions, and rumen functions, subsequently influencing animal production performance. Indeed, comparative analysis of the rumen viromes demonstrates its associations with key ruminant production traits, such as feed efficiency, lactation performance, and methane emissions. Some of the lytic viruses infecting rumen ciliates and methanogens have the potential to control these undesirable microbes for improved nitrogen utilization and reduced methane emissions.

Key Words: omics, phages, intraruminal recycling

5 Fungi, archaea, and their interactions. T. McAllister*, K. Thomas, and R. Gruninger, *Agriculture and Agri-Food Canada Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada.*

Anaerobic fungi were the last kingdom to be identified within the rumen microbiome and account for 7% to 9% of microbial biomass. They produce potent lignocellulases that degrade recalcitrant plant cell walls and rhizoids that can penetrate the cuticle of plant cells, exposing internal components to other microbiota. Interspecies H_2 transfer is enhanced, increasing the reduction of CO_2 to CH_4 by methanogens. This symbiotic relationship is bolstered by hydrogensomes, fungal organelles that generate H_2 and formate. The extent to which hydrogenosomes serve as a conduit for H_2 flow to methanogens is unknown, but it is likely greater with low-quality forages. Strategies that alter

the production of CH_4 could also have implications for H_2 transfer by anaerobic fungi. Understanding the factors that drive these interactions and H_2 flow could provide insight into the effect of reducing CH_4 production on the activity of ruminal fungi and the digestion of low-quality feeds.

Key Words: rumen fungi, methanogens, hydrogensomes

Session 3: Whole Animal Microbiome and Integration of Effects Across Systems Including the Central Nervous System, Lactation, Reproduction, Respiration, and Immunity

6 The holobiont concept in ruminant and human physiology—More of the same, or something new and meaningful to food quality, security, and health? T. Callaway*[1] and M. Morrison[2], [1]*Department of Animal and Dairy Science, University of Georgia, Athens, GA,* [2]*Frazer Institute, University of Queensland, Brisbane, Australia.*

The holobiont concept has emerged as an attempt to recognize and describe the myriad interactions and physiological signatures inherent to a host organism, as impacted by the microbial communities that colonize and/or co-inhabit the environment within which the host resides. The field acknowledges and draws upon principles from evolution, ecology, genetics, and biology, and in many respects has been "pushed" by the advent of high-throughput DNA sequencing and, to a lesser extent, other omics-based technologies. Despite the explosion in data generation and analyses, much of our current understanding of the human and ruminant holobiont is based on compositional forms of data and, thereby, restricted to describing host phenotypes via associative or correlative studies. So, where to from here? We will discuss some past findings arising from ruminant and human gut microbiota research and seek to evaluate the rationale, progress, and opportunities that might arise from the holobiont approach to the ruminant and human host. In particular, we will consider what is a "good" or "bad" host (gut) microbiome in different scenarios, as well as potential avenues to sustain or alter the holobiont. Although the holobiont approach might improve food quality, security, and health, these benefits will most likely be achieved via a judicious and pragmatic compromise in data generation, both in terms of its scale, as well as its generation in context with the "forgotten" knowledge of ruminant and human physiology.

Key Words: microbial interactions

7 Stochastic and deterministic factors that shape the rumen microbiome. S. C. Fernando*, M. L. Spangler, S. Adams, and A. Lakamp, *University of Nebraska–Lincoln, Lincoln, NE.*

At the heart of degradation of complex organic matter within the rumen are its microbes. These microbes can convert unusable organic matter into useable protein and energy and therefore can directly influence the animal's health, performance, and feed utilization. As such, understanding stochastic and deterministic factors that contribute toward rumen microbial assembly and persistence can provide valuable information into developing methods to increase animal health, performance, and nutrient utilization. Here we describe how host genetics affect rumen microbiome composition and function. Additionally, we will discuss how ecological theory such as colonization history and founder hypothesis can be used to help understand rumen microbial community assembly and provide strategies for microbiome manipulation using early colonization. Finally, we will discuss how microbiome-wide association studies can be used to understand the role of the microbiome and how such information can be used to develop novel biotherapeutics to improve animal health and productivity.

Key Words: genome-wide associations, microbiome-wide associations, founder effects

8 Understanding the rumen microbiome to develop methane mitigation strategies for ruminants. S. M. Waters*[1], P. E. Smith[2], D. A. Kenny[2], M. Popova[3], M. Eugène[3], and D. P. Morgavi[3], [1]*School of Biological and Chemical Sciences, University of Galway, Galway, Ireland,* [2]*Teagasc Grange, Animal and Bioscience Research Department, Dunsany, Co. Meath, Ireland,* [3]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champanelle, France.*

The rumen microbial community provides ruminants with a unique ability to convert human-indigestible plant matter into high-quality proteins. However, CH_4 produced in the rumen is both a potent greenhouse gas and a metabolizable energy loss for ruminants. As the rumen microbiome constitutes 15%–40% of the inter-animal variation in enteric CH_4 emissions, understanding the microbiological mechanisms underpinning ruminal methanogenesis is crucial for developing CH_4 mitigation strategies. Differences in the rumen microbiome composition were observed in cattle with contrasting residual CH_4 emission and CH_4 yield. Low emitters had an increased abundance of bacteria producing lactic acid, succinate, and propionate, whereas in high emitters, H_2-producing microbes were increased. Indeed, the relative abundance of 3 ruminal bacteria and the *Methanobrevibacter* SGMT clade, accounted for 20% of the variation in CH_4 emissions. However, the demonstration of ruminotypes associated with high or low CH_4 emissions suggests that interactions within complex microbial consortia are a major source of variation in CH_4 emissions. Consequently, microbiome-assisted genomic approaches are being developed to select low CH_4-emitting cattle, with breeding values for enteric CH_4 being included as part of national breeding programs. Generating rumen microbiome data for use in selection programs is expensive, so identifying microbial biomarkers in milk or plasma to develop predictive models

and including microbial predictors in equations based on animal-related data is investigated. Understanding the rumen microbiome has also aided the development of anti-methanogenic feed additives. However, strategies that mitigate CH_4 unfortunately increase emission of H_2 rather than directing reducing equivalents to propionate or higher-chain VFA. Current research aims to provide alternative hydrogen sinks and to stimulate activity of commensal microbes or the direct supplementation of DFM to capture this lost energy. Furthering our knowledge of the rumen microbiome aids in the development of methane mitigation strategies for ruminant livestock.

Key Words: enteric methane, rumen microbiome, cattle

Session 4: Post-Absorptive Physiological Impacts of Nutrients on Cellular Signaling

9 Nutrient signaling to skeletal muscle and adipose tissue. B. J. Johnson*, *Texas Tech University, Lubbock, TX.*

Accretion of key carcass tissues such as skeletal muscle and adipose tissue is a direct effect of the specific nutrients available to support the growth and development of these key tissues. In turn, these specific nutrients are often key regulators of important cell signaling pathways that regulate the growth and differentiation of these tissues. Postnatal skeletal muscle growth is a result of hypertrophy of the existing skeletal muscle fibers in animals. A major driver of skeletal muscle hypertrophy is protein accretion in the existing muscle fibers. This is a result of the balance between rate of protein synthesis and rate of protein degradation. Certain signaling pathways can alter the myosin heavy chain isoform (MyHC) type in postnatal skeletal muscle. Alterations in the various MyHC isoforms result in different degrees of postnatal skeletal muscle hypertrophy. An enzyme, AMP-activated protein kinase α (AMPK-α), has been shown to affect expression of MyHC depending on sources and availability of energy to the skeletal muscle. The mammalian target of rapamycin (mTOR) signaling pathway has been shown to be a critical regulator of protein synthesis in tissues such as skeletal muscle. Different components of this pathway are regulated by key nutrients such as individual amino acids. In many meat animals, such as cattle and sheep, there are different types of adipose tissue depots that grow and differentiate differently from each other. Two key types of adipose tissue in ruminants that have economic importance are subcutaneous and intramuscular adipose tissue. Many of the genes that control preadipocyte differentiation of these different adipose tissues in cattle are regulated by fatty acids circulating in plasma. It appeared that various fatty acids could affect cell signaling through a membrane-bound GPR signaling pathway. Research indicated that expression of GPR-43 and −120 was different for intramuscular as compared with subcutaneous adipose tissue, and these differences were a result of responses to various fatty acids.

Key Words: adipose tissue, GPR, skeletal muscle hypertrophy

10 Advances in trace mineral and vitamin nutrition. S. Hansen*, *Iowa State University, Ames, IA.*

This presentation explores the evolution of trace mineral and vitamin nutrition in beef cattle, shifting focus from mere deficiency prevention to their roles in supporting growth, immunity, reproduction, and stress response. Many of the current nutrient recommendations for beef cattle are based on studies conducted decades ago, with animals of very different intake patterns and growth capacity. Although trace minerals and vitamins are often squeezed into less than 2% of a ration, they critically support nearly every biological process in the body. This presentation will highlight the evolution of our understanding of the needs for these micronutrients to support optimal cattle performance, be it reproduction, health, or growth. For example, although the recommendation for zinc of 30 mg/kg DM likely prevents overt signs of zinc deficiency, better foot health, reproduction, growth, stress response, and immunity have been linked to increased rates of supplementation. Similarly, recommendations for vitamin A may differ for the modern cow, who, depending on timing of production cycle, drought, and forage availability, may not have the reserves coming into winter that were once assumed and require additional supplementation. Further, the B vitamins and vitamin C have largely been ignored because the ruminal microbes or the animal itself make these, but today's beef animal may have outpaced endogenous synthesis of these essential nutrients. There are also cases of clear oversupplementation, as with Cu; this is increasingly problematic with the slew of dairy-beef calves entering feedlots. This presentation culminates with forward-looking recommendations, emphasizing the need to evolve our approach to trace mineral and vitamin supplementation in beef cattle, ensuring that it keeps pace with the changing demands of modern cattle production and contributes to the overall sustainability of the beef industry.

Key Words: cattle, mineral, vitamin

11 One-carbon metabolism in beef cattle throughout the production cycle. M. S. Crouse*[1], R. A. Cushman[1], C. A. Redifer[1], B. W. Neville[1], A. K. Ward[2], C. R. Dahlen[3], and J. S. Caton[3], *[1]U.S. Meat Animal Research Center, Clay Center, NE, [2]University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [3]North Dakota State University, Fargo, ND.*

One-carbon metabolism (OCM) is a series of connected pathways involving the methionine-folate cycles, transsulfuration, polyamine synthesis, nucleotide synthesis, free-radical scavenging, and energy metabolism pathways. These pathways functionally depend upon amino acids (methionine, glycine, and serine), vitamins (folate, B_2, B_6, and B_{12}), and minerals (sulfur and cobalt). Growing bodies of research indicate that physiological stage, nutritional plane, diet, species (*taurus* vs. *indicus*), rumen protected versus not, individual versus combination

supplementation, and method of delivery all affect the efficacy of one-carbon metabolite supplementation in beef cattle. Infusion studies showed that supplementing methionine to growing steers improves N retention and alters hepatic activity of methionine synthase; however, only supplementing methionine without folate decreases folate concentrations in circulation. When heifers were supplemented with methionine, choline, folate, and B_{12} for the first 63 d of gestation, metabolomic analysis revealed increasing OCM analytes to the heifer, but a buffering effect with minimal changes seen in fetal hepatic metabolite abundance. Methionine supplementation to heifers during the periconceptual period increased heifer circulating methionine but shifts fetal hepatic metabolism toward the transsulfuration pathway. In vitro supplementation of choline to beef cattle embryos results in calves of increased birth and weaning weights. Periconceptual methionine supplementation to cows increased gain and total-tract digestibility of calves. Overall, these data demonstrate that OCM is altered in those cattle receiving one-carbon metabolites, and that a metabolic programming response is elicited in offspring receiving supplements in vitro or during early gestation. Research should be considered to maximize efficiency of beef cattle production at all stages by identifying limiting metabolites or enzymes to maximize efficiency of OCM in beef cattle, as well as to understand the concerted effects of multiple one-carbon metabolites to balance the stoichiometry of the pathway. USDA is an equal opportunity provider and employer.

Key Words: developmental programming, methyl donors

Session 5: Inflammation, Metabolic Endocrinology, Metabolomics, and Novel Regulators of Physiology and Metabolism

12 Endocrine adaptations to lactation: IGF, FGF21, and beyond. Y. R. Boisclair*, *Cornell University, Ithaca, NY.*

Modern dairy cows experience a substantial nutritional deficit in early lactation as a consequence of rapidly rising milk production occurring in the absence of an appropriate compensatory increase in feed intake. Dairy cows maintain productivity during this period by engaging adaptive metabolism, a response aimed at preserving metabolic well-being, and consisting of hormonally-driven adaptations such as mobilization of endogenous reserves, improved energy efficiency, and tissue-specific insulin resistance. For example, research in the 1980s and 1990s identified the combination of elevated plasma growth hormone and hypoinsulinemia as key endocrine adaptations limiting glucose utilization in insulin-responsive tissues and favoring the diversion of available glucose to the mammary gland where uptake is independent of insulin. The following decades have seen the discovery of over 20 signals secreted almost exclusively by adipose tissue, skeletal muscle, or liver, dynamically regulated by metabolic challenges, and engaged in cross-organ communication. The significance of these signals in the coordination of metabolism in ruminants has been limited by the availability of assays to measure their circulating concentrations and materials to perform functional studies. Nevertheless, emerging data point to their importance during demanding physiological states in ruminants, including early lactation in dairy cows and late pregnancy in sheep. Examples include the regulation of energy efficiency by the action of the adipose-derived hormone leptin via its ability to control the hypothalamic-pituitary-axis and modulation of insulin action by the liver-derived fibroblast growth factor-21 via its ability to increase adipose tissue production of the insulin-sensitizing hormone adiponectin. Recent studies investigating the regulation and action of leptin, adiponectin, and fibroblast growth factor-21 in dairy cows and sheep will be used to illustrate the potential of recently discovered signals to coordinate metabolism during physiologically demanding states such as early lactation.

Key Words: metabolism, liver, adipose

13 Role of the endocannabinoid system in metabolic and inflammatory responses in cows in negative energy balance. M. Zachut*, *Agriculture Research Organization, Volcani Institute, Rishon Lezion, Israel.*

The endocannabinoid system (ECS) regulates energy metabolism and immune function in mammals. The ECS components are the endocannabinoid (eCB) ligands, the cannabinoid receptors, and the enzymes that synthesize and degrade eCBs. In mammals, cannabinoid-1 receptor (CB1) is expressed in the central nervous system and in peripheral tissues; its activation increases feed intake and lipogenesis, and promotes energy storage. The cannabinoid-2 receptor (CB2) is expressed in immune cells, and exerts mostly anti-inflammatory effects. Peripartum dairy cows experience vast changes in energy metabolism and in immune function, and the involvement of ECS elements is emerging; adipose of cows with increased lipolysis postpartum (PP) shows increased eCBs and inflammatory mediators, coupled with higher expression of CB2 and ECS enzymes. Levels of blood eCBs are increased PP, and may affect feed intake. Treating transition cows with eCB injections has increased adipose deposition, but may not affect feed intake. Modulation of ECS activation is possible via supplementation of omega-3 (n-3) fatty acids, which reduce the availability of the eCB precursor arachidonic acid. In peripartum dairy cows, n-3 supplementation partly modulated ECS components in blood, adipose tissue, and liver, and was associated with moderate changes in lipid metabolism in adipose and inflammation in liver. Reducing ECS activation by n-3 also improved systemic insulin sensitivity and immune function, and reduced insulin sensitivity in adipose of dairy cows. Thus, modulation of the ECS seems to be associated with metabolic and immune function PP. In vitro models showed that adipose lipolysis, adipogenesis, and lipogenesis were differentially affected by cannabinoids in nonlactating non-gestating compared with PP cows. Together, the ECS has a complex role in the physiology of dairy cows in negative energy balance. Cannabinoids and pharmacological ECS modulators are expected to become more available and accepted for use in animals. Selective activation of CB1 and CB2 in tissues could have beneficial effects on metabolic and immune function.

Key Words: endocannabinoids, negative energy balance, dairy cow

14 The immunometabolism of transition dairy cows: Lights and shadows. E. Trevisi*[1,2], L. Cattaneo[1], F. Piccioli-Cappelli[1], M. Mezzetti[1], and A. Minuti[1], *[1]Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy, [2]Romeo and Enrica Invernizzi Research Center for Sustainable Dairy Production of the Università Cattolica del Sacro Cuore (CREI), Piacenza, Italy.*

Research on the complexity of the transition period in dairy cows has focused mainly on the mismatch between nutrient

intake from the diet and output by the mammary gland. This gap causes a negative energy balance, that, if excessive, promotes several metabolic disorders. Other relevant phenomena occur during transition, such as inflammation at calving and changes in immunocompetence, redox balance, and mineral metabolism. Despite the efforts made, some aspects of the adaptive mechanisms observed in the transition period still need to be clarified. For instance, alterations of the physiological responses even before the dry-off or during the dry period can affect the success of the whole transition period in certain cows. In this context, the mechanism regulating the inflammatory-like response around calving may play a pivotal role, as suggested by the variety of factors influencing it and its consequences, as, in particular, feed intake depression, which can amplify and anticipate the negative energy balance. When this mechanism derails is still unclear, but detecting the triggers of diverted or abnormal physiological responses and where they stem (e.g., liver, rumen, and gut epithelia, uterus, or mammary gland) will help to reveal the weak points in the immune system and the possible ways to restore it. Furthermore, the postpartum healthy cow appears to have an acute phase response at liver level, despite a decrease in circulating proinflammatory cytokines. What is physiological and what is pathological in this context? To understand the latter, it would be advisable to find markers of unsuccessful transition period, that go beyond the energy deficit. Future efforts should be dedicated to clarifying the causes of the acute phase response at calving, exploiting the potential of system biology. Moreover, it would be helpful for both research and on-field purposes to define biomarkers associated with pathological responses, and to introduce into genetic selection phenotypes related to the ability of cows to adapt to the immunometabolic stress typical of the transition period.

Key Words: inflammation, immune system, metabolic disorders

Session 6: Integration of Environment, Physiology, and Well-Being in the Face of Climate Change and Resource Limitations

15 Integrating our understanding of stress physiology. E. Horst*[1], J. Mayorga[1], S. Kvidera[1], S. Rodiguez-Jimenez[1], J. Opgenorth[1], M. Abeyta[1], B. Goetz[1], V. Sanz-Fernandez[1], J. Selsby[1], A. Keating[1], J. Ross[1], R. Rhoads[2], and L. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*Virginia Tech University, Blacksburg, VA.*

There are numerous abiotic stressors that reduce farm animal productivity. One of the most globally studied is heat stress (HS), because it compromises almost every metric of agriculture profitability. Suboptimal production during HS was traditionally thought to result from hypophagia. However, independent of feed intake, HS affects a plethora of endocrine, physiological, metabolic, circulatory, and immunological variables. It is becoming increasingly clear that these changes are chronologically causal and that the etiological epicenter is a compromised gastrointestinal track (GIT) barrier. A hyperpermeable GIT allows luminal contents to infiltrate, and these antigens stimulate an immune response with local and systemic inflammatory implications. Once activated, most leukocytes switch from oxidative phosphorylation to aerobic glycolysis, where the glucose requirement of an intensely triggered immune system can exceed 2 kg/d in a lactating dairy cow. Whole body adjustments are coordinated to ensure glucose is prioritized for the immune system and this is primarily characterized by increased basal and stimulated circulating insulin, hypercortisolemia, hyperprolactinemia, and increased hepatic glucose output. This hormonal profile is accompanied by decreased adipose tissue mobilization and skeletal muscle insulin resistance. Interestingly, the aforementioned physiology is almost identical to distinctly different abiotic and biotic stressors. For example, feed restriction, weaning, cold stress, noise stress, and immune-challenged animals all have a similar physiological footprint. Ultimately, these stressors are “psychological” that emanate their pathology from a compromised GIT barrier. Psychological stress negatively affects GIT epithelia via at least 2 mechanisms: (1) mast cell degranulation and (2) immune cell creation of an apical prooxidant environment that paradoxically favors pathogens. Interestingly, stress is often reported to be immunosuppressive and thus the reason why morbidity increases during suboptimal conditions. However, it is not clear whether ex vivo assays reflect immunosuppression or the leukocyte dynamics of an animal in the midst of immune stimulation stemming from a hyperpermeable GIT.

Session 7: Genomics and Epigenetic Impacts on Ruminant Physiology and Efficiency

16 Maternal nutrient supply: Impacts on physiological and whole animal outcomes in offspring.

J. S. Caton*[1], M. S. Crouse[2], C. R. Dahlen[1], A. K. Ward[3], W. J. S. Diniz[4], K. M. Hauxwell[1], and L. P. Reynonds[1], *[1]North Dakota State University, Fargo, ND, [2]U.S. Meat Animal Research Center, Clay Center, NE, [3]University of Saskatchewan, Saskatoon, SK, Canada, [4]Auburn University, Auburn, AL.*

Demands for animal products are projected to increase in the future; consequently, enhancing ruminant livestock production efficiencies in sustainable ways is a major goal for the livestock industry. Developmental programming (DP) is the concept that various stressors, including compromised maternal nutrition, during critical developmental windows will result in both short- and long-term changes in the offspring. Ruminant models of DP indicate that compromised maternal nutrition, including global under- and over-nutrition, macronutrients, and specific micronutrients, including amino acids (Met and Arg), vitamins (folate, B_{12}, and choline), and minerals (sulfur, cobalt, and selenium) can alter offspring outcomes. Data indicate that maternal histotrophic composition, placental function, and likely fetal nutrient supply are altered by compromised maternal nutrition. Likewise, in offspring, visceral organ mass and function, metabolism, growth, and reproduction are affected. Findings from multi-omics approaches demonstrate that compromised maternal nutrition alters transcript abundance, metabolomic profiles, and multiple metabolic pathways. The underlying mechanisms of DP are driven by epigenetic events, which depend on one-carbon metabolism and micronutrient supply. Current findings indicate that DP in ruminants is real, that maternal nutrition can be a major driver of DP, that genomic and metabolomic changes in offspring are modulated by altered maternal nutrition during critical windows of development, and that underlying mechanisms of DP involve epigenetics. Critical research needs in the area of DP in ruminants include enhanced understanding of the underlying mechanisms, practical relevance to production segments and systems, impacts on short- and long-term animal health, and interrelationships with the host microbiome. Additionally, strategic supplementation and precision nutrition approaches should be developed to foster the positive and mitigate the negative aspects of DP to improve efficiencies and sustainability of ruminant livestock production systems. USDA is an equal opportunity provider and employer.

Key Words: developmental programming, nutrition, beef cattle

17 Paternal nutrient supply: Impacts on physiological and whole animal outcomes in offspring.

C. R. Dahlen*[1], K. A. Bochantin-Winders[1], G. D. Ramirez-Zamudio[1,2], M. S. Crouse[3], K. J. McLean[4], W. J. S. Diniz[5], S. Amat[1], A. P. Snider[3], J. S. Caton[1], and L. P. Reynolds[1], *[1]North Dakota State University, Fargo, ND, [2]University of São Paulo, Pirassununga, SP, Brazil, [3]U.S. Meat Animal Research Center, Clay Center, NE, [4]University of Tennessee, Knoxville, TN, [5]Auburn University, Auburn, AL.*

Recent evidence suggests that environmental factors regarding sires can be transmitted through the ejaculate into the female reproductive tract, influencing fertilization, embryo development, and postnatal offspring outcomes. This concept is termed paternal programming, and in rodent models, it has been shown that nutrition can implicitly alter offspring outcomes through sperm epigenetic signatures, DNA damage and oxidative stress, cytokine profiles, and the seminal microbiome. In ruminants, dietary ingredients prompt programmed responses, such as supplementing bulls with omega 3 fatty acids, impacting blastocyst development and transcript abundance. Feeding bulls high-grain diets reduced the proportion of IVF embryos growing to the blastocysts stage, and feeding rumen-protected methionine to breeding rams during the prepubertal period influenced sperm DNA methylation and performance characteristics all the way to the F_3 generation. Global undernutrition or excess in bulls led to changes in sperm morphology, differential expression of mRNA and miRNA, and cytokine profiles, which could subsequently alter the uterine environment, immune response to mating, and embryo growth. Divergent planes of nutrition in breeding rams influenced body weight and morphometric characteristics of the offspring at birth that persisted into the postnatal development period of the lambs, affecting growth rate and feed intake. Interestingly, sire nutrition also resulted in alterations in concentrations of IGF-1 and insulin in response to a glucose challenge. The field of animal science is on the precipice of understanding the paternal contribution to developmental programming in livestock species. Current models are in place to address critical questions such as whether and the extent to which paternal programming effects are present within the bounds of common management scenarios, and whether effects of paternal nutrition interact with effects of maternal nutrition to influence offspring physiology, whole animal outcomes, and herd or flock productivity. USDA is an equal opportunity provider and employer.

Key Words: paternal programming, offspring outcomes

18 Early regulation of mammalian development: Establishing the full extent and mechanistic basis of environmental effects. K. D. Sinclair*, *University of Nottingham, Loughborough, Leicestershire, UK.*

The developmental integrity and well-being of mammalian offspring are influenced by the environment encountered in utero and during infancy. Data exist on the effects of parental body composition and diet, exposure to environmental chemicals and stress (including heat stress) at different stages of early development. Procedures used in assisted reproduction can also influence pregnancy outcomes and well-being of offspring. While most studies report sexually dimorphic responses to these environmental factors and interventions, often there is a lack of consensus on the nature and magnitude of responses. This could in part be explained by specific experimental conditions, although other factors such as genotype and stage of development at point of insult are likely to be important. Although nutrient requirements of the conceptus increase during pregnancy, it is the maturing gamete (sperm and/or egg) and the rapidly proliferating pluripotent cells of the preimplantation embryo that are most sensitive to environmental cues (including parental nutrition), as sweeping epigenetic changes required to facilitate syngamy and subsequent lineage specification occur during these early stages. Nevertheless, with few notable exceptions, reported effects in offspring are subtle, frequently relying on detailed physiological assessments or histological/molecular measurements of altered structure and function to determine consequence. Studies exploring the full extent of exposure to environmental insults are largely absent in farm animals. Such studies would assess the combined effects of "insult" on both parents and cumulative effects of continuous "insult" across the life course of an individual and across successive generations. Simultaneous epigenetic and metagenomic analyses would provide comprehensive insight into cumulative effects and underlying mechanisms, particularly if combined with individual genetic analyses. This would explain variance in responses and help develop personalized mitigation strategies. Funding: R01 ES030374/ES/NIEHS NIH HHS/US; BBSRC BB/R007985/1.

Key Words: gamete, embryo, epigenetics

Poster Session 1: Gastrointestinal Microbiology

P1 Plant extracts of *Tithonia diversifolia* and *Senna spectabilis* are viable alternatives to ionophore antibiotics in improving rumen microbial fermentation. O. Barreto Cruz[1,2] and V. Fellner*[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Universidad Cooperativea de Colombia, Sede Ibagué, Espinal, Colombia.*

Ionophores improve feed efficiency but may result in antibiotic-resistance. Natural plant extracts have similar effects as ionophores to lower microbial methane production and enhance feed efficiency. The objective of this study was to determine the effect of 2 natural plant extracts *Tithonia diversifolia* (*T*) and *Senna Spectabilis* (*S*) as alternatives to antibiotics in ruminant diets. Plant extracts were inoculated for 24 h with mixed rumen microbes in an in vitro batch culture to assess the effect on fermentation. Basal diet consisted of alfalfa hay and concentrate mix in a 60:40 ratio without (C) or with 22 ppm monensin (M) or 4.24 g/kg T and S. Data were analyzed as a completely randomized block design with 2 replications using Proc Mixed procedure of SAS. Total short-chain fatty acids and acetate (A) were not affected by treatment. Compared with C, both M and *S* reduced methane ($P < 0.01$) and increased propionate ($P < 0.02$), resulting in a reduced A:P ratio ($P < 0.01$). M lowered pH compared with C, *T*, and *S* (Table 1). Plant extracts supported favorable fermentation, with S showing a greater improvement in feed carbon capture and increased energetic efficiency. Additional research to determine optimal level of plant extract inclusion is warranted. GC-HPLC-MS analyses to identify metabolites in the plant extracts and their impacts will also be presented. Based on our data, plant extracts reduced methane, a greenhouse gas, and improved fermentation efficiency of rumen microbes.

Key Words: plant extracts, methane, rumen microbes

P2 Rumen microbes associated with the digestion of plant secondary metabolites of willow *leaves* fed to cattle. C. B. M. Müller*[1,2] and B. Kuhla[2], [1]*University Clinic for Ruminants, University of Veterinary Medicine Vienna, Vienna, Austria,* [2]*Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Tree leaves are widely established as N-rich feed supplement in extensive cattle farming. However, the effects of willow (*Salix* spp.) leaves and their plant secondary metabolites (PSM) on rumen microbes and total-tract digestibility have not been investigated. The aim of this study was to identify rumen microbes associated with ruminal NH_3 concentrations and the digestion of willow leaf PSM in cattle. Eight weaned Holstein bull calves had free access to grass clippings and were supplemented with willow leaves (SAL) or alfalfa hay (CON) in a crossover design. Both diets were formulated isoenergetic (9.7 MJ ME/kg DM) and isonitrogenous (134.5 g CP/kg DM). On each diet, calves were adapted for 2 weeks. Subsequently, feed intake and fecal excretions were quantified for 4 d. Feed and feces were analyzed for concentrations of N, phenols, condensed tannins (CT), and total tannins. Rumen fluid was sampled and analyzed for NH_3 concentration and microbial DNA sequencing. The rumen microbial composition was taxonomically assigned by mothur and Silva databases and analyzed in R. Remaining data were analyzed in a mixed model (fixed effects: diet and block) and correlated in SAS. Feed intake and average daily gain were comparable between groups. The SAL diet contained 5 times more phenols and tannins than the CON diet ($P < 0.001$). The apparent digestibility (AD) of N was 14% diminished in SAL calves ($P < 0.01$) accompanied with reduced rumen NH_3 concentrations. The latter negatively correlated with *Desulfobulbaceae* ($r = -0.5$, $P < 0.1$). The AD of phenols and total tannins increased by 44% and 84% on the SAL than on the CON diet ($P < 0.001$). Ten PSM-degrading rumen microbial taxa were more abundant

Table 1 (Abstr. P1). Effect of *Tithonia diversifolia* and *Senna spectabilis* plant extracts on fermentation after 24 h of incubation in in vitro rumen cultures

	Treatments					
Item	Control	Monensin	*T. diversifolia*	*S. spectabilis*	SE[1]	*P*-value
Methane, mmol/L	5.25^{bc}	4.46^{a}	5.66^{c}	4.99^{ab}	0.22	0.007
pH	5.56^{ab}	5.53^{a}	5.59^{b}	5.60^{b}	0.02	0.021
Total VFA, m*M*/L	106.80	113.08	110.90	107.56	7.91	0.938
Acetate (A)	61.04	59.06	57.28	59.20	2.15	0.699
Propionate (P)	17.87^{a}	24.25^{b}	18.81^{a}	22.54^{b}	1.32	0.018
Butyrate	18.52	14.05	20.46	16.24	2.37	0.334
Isovalerate	1.44	1.45	2.11	1.51	0.33	0.365
Valerate	1.13	1.19	1.34	0.88	0.19	0.451
A:P	3.48^{c}	2.45^{a}	3.03^{bc}	2.66^{ab}	0.19	0.012

[1]SE = standard error.

in SAL than in CON calves. From these, *Eggerthellaceae*, *Rhodocyclaceae* ($P < 0.05$), *Butyrivibrio 2*, and *Denitrobacterium* ($P < 0.1$) correlated positively with AD of phenols. *Tyzerella 3* correlated with AD of CT on the SAL diet (r = 0.87, $P < 0.05$). In conclusion, this study demonstrates that the supplementation of willow leaves induces rumen microbial adaptation processes involving the decrease of NH_3 concentrations and PSM digestion.

Key Words: rumen microbes, phenols, tannins

P3 Energy utilization and rumen microbial composition of phenotypically low and high methane intensity dairy cows. H. N. Cahyo*, P. Niu, V. T. E. Aho, B. Heringstad, P. B. Pope, and A. Schwarm, *Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway.*

Objectives: The study aimed to investigate the difference in energy utilization and rumen microbial composition between phenotypically low and high methane intensity dairy cows. Material and methods: A total of 16 Norwegian Red dairy cows in late lactation (days in milk 251 ± 30) were used in a continuous experimental design. Feces, urine, milk, and rumen fluid were sampled. Methane (CH_4) emissions from individual cows were measured using GreenFeed system (C-Lock Inc., Rapid City, SD). Based on the methane intensity (g CH_4 per kg energy-corrected milk [ECM]), cows were categorized into low (LM) and high (HM) phenotypes, averaging 21 ± 1 and 28 ± 1 g/kg ECM, respectively. Cow breeding values of relevant traits were available from official genetic evaluation in Norway (Geno SA, Norway). Compared with HM cows, LM had on average higher breeding values for 305-d milk yield (111 vs. 93) and 305-d milk protein yield (105 vs. 91). Extracted metagenome-assembled genomes (MAGs; bacteria and archaea) and single amplified genomes (SAGs; protozoa: *Isotricha* spp.) were used to analyze the rumen microbial community structure in LM and HM cows. Statistical analysis was performed using R. Results: The gross energy intake, metabolizable energy intake, and energy loss in the feces, urine, CH_4, and heat were not significantly different between LM and HM, but LM cows had higher energy retention in milk ($P < 0.05$). The metagenomic approach revealed fiber-degrading bacteria in the genera *Ruminococcus* and *Anaerobutyricum* showed greater relative abundance in LM compared with HM ($P < 0.05$). For the archaeal community, one *Methanobrevibacter A* sp. was observed to be less abundant in LM than HM cows ($P < 0.05$). For holotrich protozoa, no significant difference in relative abundance between groups was observed. Conclusions: At a similar level of CH_4 emissions, low CH_4 intensity cows seem to have a greater milk production efficiency, which can only partly be explained by microbial group differences. The major part might be explained by the host genetic merit or metabolic pathways of rumen microbiota.

Key Words: methane intensity, efficiency, microbiome

P4 Rumen microbiome reconstruction following rumen content exchange: Low methane emitters reconstitute while high emitters inherit. P. Niu*[1], C. Kobel[1], V. Aho[1], C. Alvarez[2], E. Prestløkken[1], P. Lund[3], B. Heringstad[1], P. B. Pope[1], and A. Schwarm[1], [1]*Norwegian University of Life Sciences, Ås, Norway,* [2]*Yara International ASA, Oslo, Norway,* [3]*Aarhus University, AU Viborg, Tjele, Denmark.*

Objective: This study aimed to examine the reconstitution of the rumen microbial community after total exchange of rumen contents between low and high methane (CH_4) yielding Norwegian Red dairy cows. Materials and methods: Two low and two high emitters in their first lactation with on average 616 kg BW, 25.2 kg DMI, and 39.0 kg milk yield, were selected from a group of 20 cows. Methane yields measured with GreenFeed of the 2 low and 2 high emitters were 21.7 and 26.0 g CH_4/kg DMI, respectively, before the rumen cannulation. The total rumen contents were exchanged between the 2 groups within 2 h, and rumen content samples were collected on days −5, 0, 2, 7, 20, 25, 50, and 53 from the day of exchange. Shotgun metagenomics and 16S rRNA gene amplicon analysis were employed to assess microbial community α-diversity using the Wilcoxon test and β-diversity through Bray-Curtis distance in R. Results: At the end of the trial, CH_4 yield decreased to 12.4 g/kg DMI in low emitters due to the drop in absolute CH_4 production and remained unchanged in high emitters (25.0 g/kg DMI). Before the exchange, the low emitters exhibited higher ($P < 0.05$) Shannon diversity and higher relative abundance ($P < 0.05$) of species classified as genus *Prevotella* and *Ruminococcus* (shotgun metagenomics) compared with high emitters, whereas no significant differences were observed between the 2 groups after the exchange. β-Diversity analysis showed that post-exchange, the microbiome of low emitters gradually reverted to their original profiles. In contrast, the microbiome of high emitters mirrored that of low emitters and did not revert to its original state. Genome-centric metaproteomics is underway to unveil the microbial community response and its impact on CH_4 yield post microbial exchange. Conclusion: Low emitters demonstrated reconstitution in rumen microbial community structure after significant microbial disturbance, with a significant decrease in CH_4 yield, whereas high emitters retained the donor's rumen microbial community without transitioning into low CH_4 yielders.

Key Words: rumen transfaunation, enteric methane, metagenomics

P5 What is normal? Evaluation of core microbial communities along the gastrointestinal tract of feedlot cattle.

J. D. Young*[1,2], L. J. Pinnell[2], C. Wolfe[2], M. A. Scott[2], T. E. Lawrence[1], J. P. Cavasin[3], J. T. Richeson[1], and P. S. Morley[2], [1]*West Texas A&M University, Canyon, TX,* [2]*VERO Program, Texas A&M University, Canyon, TX,* [3]*College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.*

The gastrointestinal tract (GIT) is a complex organ system that hosts a diverse microbial population. Although the rumen has been extensively studied and its core microbial taxa in cattle have been well characterized, there have been few studies investigating microbial populations within the hindgut. The objective of this study was to identify core taxa within the microbial communities from the entire GIT of feedlot cattle using a unique sample population. Twenty-one steers from 21 feedlot locations throughout the Texas Panhandle were harvested at USDA Establishment #7124. Cattle were representative of populations in the region (live weight = 647 kg ± 46, marbling score = 513 ± 110, yield grade 3.6 ± 0.7). Fecal samples, luminal contents, and epithelial tissue from the rumen, jejunum, and colon were collected from each animal immediately post-evisceration. Microbial populations were investigated via 16S rRNA gene sequencing, and sequenced reads were processed using DADA2. The R packages phyloseq, microbiome, and vegan were used to analyze microbial diversity and composition, core taxa, and statistical analysis. Despite different origins and environmental influences, the ordination of microbial communities measured by NMDS was not different ($P \geq 0.05$) by animal, but community structures were different ($P < 0.001$) between sample sites. Core microbial families were evaluated for each GIT location. *Lachnospiraceae*, *Atopobiaceae*, *Erysipelotrichaceae*, *Ruminococcaceae*, *Anerovoracaceae*, and *Methanobacteriaceae* were determined to be core families along the entire GIT with a relative abundance of 0.1% in 100% of the samples. In the rumen, small intestine, large intestine, and feces, 18, 10, 15, and 20 families were identified as core families, respectively (relative abundance ≥0.1%). This work represents one of the most in-depth investigations into the microbial community structure of feedlot animals' GIT. Given the different feedlot origins, families identified as core are likely important homeostatic elements to the GIT. These data provide valuable comparisons for future work regarding the health and metabolism of feedlot cattle.

Key Words: 16S rRNA, microbiome

P6 Validation of rumen sampling via stomach tubing to inoculate mini dual-flow fermenters.

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Table 1 (Abstr. P6). SCFA pattern in inocula, amounts of hexoses fermented (HF) and fermentation pattern in fermenters at days 6-7

SCFA in inocula	RC	ST	RMSE	*P*
Total (m*M*)	46.9	33.4	6.84	0.008
Acetate, C2 (%)	71.3	70.8	0.92	0.73
Propionate, C3 (%)	19.9	20.1	1.12	0.79
Butyrate, C4 (%)	8.7	9.1	1.39	0.99
HF (mmol/day)	4.52	4.54	0.333	0.57
Specific productions (mol/100 mol HF)				
C2	115.7	116.7	3.41	0.45
C3	41.6	41.2	2.15	0.70
C4	21.4	21.1	1.88	0.64
CH_4	55.5	55.5	0.98	0.98

Validating alternatives to rumen cannulation (RC) to obtain rumen contents for inoculating in vitro devices is necessary due to societal demand for less-invasive techniques in animal experimentation. Our study aimed to compare stomach tubing (ST) and RC for fermentation parameters, methanogenesis, and microbiota composition in mini dual-flow fermenters (working volume of 80 mL, liquid and solid turnover rates of 7%/h and 3%/h). At the start of four 7-d periods, the rumen contents of 2 cannulated dry goats were sampled via ST and RC before feeding, and each condition for each goat inoculum was tested in duplicate on a diet (hay 0.6; barley 0.2, soybean meal 0.2) given at 0830 h and 1530 h. At the end of each period, all 8 fermenters were sampled for fermentation end product production and microbiota composition using 16S metabarcoding (V4/V5 regions). The short-chain fatty acid (SCFA) concentration of the rumen inocula was lower with ST than with RC ($P = 0.008$), with similar proportions of individual SCFA. After 5 adaptation days, fermentation parameters were not significantly different between ST and RT (Table 1). The Chao1 and Shannon indices on 1285 ASV were 1,078 and 5.22 with SR, and 1,069 and 5.22 with RT, respectively, showing no difference in α-diversity between microbiota ($P = 0.42$ and $P = 0.96$). Regarding β-diversity, using Bray-Curtis distance, community structures were similar between SR and RT ($P = 0.36$). In conclusion, the activity and structure of microbiota in fermenters inoculated with ST and RC were similar. Therefore ST sampling is a good alternative to RC.

Key Words: fermenters, microbiota, fermentation

P7 Effect of abrupt transition from high-concentrate diets to endophyte-free tall fescue and alfalfa mixed hay in beef cattle.

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The effects of abruptly transitioning beef cattle consuming 0%, 20%, or 40% distillers grains with solubles to 100% endophyte-free tall fescue and alfalfa mixed hay were evaluated on *Escherichia coli* shedding, ruminal pH, feed intake, water consumption, and growth. Thirty-two Angus-crossbred animals (BW = 359 ± 62.2 kg) in a randomized complete block design were allocated into pens equipped with SmartFeed (C-Lock; n = 8 animals/treatment). Cattle consumed a high concentrate-based diet ad libitum for 35 d on the following treatments: (1) Basal diet without distillers grains with solubles (CTRL), (2) 20% distillers grains with solubles (20DGS; DM basis), (3) 40% distillers grains with solubles (40DGS), (4) positive control = DGS 40% distillers grains with solubles (PCTRL). Then, all treatments except PCTRL were abruptly changed to 100% endophyte-free tall fescue and alfalfa mixed hay for 18 d. Fecal samples, ruminal pH, and body weights were collected on d 0, 4, 11, and 18. 3M EC-Petrifilm was used to enumerate fecal *E. coli* and coliforms. Water and feed intake data were collected daily using the SmartFeed (C-Lock) system. Data were analyzed using the MIXED procedure of SAS, and animal was considered the experimental unit. Cattle on PCTRL had greater ($P \leq 0.0003$) DMI and water intake than other treatments. No treatment × day interactions ($P \geq 0.226$) were observed on *E. coli*, coliform counts, and ruminal pH. However, when comparing the PCTRL to 40DGS on d 18, *E. coli* counts were decreased by 0.81 Log_{10} cfu/g (6.26 vs. 5.46, respectively). Ruminal pH had a day and treatment effect, being lower ($P \leq 0.0001$) on the PCTRL compared with other treatments on d 18. Moreover, treatments did not affect ($P \geq 0.7631$) the BW and ADG of steers. Although an abrupt shift from a high-concentrate diet to endophyte-free tall fescue and alfalfa mixed hay may not be a practical strategy for reducing real-world *E. coli* shedding, this experiment highlights potential avenues to decrease *E. coli* shedding. It also shows a decrease in DMI and water consumption, alongside an increase in ruminal pH, without affecting overall animal performance.

Key Words: *E. coli*, shedding

P8 Identifying microbial biomarkers in the gastrointestinal tract of cattle associated with the presence of liver abscess and the onset of bovine respiratory disease. L. J. Pinnell*[1], J. D. Young[1,2], E. Doster[1], C. A. Wolfe[1], T. W. Thompson[3], T. C. Bryant[4], M. N. Nair[3], J. T. Richeson[2], and P. S. Morley[1], [1]*Texas A&M University, Canyon, TX,* [2]*West Texas A&M University, Canyon, TX,* [3]*Colorado State University, Fort Collins, CO,* [4]*Five Rivers Cattle Feeding, Johnstown, CO.*

Liver abscesses (LA) and bovine respiratory disease (BRD) represent 2 of the largest economic and animal welfare problems facing cattle producers. As the importance of the gut-lung and gut-liver axes in animal health continues to emerge, research targeting the microbiota of the gastrointestinal tract (GIT) within the context of these 2 important diseases is of critical importance. The objective of this work was to identify microbial biomarkers throughout the GIT and in feces that predict the presence of LA or the onset of BRD, or both. Here, we collected samples from the rumen, ileum, colon, and feces from cattle (n = 60) with known LA and BRD status (i.e., healthy or diseased). All samples were analyzed using 16S rRNA gene sequencing and the 341F/785R primer pair targeting the V3–V4 region of the 16S rRNA gene. A combination of DADA2 within the program QIIME2 and the R package phyloseq were used to analyze sequence data. Microbial community composition was compared using generalized UniFrac distances and the R packages phyloseq and vegan. Both LEfSe and ANCOM-BC were used to identify microbial taxa discriminant of disease states. Although overall differences in community composition were not identified, multiple microbial taxa including *Bifidobacterium* spp.—widely considered beneficial to gut barrier function in humans—were differentially abundant in both the foregut and hindgut of cattle that did not have LA. Similarly, multiple fecal taxa were discriminant of cattle that developed BRD, including *Odoribacter* spp., *Parabacteroides* spp., *Oscillospira* spp., and *Prevotellaceae* UCG-003. Together, these results provide support for the importance of the gut-lung and gut-liver axes in cattle health. Further, the establishment of biomarkers within microbial communities from easily accessible sample types (e.g., feces) in predicting disease status in cattle will aid producers in effectively managing these crucially important diseases.

Key Words: microbial biomarker, BRD, liver abscess syndrome

P9 Composited rumen enhancer in milk replacer improves feeding transition of neonatal calves. O. O. Adelusi*[1], D. P. Casper[1,2], and U. Y. Anele[1], [1]*North Carolina Agricultural and Technical State University, Greensboro, NC,* [2]*Casper's Calf Ranch, Freeport, IL.*

Neonatal calves suffer various pathogenic infections that cause enteric and respiratory problems, with disease treatment costs exceeding $250 million annually. Antibiotics are known to lower mortality and calf scours and improve performance; however, their use is federally restricted due to the linkage of at least 2 million annual infections in the US to antimicrobial resistance. It is hypothesized that supplemental formulation of milk replacers with rumen enhancers will improve calf feeding transition, minimize diarrhea, and promote improved animal health and performance. Holstein dairy bull calves (n = 60; ages 2 to 5 d) were grouped into 3 treatments of milk replacers: Control (no rumen enhancer), Added (rumen enhancer added at milk mixing), and Composited (rumen enhancer supplied with milk replacer). The study lasted for 10 weeks, with 6 wk of milk-feeding with starter and 4 wk of only starter feed. Data were collected on starter intake, body weight, linear body measurement, and rumen ammonia, and analyzed using one-way ANOVA. Results showed that calves fed Composited milk replacer had significantly higher weight gain and final body weight when compared with those on Added milk replacer, with the values also tending to be higher than those obtained from the calves in the Control treatment. There were significant reductions in starter feed intake and average daily gain (ADG) of Control calves during weaning (wk 6 and 7), with calves fed Composited milk replacer having the highest starter intake and ADG during the period. Treatments did not significantly affect the overall calf starter intake, linear body measurement, or rumen ammonia. In conclusion, feeding calves with composited rumen enhancer in milk replacer improve calf performance by promoting better calf transition from milk feeding with increased starter feed intake and average daily gain during weaning, and overall body weight of neonatal calves.

Key Words: rumen enhancer, starter feed, average daily gain

P10 Difference of feeding concentrate- versus fermented forage-based starter diets in weaning dairy calves under intensive milk feeding program: Rumen fermentation and microbiome. K. Masuda*[1], M. Nakano[2], H. Kobayashi[2], M. Tohno[2], H. Ishizaki[2], Y. Suzuki[3], H. Lee[1], S. Roh[1], and S. Haga[1,2], [1]*Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan,* [2]*Institute of Livestock and Grassland Science, NARO, Nasushiobara, Tochigi, Japan,* [3]*Graduate School/Research Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan.*

Rearing methods that use forage as a starter are attracting attention. The aim of this study was to compare the effects of feeding concentrate- (C) or fermented forage-based (F) starter diets on ruminal fermentation and microbiome in weaning dairy Holstein calves under intensified milk feeding. Holstein calves were allocated to C or F diets (n = 9, 10). They were fed increasing amounts of each starter combined with intensive milk feeding program until 56 d of age (weaning day). At weaning, rumen fluid samples were collected and rumen fermentation and microbiome measured (16S rRNA metagenomic analysis). We hypothesized that fermented-forage feeding will develop different rumen microbial composition compared with concentrate feeding. The concentration of acetate in the rumen fluid was significantly higher and that of propionate significantly lower in the F group ($P < 0.05$). The F group showed significantly higher rumen microbial diversity than that of C groups ($P < 0.05$). At the genus level, the F group showed a higher abundance of *Rikenellaceae_RC9_gut_group*, *Bacteroides*, and *Christensenellaceae_R-7_group*, whereas in the C group, *Acetitomaculum*, *Succinivibrionaceae_UCG-001*, and *Dialister* were more abundant. *Rikenellaceae_RC9_gut_group* and *Christensenellaceae_R-7_group* showed a positive correlation with the concentration of acetate in the rumen fluid. *Acetitomaculum*, *Succinivibrionaceae_UCG-001*, and *Dialister* exhibited a positive correlation with the concentration of propionate in the rumen fluid. We predicted the functions of the bacterial community using KEGG (Kyoto Encyclopedia of Genes and Genomes), and there was significant difference in carbohydrate metabolism between the groups ($P < 0.05$). From these results, it became clear that feeding fermented forage-based starter led to the acquisition of a different rumen microbiome compared with concentrate-based starter. Our study could contribute to the developing technology to improve rumen development and function of calves.

Key Words: bacterial community, microbial function, rumen development

P11 Evaluation of antiparasitic efficacy of herbal and leguminous plants' extracts: In vitro experimentation on larvae of sheep fecal cultures. C. Ke*[1,2], T. Wuliji[1,2], and T. McFadden[1], [1]*University of Missouri in Columbia, Columbia, MO,* [2]*Lincoln University, Jefferson City, MO.*

Natural plant bioactive compounds have gained greater attention as potential anthelmintic agents due to their sustainable and effective properties. This study aims to evaluate the anthelmintic efficacy of 24 herbal and leguminous plants containing bioactive compounds through in vitro experiments. The following plants, including common chicory (CC), amaranth plant leaves (AL), birdsfoot trefoil (BD), jicama leaves (JL), sericea lespedeza (SL), mimosa tree leaves (ML), narrowleaf plantain (NP), purple prairie clover (PP), sainfoin (SF), hemp leaves (HL), sunn hemp (SH), sweet wormwood (SW), blue sage (BS), broadleaf plantain (BP), basil (BS), goat weed (GW), goldenrod (GR), goosefoot (GT), jicama beans (JB), pokeweed (PW), spurge weed (SW), stinging nettle (SN), tall boneset (TB), and velvet leaves (VL), were freeze-dried, and prepared for in vitro experimentation. Bioactive compounds were extracted by soaking 3 g of dry matter-based samples in 30 mL of deionized water for 24 h; the supernatant was obtained after centrifugation. Sheep fecal samples were incubated at 27°C for 7 d to obtain nematode larvae. Treatment involved administering 3 mL (high) and 1 mL (low) of each extract, along with 3 mL of the dewormer Cytectin (high: 2 mL/100 mL; low: 0.5 mL/100 mL) and Eprinex (high: 1.6 mL/100 mL; low: 0.4 mL/100 mL). Additionally, a control group received 3 mL of water. Approximately 200 larvae were exposed in each treatment and control filtrate medium. The living larvae were recorded at 12 h, 24 h, and 48 h. Statistical analyses were performed using PROC GLM and the Tukey test on SAS. BS, BLP, AL, PW, HV, and SL failed to induce lethal dose at high dosages for 24 h. GW, SW, GF, and TB demonstrated high motility rates, surpassing Eprinex at 24 h in high dosage. An upward trend in the number of deceased larvae during 3 time intervals was demonstrated. Extracts with high dosages showed significant efficiency ($P < 0.001$) compared with those with low dosages. The study highlights the potential of natural plants as effective anthelmintic agents.

Key Words: in vitro experiment, gastrointestinal nematodes, natural plant extracts

P12 Assessment of acidosis risk in beef steers: Relationship to intake, ruminal pH, and the microbiome during the late-finishing phase. C. W. Dornbach and J. C. McCann*, *University of Illinois Urbana-Champaign, Urbana, IL.*

The objectives were to assess acidosis risk during the late-finishing phase and determine the relationship to intake, ruminal pH, and ruminal microbiome composition in beef steers. Dry matter intake (DMI) data from beef steers (n = 733) in 4 contemporary groups were collected during the final 56 d of the finishing phase. Within each contemporary group, steers were fed a common finishing diet consisting of dry rolled corn, modified wet distillers grains with solubles, and corn silage. Twenty steers were selected from the 4 contemporary groups (n = 80) based on divergent classification of acidosis risk. Acidosis risk was determined based on the frequency and severity of DMI fluctuations during the feed intake evaluation period. Before slaughter, rumen samples were collected via esophageal tubing to determine ruminal pH and microbiome composition. Bacterial DNA was extracted from rumen fluid and the 16S rRNA amplicons were sequenced on the Illumina MiSeq platform to generate 2 × 300 bp paired-end reads. Sequencing reads were analyzed using QIIME2. Statistical analyses were conducted using SAS 9.4. Steers considered high-risk for acidosis due to greater DMI fluctuation had decreased ($P = 0.02$) ruminal pH compared with low-risk steers (6.35 vs. 6.50, respectively). Risk classification affected average daily gain ($P < 0.01$), as high-risk steers gained less weight (1.72 kg) compared with low-risk steers (1.94 kg) over the final 56 of the finishing phase. Within the ruminal microbiome, α diversity and richness were not affected ($P \geq 0.37$) by acidosis risk classification. The most abundant genus, *Prevotella* 1, was increased ($P < 0.01$) in high-risk classified steers. Relative abundances of *Methanobrevibacter* and *Succiniclasticum* were also greater ($P \leq 0.03$) in steers classified as higher risk for acidosis. In contrast, steers classified as low-risk had greater relative abundance ($P \leq 0.01$) of *Succinivibrionaceae* UCG-001. Greater acidosis risk based on fluctuation in feed intake was related to decreased average daily gain and ruminal pH, as well as alterations in the ruminal microbiome composition.

Key Words: acidosis risk, rumen microbiome

P13 Evaluation of an exogenous fibrolytic enzyme combination in forage-based growing diets fed to growing beef cattle. M. Nichols*[1], G. Gullickson[1], Y. Entzie[1], S. Underdahl[1], L. Hansen[1], J. Syring[1], J. Wianecki[1], D. Jensen[1], K. Hauxwell[1], T. Winders[2], and Z. Carlson[1], *[1]Department of Animal Sciences, North Dakota State University, Fargo, ND, [2]Danisco Animal Nutrition and Health, Wilmington, DE.*

A potential strategy to improve growth performance in forage-based growing diets is the use of exogenous fibrolytic enzymes (EFE). To examine the effects of supplementation with a blend of fiber-degrading enzymes on growth performance of steers fed forage-based growing diets, 73 crossbred steers (287 ± 19 kg initial BW) were fed 1 of 4 treatment diets for 84 d. Treatments were arranged as a 2 × 2 factorial, with 2 levels of wheat straw (WS) inclusion, 5% (LS) or 15% (HS) of diet dry matter; and 2 levels of EFE inclusion, 0 (N) or 750 g/ton (E; Bovizyme CX, Danisco Animal Nutrition & Health, Wilmington, DE) of diet. Body weights and blood samples were collected every 28 d. Individual intake data were collected using an automated feeding system. Average daily gain (ADG), dry matter intake (DMI), gain:feed (G:F), plasma urea nitrogen (PUN), plasma glucose (GLC), and plasma nonesterified fatty acids (NEFA) were analyzed using PROC MIXED. Animal was the experimental unit, and fixed effects were blocks of body weight (n = 3), source of steers (n = 2), WS inclusion level (n = 2), EFE inclusion level (n = 2), and WS × EFE interactions. Statistical analysis showed no WS × EFE interactions ($P \geq 0.18$) for all evaluated traits. The addition of E was not found to affect final BW, ADG, DMI, G:F, or PUN ($P \geq 0.15$). Steers receiving E had greater GLC than those receiving N (4.92 vs. 4.78 mmol/L. respectively; $P = 0.04$). Steers receiving E also had lower NEFA than those receiving N (326.27 vs. 359.71 µmol/L, respectively; $P = 0.05$). Steers consuming LS diets had greater ending BW (413 vs. 393 kg, respectively; $P < 0.01$), ADG (1.50 vs. 1.27 kg, respectively; $P < 0.01$), DMI (8.92 vs. 8.22 kg, respectively; $P < 0.01$), and G:F (0.168 vs. 0.153, respectively; $P < 0.01$) than those consuming HS diets. Steers fed LS treatment had lower NEFA compared with the HS treatment (310.32 vs. 375.66 µmol/L, respectively; $P < 0.01$). This study concluded that increasing WS inclusion level negatively impacted the performance of growing steers, and the inclusion of EFE did not influence growth performance.

Key Words: enzymes, forage, steers

P14 Longitudinal assessment of *Salmonella enterica* prevalence and concentration throughout the gastrointestinal tract of finishing beef steers with and without liver abscesses.

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Our objective was to longitudinally assess the presence and quantity of *Salmonella enterica* throughout the gastrointestinal tract of finishing beef steers with and without liver abscesses (LA). Crossbred steers (n = 225; initial body weight = 353 ± 39 kg) were used in an observational case-control design with individual steer designated as the experimental unit. Nasal, ruminal fluid, and fecal samples were collected at feedlot arrival, one week after adaptation to the finishing diet, and the day before harvest at a commercial abattoir. Healthy and abscessed liver tissue samples were collected at harvest. Steers were fed a steam-flaked corn-based diet without tylosin phosphate. After harvest, LA prevalence was determined, and steers were sorted into those with and without LA. Data were analyzed using the GLIMMIX procedure of SAS. Overall LA prevalence was 18.7% (n = 42). Nasal *Salmonella* prevalence was 34.4% but did not differ between steers with or without LA (P = 0.73), and nasal *Salmonella* concentrations did not differ between treatments (P = 0.85) or collection day (P = 0.50). Ruminal fluid *Salmonella* prevalence was 73.7% but did not differ between treatments (P = 0.83). From feedlot arrival to harvest, ruminal fluid *Salmonella* concentration and prevalence decreased (P < 0.01). Fecal *Salmonella* prevalence tended (P = 0.09) to be 6.4% greater in steers with LA. Conversely, fecal *Salmonella* concentration tended (P = 0.07) to be 5.9% greater in steers without LA. Fecal *Salmonella* prevalence was greatest after transition to the finishing diet (P < 0.01), whereas fecal *Salmonella* concentration was greatest before harvest (P < 0.01). Liver *Salmonella* prevalences were 9.8% and 6.5% for steers with and without LA, respectively, but did not differ between treatments (P = 0.47). Similarly, liver *Salmonella* concentrations did not differ in steers with or without LA (P = 0.18). Overall, these data suggest that *Salmonella* concentration and prevalence were affected by time on feed, but not indicative of LA formation.

Key Words: liver abscess, *Salmonella enterica*, finishing beef steers

P15 Chitosan nanoparticles loaded with dihydromyricetin as a novel delivery model for antibacterial and antioxidant.

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Globally, bovine mastitis mainly uses antimicrobials that significantly affect milk quality and threaten human health. The mammary gland could be damaged by the reactive oxygen species (ROS) accumulating, particularly during the perinatal period. Dihydromyricetin (DHM) has a wide range of pharmacological activities and safety. However, its poor bioavailability, solubility, and absorption have limited its widespread application. To address this issue, a novel chitosan nanoparticle (HACC-NP) loaded with DHM is proposed, which has the potential to overcome delivery problems and enhance antibacterial and antioxidant effects. First, we used the emulsification cross-linking method to load DHM into chitosan nanoparticles (DHM/HACC-NP), and then SEM images and FTIR analysis were collected and suggested that a denser and more packed matrix for DHM/HACC-NP was achieved. Specifically, the size (176.4 nm), PDI (0.21), and zeta potential absolute value (+26.3 mV) confirmed the stability of DHM/HACC-NP. More importantly, DHM/HACC-NP also exhibited fair antibacterial effects with the inhibition zone method, which had a better antibacterial effect on *Staphylococcus aureus* (MIC 0.52 mg/mL and MBC 1.02 mg/mL) than on *Escherichia coli* (MIC 1.02mg/mL and MBC 2.05 mg/mL). It is worth mentioning that DHM/HACC-NP display the ability to scavenge DPPH and hydroxyl radicals in a concentration-dependent manner. These results imply that dihydromyricetin was successfully loaded into chitosan nanoparticles, significantly enhancing antimicrobial and antioxidant effects. This suggests that DHM/HACC-NP offer a novel insight into udder-robust agents and provide a promising therapy strategy in dairy cow mastitis.

Key Words: nanoparticles, mastitis, antioxidant activity

P16 Integrated multi-omics analysis reveals ruminal microbial structure and metabolite activity in dairy cows with mastitis.

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To meet the high quality of milk products, mammary gland health is necessary for the dairy industry. Mastitis is one of the most common and difficult to prevent and treat diseases in dairy cows. Studies have shown that rumen microbiota have important effects on host health, immunity, and productivity. The rumen microbial atlas of cows with different somatic cell counts (SCC) is unknown, and the effect of rumen microbiota on the occurrence and development of mastitis in dairy cows needs further evaluation. We integrate metagenomics and 16S rRNA gene sequencing aims to clarify the mechanisms between rumen microbes and mastitis processing, as well as to show the changes in milk production performance and serum cytokines in cows with either low somatic cell counts (L-SCC group) or high somatic cell counts (H-SCC group). When mastitis occurs, the contents of lactose and fat were

decreased, whereas serum levels of IgG2, IgM, IL-1β, IL-6, and TNF-α were increased. Compared with the L-SCC group, the H-SCC group had lower rumen fermentation product content and higher abundance of *Bacteroidetes* and lower abundance of *Firmicutes*. At the genus level, the H-SCC group showed higher levels of *Prevotella* and lower levels of *Ruminiclostridium* and *Muribaculaceae*. Furthermore, the microbial functional analysis revealed that the differentially expressed rumen microbiota genes were significantly enriched among the top 20 pathways (involving glutathione metabolism, MAPK signaling, etc.). In addition, the correlation network shows that different SCC changes in bacterial genera, such as *Rikenellaceae*, *Muribaculaceae*, and *Christensenellaceae*, were associated with cytokines, rumen fermentation, and milk quality. This study highlights that there are significant differences in rumen microbes among cows with different SCC. These results suggest that modulation of rumen fermentation to improve mammary gland immune function is a strategy that may promote the green and healthy lasting development of dairy industry.

Key Words: metagenome, glutamate metabolism, feed efficiency

P17 Relationships between methane production in the rumen of dairy cows and alterations in the rumen bacterial community structure after feeding on post-extracted black tea lees.

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Objective: The demand for upcycling black tea leaves after extraction (tea lees) is increasing. Ruminal methanogenesis in cows can be mitigated by feeding tea leaves because of their rich PSM content; however, few studies exist on feeding black tea lees (BTL) to dairy cows. In this study, we aimed to elucidate how feeding BTL to lactating cows impacts methane emissions from the rumen and investigate the specific rumen bacterial population that changed in response to BTL and played a role in modifying the methane production levels. Methods: A total of 68 dairy cows were used in 3 feeding trials. Two crossover trials were conducted for 3 weeks: feeding with BTL at 2% dry matter (2% DM; Trial 1) and 1% DM (Trial 2). Trial 3 was a 12-week feeding trial of 2 groups: BTL up to 2% DM and a control. Rumen fluid was collected using a gastric tube at 3-week intervals during each trial. The fluid was used for bacterial 16S rRNA gene amplicon sequencing analysis and subjected to ex vivo cultivation, which simulates rumen fermentation, to determine methane production. Results: Methane production significantly decreased post-feeding compared with pre-feeding. Multiple linear regression analyses of methane production, amount of BTL fed, and milk production of the cows suggested that feeding 2% BTL suppressed methane production by 9% in a cow with a 40-kg daily milk yield. Principal coordinate analysis was performed to determine rumen bacterial community shifts caused by BTL ingestion; proportional changes occurred in the major bacterial phyla (*Bacteroidota*, *Firmicutes*, and *Proteobacteria*) after BTL ingestion. In conclusion, the composition of rumen microflora changed with BTL feeding, and the change depended on the proportions of major bacterial groups in the rumen of each cow, partly explaining the inconsistency in the degree of rumen methane mitigation in dairy cows that were fed BTL.

Key Words: black tea by-product, ex vivo cultivation

Poster Session 1: Precision Technology and Data Science

P18 A comparison of two remote monitoring technologies to predict feed intake of feedlot cattle. M. M. E. Luke*[1,2], J. E. M. Burgess[1,2], and L. A. Gonzalez[1,2], *[1]School of Life and Environmental Science, University of Sydney, Camden, New South Wales, Australia, [2]Sydney Institute of Agriculture, University of Sydney, Camden, New South Wales, Australia.*

Individual feed intake is required to optimize profitability, feed efficiency, and carcass endpoint, such as weight and fatness. Current methods of measuring individual feed intake for feedlot cattle are expensive and time-consuming and therefore not viable in a commercial setting. The present study used behavior collected from radio-frequency identification (RFID) tags linked to electronic feeders and smart ear tags with accelerometers to predict dry matter intake (DMI) for feedlot steers. A total of 72 steers from 3 breeds were used (Angus, Brahman, and Charolais; n = 24 per breed). Individual DMI was recorded using 10 electronic feeders over 200 d and expressed as kg DMI/d. Data were summarized per animal per day and used to draw a correlation matrix and develop various linear mixed-effects prediction models of DMI using analysis of covariance, with breed and length of the feeding period as fixed effects, and animal and date as random effects. A very low correlation was found between time at the feeders from RFID and eating time measured by smart tags ($r = 0.05$; $P < 0.001$). The RFID data showed moderate precision and accuracy on DMI (kg/d) using a single variable ($R^2 = 0.76$; RMSE = 1.49 kg DM/d) or all variables ($R^2 = 0.77$; RMSE = 1.46 kg DM/d). However, predictions of DMI from smart tags were poorer with both a single variable ($R^2 = 0.63$; RMSE = 1.93 kg DM/d) or all variables ($R^2 = 0.65$; RMSE = 1.80 kg DM/d). Combining both data sets marginally improved predictions ($R^2 = 0.79$; RMSE = 1.39 kg DM/d). The performance of DMI prediction from data provided by RFID tags and smart ear tags may be suitable for some commercial applications; however, further research is required to improve predictions.

Key Words: prediction modelling, dry matter intake, feedlot steers

P19 Improving animal health and welfare by using sensor data in herd management and dairy cattle breeding—A joint initiative of ICAR and IDF. C. Egger-Danner[1], I. Klaas[2], L. Brito[3], K. Schodl[1], J. Bewley[4], V. Cabrera[5], N. Charfeddine[6], N. Gengler[7], M. Haskell[8], M. Iwersen[9], R. van der Linde[10], K. Stock[11], A. Stygar[12], E. Vasseur[13], and M. Hostens*[14], *[1]Rinderzucht Austri, Vienna, Austria, [2]Delaval, Thumba, Sweden, [3]Purdue University, West Lafayette, IN, [4]Holstein Association USA, Brattleboro, VT, [5]University of Wisconsin–Madison, Madison, WI, [6]Confederación de Asociaciones de Frisona Española, Madrid, Spain, [7]Gembloux Agro-Bio Tech, Gembloux, Belgium, [8]Scotland's Rural College, Edinburgh, UK, [9]Veterinärmedizinische Universität Wien, Vienna, Austria, [10]International Committee for Animal Recording, Utrecht, the Netherlands, [11]Vereinigte Informationssysteme Tierhaltung, Verden, Germany, [12]Natural Resources Institute Finland, Helsinki, Finland, [13]McGill University, Montreal, Canada, [14]Cornell University, Ithaca, NY.*

Digitalization is advancing with rapid developments in farm technologies, which has the potential to revolutionize and improve the long-term sustainability of dairy production. Farmers are increasingly implementing sensors and other technologies that monitor various parameters. Large amounts of data are collected, but just a small fraction is currently used along the dairy value chain. This has motivated the International Committee of Animal Recording (ICAR) and the International Dairy Federation (IDF) to start a joint initiative aimed at providing guidelines and best practices for using data from sensors across systems and applications, with a focus on functional traits such as health and animal welfare. The key partners are the ICAR Functional Traits Working Group and the IDF Standing Committee of Animal Health and Welfare, who have formed a network of representatives from various stakeholders and leading scientists. Research and approaches to improve the usability of data are discussed to promote knowledge transfer and practical implementation in the dairy industry. Experiences and best practices are exchanged, and recommendations for the use of sensor data are being elaborated. The results will be broadly disseminated through ICAR and IDF avenues. Furthermore, the collaborations among multidisciplinary experts are enabling a holistic approach to the current challenges faced by the worldwide dairy industry and will facilitate cutting-edge research and innovation. The initiative will be presented, with a progress report on reference standards, harmonized definitions and terminology, as well as recommendations and best practices regarding data cleaning and editing and definition of novel traits using data from sensor technologies in herd management and genetic evaluations.

Key Words: sensors, ICAR-IDF, welfare

P20 Investigation of the efficacy of accelerometers for identifying behavioral estrus in crossbred beef heifers on pasture. R. A. Cushman*, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Knowing estrous status of females can improve reproductive management, but these data are labor intensive to collect. Accelerometers automate collection of timing and intensity of estrus in dairy cows in confinement or on pasture, but the adoption of this technology to range beef cows is slow. The current study was designed to compare accelerometer data against ultrasonographic determination of ovulatory status and visual detection of behavioral estrus in crossbred beef heifers to evaluate the efficacy of the system in correctly identifying behavioral estrus on pasture. Over 3 years, yearling heifers (n = 1,349) were fitted with accelerometers and continuously monitored for 81 d. After 45 d, heifers were submitted for an ultrasonographic examination to determine ovulatory status. Examinations were performed by a trained technician with no knowledge of sensor data. Data were analyzed using the GLM procedure of SAS. At d 60, twice-daily detection of behavioral estrus was initiated and continued for 21 d with no access to the sensor data. In all, 78% (n = 1,049) of heifers had a corpus luteum (CL) at ultrasonographic examination. A greater percentage of the heifers identified with a CL exhibited regular estrous cycles (78.2 ± 1.3% vs. 51.3 ± 2.9%, $P < 0.01$) according to sensor data. Inter-estrous interval was shorter in heifers with a CL (21.0 ± 0.2 d vs. 25.2 ± 1.2 d, $P < 0.01$), but peak intensity of estrus, when confirmed by visual observation, did not differ between heifers with or without a CL (84.8 ± 0.6 au vs. 83.2 ± 1.4 au, $P = 0.28$). Sensor-based estrus and visual estrus agreed in a greater percentage of heifers with a CL than heifers without a CL (80.0 ± 1.2% vs. 56.0 ± 2.9%, $P < 0.01$). In all disagreements (n = 342), there was not a sensor-based estrus reported, although behavioral estrus was identified by visual observation. Much of the disagreement appears to be in females demonstrating abnormal behavior that does not trigger an event with sensors. Automated interfacing of accelerometer data and ultrasound data could improve diagnosis of estrous status.

Key Words: beef heifers, accelerometers, ovarian function

P21 Surrogate modeling to explore associations between stocking density and environmental footprints.

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As we explore strategies to incentivize adoption of climate-smart grazing systems, there is a need for producer-friendly guidance on how management choices influence the environmental impact of their operations. The objective of this study was to use Bayesian network analysis as a tool for surrogate modeling of associations between stocking density and predicted environmental footprints. The hypothesis was that the network could provide a simplified tool to identify changes in environmental footprints linked to varying stocking densities, aiding on-farm decision making. The USDA Integrated Farm System Model (IFSM) version 4.6 was used to run 576 simulations varying in inputted stocking densities, grass types, soil types, topography, and fertilizer applications. Data from these simulations, involving 22 variables (animals, acreage, crop and soil variables, as well as feed and grazing variables), were used for network derivation. Bayesian learning network (BLN) analysis, using the bnlearn package of R, was leveraged to identify the graphical structure and derive the network parameters. To explore capacity of the network to serve as a surrogate model for the more complicated IFSM, the root mean squared error (RMSE) was estimated for each environmental impact output targeted (carbon, reactive nitrogen, blue water and green water footprints, along with carbon dioxide, methane, manure, animal, and land emissions). The BLN revealed the relationships between variables. The model's predictions, with root mean squared errors (RMSE) ranging from 1.99% to 19.8%, reflected a high level of accuracy in serving as a surrogate for IFSM, while packed within a tool that is considerably simpler to use. This study demonstrated the opportunity to leverage BLN for surrogate modeling of environmental responses to management choices, which may be appropriate and useful in extension and education contexts where accurate directionality of predictions is sufficient to support user understanding of the linkages between management and environmental outcomes.

Key Words: IFSM, Bayesian environment

P22 Predicting and forecasting fluctuations in dry matter intake in Holstein cows using machine learning.

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A data set was compiled to include variables of interest from experiments conducted at the University of Illinois, including a total of 510 dairy cows from 12 different experiments. Cows were identified as being in stage 1 (−30 to −1 d relative to calving [DRC]), stage 2 (1 to 30 DRC), or stage 3 (31 to 350 DRC), and DMI curves were smoothed to decrease noise. Peaks and valleys were identified for individual cows on smoothed DMI. Machine learning was used for predicting and forecasting DMI. Regression models were used to predict DMI and long-short-term memory deep learning time series were used to forecast 7 d of DMI based on a 7-d observation period from either actual or predicted DMI. Two regression models, fine tree and Gaussian process regression (GPR), were compared to determine accuracy. The GPR rational quadratic model was most accurate compared with the fine tree, with root mean square error (RMSE) of 5.98 and 6.98 and R^2 of 0.82 and 0.76, respectively, when the model included days in milk, body condition score, body weight, and diet components. The average lengths from peak to peak were identified as 5.77, 7.01, and 7.40 d for stage 1, 2, and 3, respectively. The average lengths from peak to valley were 3.04, 2.24, and 3.71 d for stage 1, 2, and 3, respectively. The RMSE of the forecasting model for actual, smoothed DMI was 6.15, whereas it was 5.67 when predicted, smoothed DMI was used. The forecasted days at peak, DMI at peak, difference between forecasted days at peak and observed days at peak, and difference between forecasted DMI at peak and observed DMI at peak were not statistically different when the actual or predicted DMI was used as the input for the forecasting model. In conclusion, a model to predict and forecast DMI was developed through machine learning. Therefore, commercial farms that are not able to measure individual DMI will be able to obtain it using the model and dietary information. Additionally, farmers could count on the forecast prediction to identify potential cows that may get sick by deviating from their usual DMI pattern.

Key Words: feed intake, machine learning, forecast

P23 CalfSim tool: A customizable model and online tool to simulate calf performance under different nutritional strategies.

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Developing effective nutritional strategies for preweaning calves is fundamental for ensuring their health, welfare, and future performance. The availability of tools for designing customized nutritional plans based on farm-specific conditions is often limited. Therefore, we developed the CalfSim tool, a free and user-friendly tool that aims to allow consultants and researchers to customize nutritional models and benchmark performance under different nutritional strategies. As a basis for developing the tool, we used the equations for calf starter intake and nutritional requirements presented in the National Academies of Sciences, Engineering, and Medicine (NASEM, 2021). In determining the metabolizable energy (ME; Mcal) of the calf starter, in addition to its chemical composition, we used the cumulative intake of non-fibrous carbohydrates (NFC) as an indicator of gastrointestinal tract maturity. We developed the CalfSim tool using the package and framework for web application development called Shiny as a backbone, available in the R programming language. In the CalfSim tool, the user is required to input information regarding calf initial weight (kg), weaning age (days), average temperature (°C), nutritional composition of whole milk or milk replacer, and number of scenarios to be simulated (from 1 to 4). Within each scenario, the user has the flexibility to specify the daily quantity of milk or milk replacer provided to the calves. The user must enter the nutritional composition of the calf starter to estimate its ME value. After entering the information, the user must run the model and analyze the outcomes summarized in a dashboard, comparing each simulated scenario in terms of key performance indicators, such as final weight (kg), average daily gain (kg), and age at accumulated consumption of 15 kg of NFC. Thus, the CalfSim tool can help farmers and consultants to simulate and optimize nutritional plans for calves, guaranteeing performance based on farm-specific conditions.

Key Words: nutrient requirements, modeling

P24 Prediction of ruminal VFA concentrations from sensing of rumen environment.

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Traditional methods for monitoring ruminal volatile fatty acid (VFA) concentrations require specialized experimentation and are often costly. This study assessed the feasibility of using a sensor suite to predict ruminal VFA dynamics. The sensor suite included a DF Robot Bluno microcontroller, SD card reader, and battery, housed within a waterproof junction box, and wired, waterproof sensors extending from the junction box to monitor aqueous temperature, dissolved oxygen (DO), oxidation reduction potential (ORP), and pH. During sample collection the junction box was attached to a harness outside the cannula, and the sensors extended into the rumen through a hole in the cannula plug. Data were collected through a crossover experimental design with 4 ruminally cannulated cows (3 Holstein, 1 crossbreed) consuming 2 dietary treatments over two 3-d periods. Diets included either a high-forage or a high-concentrate diet. Cows were fed twice daily (0600 h and 1800 h). Dietary treatments were switched between periods. Rumen fluid samples were collected hourly between 0600 h and 1800 h and analyzed via gas chromatography for VFA concentrations. Sensors were placed before the first sample collection and removed following the final sample collection on d 3, logging at 0.1 Hz. Data were prepared for analysis by omitting sensor measurements outside the physiological range and aligning sensor readings with VFA measurements by time of sample collection. These data were split into 3 parts with 15% for hyperparameter tuning, 55% for model training, and 30% for independent evaluation. Random forest regressions were fit using temperature, DO, ORP, and pH to predict concentrations of acetate, propionate, or butyrate. The concordance correlation coefficient (CCC) was calculated from model predictions and evaluation data to explore the ability of the sensing system to predict VFA concentrations. The CCC of acetate, propionate, and butyrate were 0.967, 0.961, and 0.976, respectively. Based on the success of this system to predict VFA concentrations, future work will focus on more comprehensive data collection to support refining prediction algorithms.

Key Words: VFA, sensing

P25 Individual variation in eructation frequency during robotic milking: Detection by applying the sniffer method.

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Frequent release of methane (CH_4) by eructation plays an important role in rumen function and homeostasis; however, the differences in eructation frequency among individuals have not been well known, due to the limited methods to easily measure eructation frequency from ruminants. This study aimed to analyze eructation frequency using gas concentration data obtained via the sniffer method, and to evaluate individual variation in eructation frequency. The CH_4 and carbon dioxide (CO_2) concentrations were measured from individual dairy cattle during milking in automatic milking system (AMS). The measurements were conducted for 7 consecutive days per month over 12 mo. The eructation frequency per AMS visit was determined as the peak frequency using 1-s values of the CH_4/CO_2 ratio. Eructation frequency for each individual was averaged over the 7-d observation period. A total of 291 records (152 ± 80 d in milk and 1.9 ± 1.0 parity, mean ± SD) of 45 animals were used for the statistical analysis. The variance components of the eructation frequency were estimated with a linear mixed model including lactation stage (20-d blocks from 1 to 300 d), parity (1, 2, >2), and measurement month as fixed effects, and animal ID as a random effect. The eructation frequency averaged 0.89 times per minute and appeared to be distributed within a biologically reasonable range (Table 1). Notably, individual variance accounted for 64% of the total variance (sum of the inter-individual and intra-individual variances) in eructation frequency, suggesting that gas concentration data obtained via the sniffer method can be used to detect individual differences in eructation frequency. The results also indicated that the rank of eructation frequency among animals was moderately maintained through lactation stages.

Table 1 (Abstr. P25). Descriptive statistics of eructation frequency (n = 291)

Unit	Minimum	1st quartile	Median	Mean	3rd quartile	Maximum
Times/min	0.52	0.83	0.89	0.89	0.95	1.13

Key Words: dairy cattle, gas concentration peak, lactation stage

Poster Session 2: Whole Animal Microbiology

P26 Whole blood transcriptome and microbial abundance changes in transition cows.

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Limited biomarkers are available for the management of dairy cows during the transitional period, at which dairy cows are challenged with imbalanced metabolism regulation, a dysfunctional immune system, and an increased chance of microbial infection. Blood is easily accessible for molecular marker development. Thus, we evaluated the combined effects of calving and diet changes on the host transcriptome and bacterial load in whole blood from 8 cannulated Holstein cows at 3 time points: 14 d before calving (−14), 2 d (±1 d) postpartum (PP), and 14 d after calving (+14). Before calving, cows were fed a high-forage close-up diet (containing 35.92% corn silage, 37.10% wheat straw, and 26.98% concentrate), and after calving they received a common high cow diet (BMR 31.67% corn silage, 28.00% alfalfa silage, and 40.33% concentrate). Blood samples were collected from the tail vein for transcriptome and microbial abundance analysis by RNA sequencing. We used DEseq2 for host differentially expressed gene analysis (DEG; adj. $P < 0.05$) and DAVID for Gene Ontology (GO) analysis using the top 5% DEGs determined by P-value. Microbial abundance was analyzed by Kraken2 using rRNA microbial sequencing reads. In total 588 DEGs were identified in +14 vs. PP, 545 in −14 vs. +14, and 295 in PP vs. −14. The DEGs between +14 and PP were enriched in biological pathways related to immunity (e.g., GO:0006953, GO:0042098, GO:0018101) and inflammatory response (GO:0006954). We observed no enriched GO terms for DEGs between +14 and −14, or between PP and −14. Compared with −14 or +14, we observed in PP a nearly 5-fold increase in the abundance of *Mycoplasma*, a pathogenic and opportunistic bacterium. The upregulation of inflammatory response pathways postpartum coincides with increased pathogenic abundance in the blood. The DEGs and differential microbial abundances identified in our study lay the foundation for further large-scale study to assess their efficacy as a molecular biomarker for transition cow management.

Key Words: blood transcriptome, microbial community, transition cows

P27 Rumen microbial inoculation effects on the liver transcriptome and meta-transcriptome in weaned calves.

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Rumen microbial inoculation in calves may influence rumen fermentation and animal metabolism, but the effects on the liver are unexplored. Our objective was to evaluate the effects of dosing 3 types of rumen microbial inoculum on the liver tissue transcriptome (LT) and meta-transcriptome (LM) of dairy calves. Fifteen Holstein bull calves were enrolled at birth and randomly assigned to 1 of the 3 inoculum treatments dosed once weekly from 3 to 6 wk of age. Calves were euthanized at 9 wk, with liver tissue collected for LT and LM. Rumen fluid was collected from 4 adult cows and processed as autoclaved (control, C), bacteria-enriched (BE), or protozoa-enriched (PE) inoculum. We used Cufflinks for differentially expressed gene (DEG) analysis ($P < 0.05$, fold-change >1.5), DAVID for Gene Ontology (GO), LEfSE for microbial signatures unique, sPLS-DA for microbial community structure analysis, and the rarefied read count to calculate the diversity. We found 132 genes differentially expressed between C and BE, 112 between C and PE, and 67 between BE and PE. For downregulated genes between BE and C, several GO terms were related to response to microbes and immune response (e.g., GO:0009617), and between C and PE most of the GO terms were in the biological process category (47%; e.g., GO:0005520). For the upregulated genes, between C and both BE and PE, the enriched GO terms were related to cellular components (e.g., GO:0001558). For the LM analysis, we found a lower α-diversity in PE compared with the other groups (Simpson's index, $P < 0.05$). The LEfSe detected microbial signatures specific to C composed of *Acinetobacter* and *Hydrogenophaga*. No unique microbial signature was detected for BE and PE. We found a higher abundance of *Clostridium* in BE and PE and a higher abundance of *Paraglaciecola* in BE. The sPLS-DA showed that BE community structure largely overlapped with C and PE, whereas C and PE showed nearly distinct microbial profiles. Our findings suggest that dosing differentially enriched ruminal microbes can affect LT expression profile and potentially introduce disruption to its native microbial communities.

Key Words: liver microbiome, transcriptome, metatranscriptome

P28 A meta-analysis of 16S rRNA gene amplicon sequencing datasets reveals core vaginal microbiota of ruminant livestock.

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The reproductive tract microbiota is a research area of high interest due to its connection with breeding efficiency and pregnancy outcome. Over the last few years, several studies describing the composition of the vaginal microbiota in various ruminant livestock species have been published.

These studies revealed variability in the vaginal microbial community composition and structure, but they are difficult to compare without a combined sequence analysis. The aim of this study was to systematically compare the vaginal microbiota of sheep and cattle to discover the core vaginal microbiota by performing a meta-analysis of available 16S rRNA gene amplicon sequencing data sets. A literature review was conducted for investigations of the vaginal microbiota of sheep and cattle. Only samples that sequenced the V4 region of the 16S rRNA gene with an Illumina platform were used. The analysis included 968 sheep and 386 cattle samples with a total of 12,845,401 reads from 13 studies (6 sheep and 7 cattle) originating from 5 countries. Examining the total data set, the most abundant phylum was *Firmicutes* at 42.1% relative abundance, followed by *Proteobacteria* with 24.2% and *Actinobacteria* at 8.4%. The most abundant genera in this data set were *Ureaplasma*, unclassified *Pasteurellaceae*, *Histophilus*, and unclassified *Leptotrichiaceae.* Beta diversity showed a distinct separation between sheep and cattle samples. The core vaginal microbiota of cattle included 24 bacterial genera that were at least 70% prevalent across samples, whereas the sheep samples had 8 core genera. The core vaginal microbiota of both cattle and sheep comprised *Bacteroides*, *Corynebacterium*, unclassified *Lachnospiraceae*, and *Treponema.* This is the first study performing a meta-analysis of the ruminant vaginal microbiota. Interestingly, some core microbes are often regarded as pathogenic, namely *Campylobacter*, found in the sheep core microbiota. These results highlight organisms of interest for future functional investigation of the vaginal microbiota in livestock and its associations with pregnancy outcomes.

Key Words: vaginal microbiota, cattle, sheep

Poster Session 3: Post-Absorptive Physiology and Cell Signaling

P29 Serotonin ameliorates ergot alkaloid-induced vasoconstriction of the isolated bovine lateral saphenous vein via 5-HT$_4$ activation. R. J. Trotta*[1], D. L. Harmon[1], H. B. Scroggins[1], H. Ji[2], and J. L. Klotz[3], [1]*Department of Animal and Food Sciences, University of Kentucky, Lexington, KY,* [2]*Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY,* [3]*USDA-ARS, Forage-Animal Production Research Unit, Lexington, KY.*

Ergot alkaloids, such as ergovaline (ERV), can cause potent vasoconstriction through interactions with vascular serotonin (5-HT) receptors. Ingestion of ERV from toxic endophyte-infected tall fescue seed decreases serum 5-HT concentration in cattle. However, postruminal infusion of 5-HT precursors (either tryptophan or 5-hydroxytryptophan) can increase serum 5-HT concentration in cattle. The objectives of the current study were to evaluate the response of ERV pre-contracted bovine lateral saphenous veins when exposed to 5-HT and determine whether the 5-HT$_4$ receptor mediated the response. Cranial branches of lateral saphenous vein were collected from crossbred steers (n = 4), suspended in chambers of a multi-myograph, and equilibrated to 1 g tension for 90 min. Vessels were exposed to either DMSO or a selective 5-HT$_4$ receptor antagonist (GR113808;1×10^{-5} *M*) for 5 min. All vessels were then pre-contracted with toxic endophyte-infected tall fescue seed extract to provide 1×10^{-6} *M* ERV for 30 min. Vessels were exposed to increasing concentrations of 5-HT or a selective 5-HT$_4$ receptor agonist (BIMU-8), and the response was recorded for 5 min. Vasoactive response data were normalized as a percentage of the maximum contractile response induced by the ERV pre-contraction. As 5-HT concentrations increased, there was a dose-dependent relaxation ($P < 0.01$) of the ERV-pre-contracted lateral saphenous vein, with maximal relaxation occurring at 1×10^{-6} *M* 5-HT. As BIMU-8 concentrations increased, there was a dose-dependent relaxation ($P < 0.01$) of the ERV pre-contracted lateral saphenous vein, with maximal relaxation occurring at 1×10^{-4} *M* BIMU-8. Antagonism of the 5-HT$_4$ receptor attenuated ($P < 0.01$) the relaxation induced by 5-HT and BIMU-8. These data provide strong evidence that 5-HT can mitigate vasoconstriction caused by previous short-term ERV exposure via 5-HT$_4$ activation in the isolated bovine lateral saphenous vein. Further research is necessary to determine whether increasing 5-HT bioavailability via 5-HT precursors could mitigate ergot alkaloid-induced vasoconstriction in vivo.

Key Words: ergot alkaloids, serotonin, vasoconstriction

P30 Effects of imidazole dipeptides on myogenic differentiation and proliferation in bovine skeletal muscle-derived cells. J. H. Lee*, X. C. Jin, B. M. Kim, J. S. Park, and H. G. Lee, *Department of Animal Science and Technology, Sanghuh College of Life Science, Konkuk University, Seoul, Republic of Korea.*

This study was conducted to examine the effects of imidazole dipeptides (anserine, balenine, and L-carnosine) on myogenic differentiation in bovine skeletal muscle-derived cells (BSMCs) obtained from cattle muscle tissue. In the initial phase of the study, we examined the impact of these dipeptides on muscle cell proliferation. Cells were treated with varying concentrations (0, 0.01, 0.1, 1, 10 m*M*) of each dipeptide and observed for 24 h. The results indicated that L-carnosine significantly enhanced cell proliferation at a 10 m*M* concentration ($P < 0.01$), whereas anserine and balenine showed no significant impact at any concentration. Further, we assessed the role of these dipeptides in myogenic differentiation. The analysis involved measuring the expression of myogenic regulatory factors (MRFs) such as Myf5, MyoD, myogenin, and Myf6. Treatment with anserine resulted in increased expression of Myf5 at 10 m*M* ($P < 0.01$), whereas the decrease in myogenin suggests an inhibition in the terminal stages of myogenic differentiation. Balenine, at its highest tested concentration, significantly affected only the expression of Myf6 ($P < 0.05$), indicating its role as a factor active in terminal differentiation. In contrast, L-carnosine promoted the expression of myogenin and Myf6 at a 1 m*M* concentration ($P < 0.01$), while also showing a decrease in Myf5 expression at all concentrations compared with the control group, highlighting its potential in enhancing muscle growth and activation. In conclusion, our study provides valuable insights into the role of imidazole dipeptides in muscle cell proliferation and differentiation. These results offer a theoretical foundation for strategies aimed at improving muscle development in cattle, utilizing the unique properties of these compounds to enhance the quality and quantity of bovine muscle.

Key Words: bovine skeletal muscle derived cells (BSMCs), myogenic differentiation, imidazole dipeptide

P31 Multiple mechanisms contribute to the lesser deposition of intramuscular fat than subcutaneous fat in cattle. Z. Tan*, B. Pokhrel, and H. Jiang, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Intramuscular fat (IF) refers to the white adipose tissue deposited between muscle fibers, and its amount and distribution directly determine the quality and value of meat. Compared with other major white fat depots such as subcutaneous fat (SF), IF develops later and grows more slowly, and as a result, the amount of IF in an adult beef is much less than that of SF. Although efforts have been made

to compare IF with other fat depots, our understanding of the biochemical and molecular differences between IF and other fat depots remains insufficient. In this study, we compared adipocyte size, lipid uptake, lipogenesis, proliferation and differentiation of stromal vascular fraction (SVF), and transcriptome between IF and SF in cattle. We collected IF and SF from mature Angus-crossbred heifers at slaughter. Histological examinations revealed that adipocytes of IF were 30% smaller and had a higher percentage of smaller cells than those of SF ($P < 0.05$, n = 6). In vitro assays using a fluorescent fatty acid analog showed that both lipid uptake and incorporation into glycerol were 50% less in IF than in SF ($P < 0.05$, n = 6). The IF SVF did not differ from the SF SVF in proliferation in vitro. However, the IF SVF had a weaker potential to differentiate into adipocytes than the SF SVF in vitro, as evidenced by the lesser amount of lipids accumulated and lesser expression of adipocyte marker genes in adipocytes differentiated from the IF than SF SVF ($P < 0.05$, n = 6). RNA sequencing revealed 539 genes downregulated in IF compared with SF (fold change ≥2, adjusted $P < 0.05$, n = 5). Notable examples of these genes were *FABP4*, *ADIG*, *ADIRF*, *PLIN2*, and *PLIN5*. Functional enrichment analyses showed that monocarboxylic acid binding, fatty acid biosynthesis, pyruvate metabolism, and PPAR signaling pathway were enriched ($P < 0.05$) in genes downregulated in IF. Collectively, these data suggest that multiple mechanisms contribute to the much lesser deposition of IF than SF in cattle, including less adipocyte differentiation, less lipid uptake, less lipogenesis, and lesser expression of genes that mediate these processes.

Key Words: adipose, cattle, intramuscular

P32 Glucose metabolism may differ in mid-lactation cows of high- or low-feed-efficient status.

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There is growing evidence that fundamental metabolic differences can contribute to the unknown variance in dairy cow feed efficiency (FE). The objective of this study was 2-fold: (1) quantify and calculate arterial-venous differences (AVdiff) of various energetic metabolites and (2) determine gene expression of pyruvate carboxylase (*PC*), phosphoenolpyruvate carboxykinase cytosolic (*PCK1*), and mitochondrial (*PCK2*) isoforms, and glucose-6-phosphatase (*G6PC*). Multiparous mid-lactation Holstein cows (n = 64; 93 ± 22 DIM) were enrolled on an 8 wk FE study and fed a diet to meet nutrient requirements. Residual feed intake (RFI) was calculated in wk 6. In wk 8, liver tissue and tail (representing arterial) and mammary vein blood were collected from the top and bottom 20% of cows based on RFI to quantify energetic metabolites. Mammary vein metabolite concentrations were subtracted from arterial blood metabolite concentrations to obtain AVdiff in nutrient absorption. Post-study, the most divergent FE cows were chosen for analysis (n = 4 highRFI [lowFE]; n = 5 lowRFI [highFE]). Data were analyzed (PROC MIXED, SAS, 9.4) with fixed effect of RFI status and random effect of sampling day; means were separated with Tukey adjustment. Expression of *PC*, *PCK1*, *PCK2*, and the ratios were not affected by RFI status ($P \geq 0.27$). Expression of *G6PC* tended to be greater ($P = 0.06$; 0.62 vs. 0.21 ± 0.001 arbitrary units) in highRFI (lowFE) cows compared with lowRFI (highFE) cows. Circulating arterial glucose (glc) tended to be greater ($P = 0.06$; 69.69 vs. 64.52 ± 1.72 mg/dL) in highRFI cows compared with lowRFI cows. No differences between RFI status were observed for mammary ($P = 0.92$; 51.88 vs. 51.54 ± 2.45 mg/dL) and AVdiff ($P = 0.13$; 17.16 vs. 12.95 ± 2.52 mg/dL) glc concentrations. No differences by RFI status were observed for arterial, mammary, and AVdiff for β-hydroxybutyrate, cholesterol, insulin, L-lactate, nonesterified fatty acid, and triglyceride concentrations ($P \geq 0.14$). Blood glc concentrations and glc sparing mechanisms are tightly regulated in ruminants and differences in arterial, but not mammary or AVdiff, may suggest that high FE cows have altered glc metabolism.

Key Words: feed efficiency, glucose metabolism, metabolite

P33 Using supply-demand analysis to investigate the role of peripheral insulin sensitivity changes in the partitioning of glucose entry between mammary and nonmammary sinks in the lactating cow.

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It is widely accepted that the sensitivity to insulin of glucose utilization (U_g) by nonmammary tissues is decreased in early lactation to favor glucose partitioning to the mammary glands. However, this hypothesis has little experimental support and there are many examples of a stronger effect of insulin in early versus late or nonlactating cows. The production (P_g) and utilization of glucose in the fasted, lactating cow can be treated as a metabolic supply-demand system under the influence of insulin concentration. Endogenous P_g constitutes the supply of glucose that is inhibited by insulin, and the sum of mammary and nonmammary U_g is the demand component that is activated by insulin. Where these 2 fluxes cross on a plot of flux versus insulin concentration indicates the steady-state insulin concentration and glucose flux that the system tends toward. To identify the system's tendencies as lactation progresses, Michaelis-Menten supply and demand curves were generated for early- and late-lactation scenarios based on published means for 11 early- and 11 late-lactation, fasted cows subjected to 2 h of intravenous insulin infusion where glucose and essential amino acids were clamped at their basal concentrations. Simulations show that changing the parameters for mammary U_g from late-lactation values to early causes an increase in mammary U_g, and decreases in glucose and insulin concentrations and nonmammary U_g. Switching parameters for nonmammary U_g from late- to early-lactation values, which makes the insulin sensitivity go down, restores nonmammary U_g and drops glucose and insulin concentrations further. Thus, the nonmammary insulin sensitivity becomes low in early lactation, not to provide more glucose for mammary use, but to maintain its own U_g in the face of low insulin. In general terms, partitioning of nutrients to the mammary glands in early- versus late-lactation cows is favored by an increase in the sensitivity of nonmammary tissues to the decrease in insulin concentration that will occur.

Key Words: insulin sensitivity, lactating cow, modeling

P34 Effect of vitamin A-enriched diet at late gestation on intramuscular fat deposition in beef offspring.

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The objective of this study was to assess the impact of vitamin A supplementation of beef cows during the third trimester of gestation on intramuscular adipogenesis and marbling deposition in the offspring. Thirty pregnant Angus cross cows were randomly assigned to 2 experimental treatments at 180 d of gestation: control group (CON, n = 15) receiving a basal diet (56.76 kIU of vitamin A per day) and vitamin A-supplemented group (VITA, n = 15) receiving the same basal diet + 170.36 kIU vitamin A per day. Calves were biopsied within 10 d of age to collect skeletal muscle samples for gene expression and protein abundance for target genes/proteins relating to adipogenesis. All calves were raised under the same conditions until slaughter. Data were analyzed using the MIXED procedure in SAS, maternal dietary treatment used as a fixed effect, and sire was considered as a random effect. Skeletal muscle from VITA calves showed increased mRNA expression of retinoic acid receptor β (*RARβ*; $P = 0.02$), while no differences ($P > 0.05$) were observed in mRNA expression of a marker for adipo/fibrogenic cells (*PDGFRα*), adipogenesis (*ZFP423* and *PPARγ*), and angiogenesis (*VEGFRα*). VITA calves showed greater protein abundance of DLK1 ($P < 0.01$) and PPARγ ($P = 0.02$) than those born to cows in the control group. No differences ($P > 0.05$) among treatments were observed in the abundance of RXR and PDGFRα. Repeated carcass ultrasound measurements of the offspring showed increased intramuscular fat content throughout all the evaluated stages of their post-natal life ($P < 0.05$), while no changes were observed within subcutaneous fat measurements ($P > 0.05$). Hot carcass weight, carcass yield, and dressing percentage and KPH fat percentage were not affected by treatment ($P > 0.05$). A lower Warner-Bratzler shear force value was observed for the control group ($P = 0.04$). These findings suggest that vitamin A supplementation during late gestation enhances intramuscular adipogenesis in offspring. However, the underlying mechanisms of vitamin A direct effects on intramuscular adipocyte population require further investigations.

Key Words: adipogenesis, fetal programming, marbling

P35 Urinary excretion of purine derivatives and microbial protein synthesis of growing steers fed corn or corn-soybean silage at different crude protein levels.

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Supplementing whole-crop cereal silages with forage legume silages can enhance ruminal microbial crude protein (MCP) synthesis, thereby augmenting beef cattle's crude protein (CP) supply. However, the physicochemical characteristics of forage during ensiling and ensiling conditions over time play a pivotal role in determining the proportion of CP fractions. This, in turn, affects the ruminally and post-ruminally available CP. Consequently, the inclusion of CP sources from concentrate may be considered beneficial. An experiment evaluated the impact of crude protein (CP) levels, silage type, and their interaction on the urinary excretion of purine derivatives (PD) and MCP synthesis in growing steers. Sixteen growing tropical crossbred steers were allocated to a 4 × 3 incomplete Latin square design over 17-d periods. The dietary treatments were organized in a 2 × 2 factorial design, featuring 2 main silages (corn silage [CS] and corn-soybean silage [CSS]) offered at 2 dietary CP levels each (115 and 135 g/kg of DM). Urine samples from steers were collected, processed, and analyzed for PD, while MCP was estimated. Data analysis was performed using the GLIMMIX SAS procedure, and least squares means were compared using the Tukey Kramer test, with significance set at $P < 0.05$. The urinary excretion of allantoin remained unaffected by silage type or CP level. However, elevated CP concentration increased urinary uric acid excretion in both silages, with CS exhibiting a higher concentration ($P < 0.05$). Notably, creatinine excretion in the urine of growing steers was lower in CSS diets but higher in CS diets ($P < 0.05$). The MCP yield was not influenced by silage type or CP level. These findings suggest that the choice of CP source in growing steer diets influences protein metabolism, as evidenced by higher creatinine and uric acid concentrations in CS diets than in CSS.

Key Words: silage, crude protein, purine derivative

P36 Metabolic responses to amino acids infused with different energy sources.

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We aimed to investigate the metabolic response of lactating cows when supplemented with amino acids (AA) and 2 energy sources (ES). Rumen-cannulated, multiparous Holstein cows (70 ± 26 DIM, n = 16) were enrolled in a replicated 4 × 4 Latin square study with 21-d periods. Treatments, arranged as a 2 × 2 ES × AA factorial, were ES, isoenergetic (2.86 Mcal/d) amounts of glucose (GLU) or buffered acetate (ACE); and AA: Ile, Leu, and Met (12, 50, and 20 g/d, respectively; ILM) or control (CON; 0 g/d), all dissolved in water and continuously infused into the abomasum, except during milkings. A diet formulated to meet 96% of the energy and 84% of metabolizable protein requirements was fed 6×/d. Ten coccygeal blood samples were collected on d 17–19 of each period, representing hourly sampling. Mammary gland (MG) biopsies of 4 cows were performed on d 21 of each period for RNaseq analysis (n = 16). Averaged cow-period results were analyzed with a mixed model containing fixed effects of ES, AA, ES × AA interaction, square, period, and random effect of cow within square. Differentially expressed genes (DEG; $P < 0.005$) of MG tissues were obtained using edgeR and pathway enrichment analysis of DEG was done with IPA (Qiagen). Plasma glucose and insulin were greater for GLU than ACE (71.5 vs. 69.0 mg/dL and 0.58 vs. 0.45 µg/L, respectively; $P < 0.001$) but acetate (1.24 vs. 0.85 m*M*) and nonesterified fatty acids (0.12 vs. 0.08 m*M*) were greater for ACE than GLU ($P < 0.001$). Insulin (+0.065 µg/L) and acetate (+0.29 m*M*) were also increased by ILM versus CON ($P < 0.004$). Relative to CON, ILM increased plasma Ile to a greater extent for ACE than GLU (+52.4 vs. +27.4 µ*M*; ES × AAL: $P = 0.009$). Plasma Met increased for ILM (46.8 vs. 17.3 µ*M*; $P < 0.001$) and Leu was only affected by ES (123.2 µ*M* for GLU vs. 155.4 for ACE; $P < 0.001$). Total essential AA (EAA) were lower for GLU vs. ACE (−77) while non-EAA (−98) and total AA (−71 µ*M*) were lower for ILM versus CON ($P < 0.008$). Downregulated genes related to lipid and ATP synthesis, and upregulated genes involved with cell structure, division, and migration signaling were observed for GLU versus ACE ($P < 0.05$). Our results suggest ES and AA effects on metabolism are driven by the anabolic effects of insulin.

Key Words: amino acid, energy, insulin

P37 Interaction of energy and amino acids on mammary and adipose gene expression in lactating dairy cows.

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Insulin and amino acids (AA) are required to activate mTOR for milk protein synthesis. The objective of this study was to investigate the effects of energy source (ES) and amino acids (AA) on mammary gland (MG) and adipose tissue (AT) gene expression in lactating dairy cows. We hypothesized that glucogenic energy would potentiate the effects of balancing for specific essential AA on gene expression in MG and AT. Four dairy cows (75 DIM) were enrolled in a 4 × 4 Latin square with four 28-d periods and 4 dietary treatments arranged as a 2 × 2 factorial. Factors were AA: 10% metabolizable AA deficient (DAA), or AA sufficient, balanced for Met, Lys, and Leu (BAA); and ES: glucogenic (GE, 29.5% starch, 3.5% fat) or ketogenic (KE, 21% starch, 6% fat, soy hulls replacing corn). Previously presented results showed independent effects of ES and AA on mammary extraction and uptake of nutrients. There was an ES × AA interaction on plasma insulin concentration ($P = 0.03$). Biopsies were taken 2 h after feeding on d 28 of each period. Extracted RNA was sequenced, aligned to a bovine reference genome, and analyzed in edgeR, with CPM >0.5, fold change = 2, and $P < 0.05$ to compile differentially expressed genes. Expression patterns were analyzed by QIAGEN Ingenuity Pathway Analysis (IPA) with absolute Z-score = 2. Based on IPA prediction in AT, KE decreased activity of 409 upstream regulators including EGR1, and increased activity of 120 regulators. The CXCR4 signaling pathway, involved in fatty acid uptake, storage, and adipocyte homeostasis, was also inhibited. In AT, BAA was predicted to decrease activity of 64 regulators and increase activity of 145. There was an ES × AA interaction for the response of 312 regulators in AT, including EGR2, CEBPA, and SREBF1, and on adipogenesis and regulation of lipid metabolism. In MG, KE inhibited 103 upstream regulators and activated 35 regulators, while BAA inhibited 11 regulators and activated 9 regulators. In MG, there was an interaction on 75 regulators, including insulin, and on the FAK signaling pathway, which activates mTOR. In conclusion, an interaction of dietary ES and AA alters gene expression in metabolic pathways of MG and AT.

Key Words: RNAseq, insulin, amino acid

P38 The effect of dietary fat level on total plasma fatty acids and mammary arteriovenous fatty acid difference in Holstein cows.

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Increasing dietary fat in dairy cow rations is a common strategy to increase energy intake and milk fat yield, but the effects of dietary fat level on circulating fatty acid (FA) concentration and mammary FA uptake are not well described. The objective was to characterize these parameters in cows fed a low- or high-fat diet. The hypothesis was that increasing dietary fat would increase mammary AV difference and extraction efficiency of palmitic acid (PA) and stearic acid (SA). Nine multiparous Holstein cows were arranged in a crossover design with two 14-d periods. Treatments were a low-fat diet (LF; ~2.5% FA) or a high-fat diet which included whole cottonseed, roasted soybeans, and a prilled FA supplement high in PA and SA (HF; ~5% FA) and was previously reported to increase milk fat yield 88 g/d. Arterial (coccygeal) and venous (mammary vein) samples were taken together at 6 time points over the last 48 h of each period to determine plasma FA. Data were analyzed with repeated measures in a mixed model that included the random effects of cow and period and the fixed effects of treatment, time, and their interactions in SAS 9.4. The HF diet increased enrichment of PA, SA, and linoleic acid (LA) in both arterial and venous plasma FA and decreased enrichment of oleic acid (OA) and α-linolenic acid (ALA). HF also increased total arterial FA by 478 mg/L, which was about a 20% increase over LF ($P < 0.0001$). Approximately half of the increase in arterial FA was due to LA (+ 232 mg/L) while smaller increases were also observed for PA, SA, OA, and ALA. There were some differences in the magnitude of the response over the day. HF also increased AV difference of total FA by 30 mg/L ($P = 0.04$), PA by 6 mg/L ($P = 0.003$), and SA by 10 mg/L ($P = 0.002$). In conclusion, increasing dietary FA increases total plasma FA, alters plasma FA profile, and results in a greater AV difference of PA and SA which mechanistically explains increased milk fat yield.

Key Words: palmitic, stearic, lipid metabolism

P39 Grazing high-sugar, low-dry matter forages maintains high rumen pH in cattle outwintering in New Zealand.

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Strip grazing of forage crops is the typical winter feeding strategy for cattle and sheep in New Zealand. A dominant forage is fodder beet (*Beta vulgaris*), a low dry matter (<15%) and high sugar content (>50%) feed of adequate protein content for grazing (10–13%). From a grazing unit of 524 steers (274 ± 14 kg), 4 animals were ruminally fistulated. The steers were fed ad libitum either fresh harvested beet (FB: 12% DM, 52% WSC, 11% CP) or ryegrass (RG: 17% DM, 8% WSC, 15% CP) in pens for 28 d, then metabolism crates for 7 d, with total fecal and urinary collection. For further 2 d indwelling rumen pH was logged from a ventral sac sensor every 15 s, then manual 50 g samples of digesta obtained every 2 h, then 2 d of evacuations every 16 h. The treatment groups were then swapped. Rumen pH was higher in the FB across the diurnal cycle, where it did not decline below 6.2. Total short-chain fatty acid concentrations of rumen fluid at 2 h intervals were higher for the RG, peaking at 10 h post feeding at 141 mmol/L, compared with 122 mmol/L at 8 h post feeding for the FB. Rumen NH_3 in the RG was higher (>150 mg/L) than the FB (<50 mg/L: range 22–48 mg/L), while the daily MCP production (g N/d: via urinary purine) of the FB was approximately double (106.4) the RG (56.7). The mean rumen fluid urea concentration for the RG 6–14 h after feeding ranged from 9 to 13 (mmol/L), greater than the 2–4 (mmol/L) of the FB. Rumen fluid turnover rate between treatments was similar (RG: 21.5%/h; FB: 20.5%/h), while mean daily total water intake was 27.6 L for the RG and 53.8 L for the FB, with mean daily urine volumes of 14.2 and 29.7 L. Despite the higher WSC and lower NDF intakes of the FB, the observed higher rumen pH compared with the RG is likely a consequence of the high rumen water load, as low ruminal NH_3 concentrations enabled the higher observed rumen wall fluid absorption, effectively removing short-chain fatty acids at a greater rate. The high urine volumes observed suggest that adaptation to this novel diet would require both rumen and circulatory changes to maintain the fluid turnovers required to regulate rumen pH.

Key Words: grazing fodder beet, rumen pH, high sugar diet

P40 Altered abundance of proteins in amino acid, fatty acid, and carbohydrate metabolism pathways contributes to variance in residual feed intake.

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Understanding the biology underlying animal variation in feed efficiency (FE) is crucial for determining the contribution of nutrient use efficiency at the tissue and molecular level to improve FE in dairy cows. The objective of this study was to determine what metabolic pathways contribute most to variance in residual feed intake (RFI) by comparing liver proteome in cows with high and low RFI. One hundred sixteen mid-lactation primi- (n = 42) and multiparous (n = 74) Holstein cows were enrolled for 42-d studies in 3 cohort groups. Each cohort received the same diet throughout the study. At the end of the experimental period, RFI for each cow was calculated by statistical determination of the deviation of actual intake of a cow from the expected intake, based on the average of the cohort. Cows within RFI values in the top and bottom 30% from each cohort were deemed most feed efficient (low-RFI) and least feed efficient (high-RFI). Twelve low-RFI (−1.58 ± 0.71) and 12 high-RFI (1.74 ± 1.00) cows with matching parity and days in milk were selected for subsequent liver sampling. Liver tissue was harvested via biopsy and stored at −80°C for subsequent protein extraction and proteomic quantification using iTRAQ-based proteomics with multidimensional LC-MS. Out of the 2,420 proteins identified, 348 were differentially abundant ($P \leq 0.05$ and fold change > ±1.5). Differentially abundant proteins were included to identify functional enriched pathways using String 12.0. Pathway analysis revealed that 32 pathways were enriched (FDR < 0.05) in low-RFI cows compared with high-RFI cows, including TCA cycle, oxidative phosphorylation, thermogenesis, biosynthesis of amino acids, bile secretion, nicotinate and nicotinamide metabolism, butanoate metabolism, and protein export. Overall, these results suggest that various aspects of hepatic amino acid, fatty acid, and carbohydrate metabolism were different between low-RFI and high-RFI cows, which likely contribute to the variance in residual feed intake and feed efficiency of dairy cows.

Key Words: feed efficiency, residual feed intake (RFI), proteomics

P41 Differences in postabsorptive fatty acid oxidation and mitochondrial uncoupling may contribute to variation in feed efficiency in dairy cows. M. Sura[1], J. Daddam[1], C. Collings[1], G. Ahmad*[1], S. Naughton[1], M. VandeHaar[1], H. White[2], and Z. Zhou[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Understanding nutrient use efficiency at the tissue and molecular level is essential for improving the selection for feed efficiency (FE) in dairy cattle. The objective of this study was to determine if hepatic lipid metabolism differed between divergent FE groups by comparing expression of key genes regulating liver fatty acid oxidation in cows with high and low residual feed intake (RFI). One hundred sixteen mid-lactation primi- (n = 42) and multiparous (n = 74) Holstein cows were enrolled for 42-d studies in 3 cohort groups. Each cohort received the same study diet. At the end of the experimental period, RFI for each cow was calculated by statistical determination of the deviation of actual intake of a cow from the expected intake, based on the average of the cohort. Cows within RFI values in the top and bottom 30% from each cohort were deemed most feed efficient (low-RFI) and least feed efficient (high-RFI). Twelve low-RFI (−1.58 ± 0.71) and 12 high-RFI (1.74 ± 1.00) cows with matching parity and days in milk were selected for subsequent liver sampling. Liver tissue was harvested via biopsy for mRNA abundance of genes regulating lipid metabolism. Data were analyzed using PROC GLIMMIX in SAS with fixed effect of RFI and parity. The expression of peroxisome proliferator activated receptor α ($P = 0.03$) and its target gene long-chain acyl-CoA dehydrogenase ($P = 0.02$) were greater in low-RFI cows, suggesting increased long-chain fatty acid oxidation compared with high-RFI cows. In contrast, although carnitine palmitoyl-transferase 1 alpha expression was greater ($P = 0.02$) in high-RFI cows, suggesting increased fatty acid transport across the mitochondrial inner membrane, only expression of uncoupling protein 2 ($P = 0.02$) was greater compared with low-RFI cows, suggesting greater energy dissipated as heat in these cows. Overall, these results indicate that changes in fatty acid oxidation and mitochondrial uncoupling activity may contribute to the variance of feed efficiency in dairy cows. Protein level results are required to support implications of these results.

Key Words: feed efficiency, fatty acid oxidation, uncoupling

Poster Session 4: Immunology, Endocrinology, and Metabolism

P42 Hematology and serum biochemistry indices of indigenous goat bucks offered *Securitas longipedunculata* leaf extracts. K. Shai, M. Mabelebele, S. Lebelo, J. Ng'ambi, and N. Sebola*, *Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Johannesburg, Gauteng, South Africa.*

Hematological analyses are a diagnostic tool for blood-related ailments and dietary toxicity. The current study aimed at evaluating the effect of *Securidaca longipedunculata* leaf extracts (SLLE) dosing level on hematological and serum biochemical indices of indigenous goat bucks. The effects of dosing varying levels of SLLE on hematology, and serum parameters of 16 indigenous goat bucks at pubertal stage (12–18 mo old) weighing about 23 ± 2.5 kg were used. The bucks were randomly allotted to 4 treatment diets in a completely randomized design with each treatment having 4 bucks replicated twice with 2 animals in each replicate, and the bucks were an experimental unit. The bucks were kept in groups A (control), B, C, and D and were dosed with SLLE in their diet at concentrations of 0, 50, 100, and 125 mg/kg body weight respectively for 4 wk. At the end of the study, the data were subjected to a one-way analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.3. Mean differences with values of $P < 0.05$ was considered statistically significant and were separated using the Duncan multiple range test. The results revealed that serum urea, nitrogen, cholesterol, and glucose were not influenced by the incremental levels of SLLE. However, repeated measures analysis revealed a significant diet × week interaction ($P = 0.006$) on total serum protein. The highest total serum protein was observed on goats reared on D_{0}SL; D_{100}SL and D_{125}SL, with the lowest being on those reared on D_{50}SL during wk 1 of the study. The highest ($P < 0.05$) red cell distribution width (RDW) was observed on goats reared on D_{100}SL. The regression analysis revealed that RDW width decreased quadratically [$Y = 24.4(\pm0.67) - 0.05(\pm0.02) + 0.0004(0.0002) X^2$; $R^2 = 0.28$] with incremental levels of SLLE. Furthermore, the platelet count decreased linearly [$Y = 691.33(\pm110.44) - 5.92(4.42)$; $R^2 = 0.45$] with the incremental levels of SLLE. Inferences can be made that SLLE can improve blood-related parameters of the indigenous goats without compromising their health performance.

Key Words: hematological analysis, medicinal plant, ruminant

P43 The effect of *Securidaca longipedunculata* leaf extract on semen quality and blood serum levels of estrogen and testosterone of indigenous goat bucks. K. Shai, M. Mabelebele*, S. Lebelo, J. Ng'ambi, and N. Sebola, *Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Johannesburg, Gauteng, South Africa.*

The current study aimed to evaluate the effect of *Securidaca longipedunculata* leaf extracts (SLLE) dosing level on the semen quality and blood serum levels of estrogen and testosterone of indigenous goat bucks. Sixteen indigenous goat bucks at pubertal stage (12–18 mo old) weighing about 23 ± 2.5 kg were used. The bucks were randomly allotted to 4 treatment diets in a completely randomized design with each treatment having 4 bucks replicated twice with 2 animals in each replicate, and the bucks were an experimental unit. The bucks were kept in groups A (control), B, C, and D and were dosed with SLLE in their diet at concentrations of 0, 50, 100, and 125 mg/kg body weight, respectively, for 4 wk. All of the data collected for semen and sperm characteristics were subjected to a one-way ANOVA using SAS version 9.3. Mean differences with values of $P < 0.05$ were considered statistically significant and were separated using the Duncan multiple range test. The current study has shown that various dosing levels of SLLE influenced semen volume, pH, and concentration except for color. At D_{50}SL and D_{100}SL SLLE favored spermatogenesis as it is envisioned by the increase in sperm concentration and semen volume with incremental levels of SLLE. The dosing levels of SLLE decreased semen volume and faded semen color and further influenced sperm motility and velocity. Moreover, D_{100}SL resulted in a higher percentage of sperm cells, while D_{125}SL had the lowest percentage of live sperm cells. Furthermore, the SLLE doses in indigenous goat bucks had no significant effect ($P > 0.05$) on estrogen and testosterone levels. However, a numerical increase was noted in both estrogen and testosterone with the incremental levels of SLLE. The use of SLLE has the potential to improve reproductive performance as seen in the improvements of semen, sperm characteristics, and estrogen and testosterone of indigenous goat bucks. Thus, SLLE can be positively used to enhance the reproductive performance of indigenous goat bucks.

Key Words: sperm cell, goat buck, reproductive hormone

P44 Effects of amount of lactose in milk replacer on gastrointestinal permeability and hepatic inflammatory-related mRNA expression in dairy calves. R. Fukumori*[1,2], T. Nakayama[1], M. Hirose[1], I. Norimura[1], K. Izumi[1], K. Shimada[3,5], H. Mineo[4], M. Steele[5], S. Gondaira[1], H. Higuchi[1], T. Watanabe[1], H. Ueda[1], T. Sano[1], K. Chisato[1], and S. Oikawa[1], [1]*Rakuno Gakuen University, Ebetsu, Hokkaido, Japan,* [2]*University of Wisconsin, Madison, WI,* [3]*ZenRakuRen, Shinjuku, Tokyo, Japan,* [4]*Hokkaido Bunkyo University, Eniwa, Hokkaido, Japan,* [5]*University of Guelph, Guelph, Ontario, Canada.*

Feeding a high amount of lactose in milk replacer (MR) is concerning, as it can cause diarrhea due to increased osmotic pressure or exceed the limit of lactose digestibility. The objective of this study was to evaluate the effects of a high amount of lactose in MR on gastrointestinal weight, small intestinal villus length, intestinal permeability, fecal properties, and inflammation-related mRNA expression in the liver of dairy calves. Fifteen Holstein bull calves were randomly assigned to one of 3 dietary treatments differing in MR lactose content (L: 38%, M: 41%, and H: 46%) and raised to 30 d of age. After birth, calves were provided colostrum with no difference in serum immunoglobulin G concentration between the treatments. Feeding of the test diets was started at 1 d of age and gradually increased to a maximum feeding rate at 20 d of age (L: 1.16 kg/d, M: 1.21 kg/d, and H: 1.26 kg/d DM). Under these conditions, the lactose dosages for the treatments were 441, 496, and 580 g/d, respectively. The MR feeding and dilution rates were prepared to ensure isocaloric and osmotic conditions. Fecal scores were recorded daily. Around 30 d of age, calves were slaughtered, and gastrointestinal tissues and livers were sampled. Intestinal permeability was evaluated using the Ussing chamber method, and the permeability in the duodenum and ileum was lower in H and M calves than in L calves; the permeability in the jejunum was also lower in H calves than in L calves. The mRNA expression of toll-like receptor-2, IL-1β, and tumor-necrosis factor-α mRNA in the liver was also lower in H and M calves than L calves. Linear and quadratic increasing trends in tissue-to-BW ratios were observed from the rumen to the abomasum and from the duodenum to the rectum. Fecal score and number of days of diarrhea were not affected by diet. These results indicate that feeding high-lactose MR may increase gastrointestinal weight and function without adversely affecting the gastrointestinal tract of calves.

Key Words: gut inflammation, lactose, milk replacer

P45 Investigations on the influence of different forage proportions in rations of fattening bulls on protein, fat and energy accretion.

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The current recommendations for energy and nutrient supply for growing male cattle of the Holstein breed are under evaluation. Therefore, the aim of this study was to investigate the nutrient and energy accretion of fattening bulls. Fifty-six fattening bulls of the German Holstein breed were slaughtered at different body weights (mean 494 kg, minimum 218 kg, maximum 787 kg). Eight animals were slaughtered at the start of the experiment and served as an initial group (IG). The remainder animals were divided into 2 feeding groups. Over the experimental period (43 wk in total) the animals were slaughtered after different weeks distributed over the entire experimental period. One-half of the animals received a ration with a high forage proportion (HF, 80% forage on dry matter (DM) basis) and the remainder of the animals received a low forage proportion (LF, 40% forage on DM basis) in the ration. During the slaughter process bull's body parts were assigned to different fractions, which were homogenized, weighted, and analyzed for DM, fat, protein, and ash content for determination of body composition (BC). The BC data were analyzed with linear or nonlinear regression models to obtain the protein, fat, and energy accretion by using the Statistica software version 13. The BC data of the IG were used as starting values for the HF and LF group. The results are related to a weight range from 200 to 800 kg live weight. Protein accretion was 116 g per kg live weight gain for the HF group and 119 g per kg for the LF group. Converted to nitrogen (N) the animals of the HF group retained 18.6 g N and the animals of the LF group retained 19.1 g N per kg of live weight gain. Fat accretion was 291 g per kg live weight for the HF group and 316 g per kg live weight gain for the LF group. Energy accretion was 13.8 MJ per kg live weight for the HF group and 14.9 MJ per kg live weight gain for the LF group. Under conditions of the present experiment, the different forage proportions in the ration influenced the accretion of protein, fat, and energy of growing male cattle only marginally.

Key Words: Holstein cattle, body composition, protein-fat-energy accretion

P46 Production and metabolic responses of periparturient cows grouped by liver activity index.

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Our objective was to relate an objective index of liver activity to production, metabolism, immune activation, and health. Multiparous Holstein cows (n = 67) from 3 experiments were classified retrospectively into tertiles based on liver activity index (LAI) as defined by Bertoni and Trevisi (Vet. Clin. North Am., 29:413) using serum concentrations of albumin, retinol, and cholesterol on d 7, 14, and 28 postpartum. By definition, cows in the upper tertile (UP) have greater liver activity than cows in intermediate (IN) or lower (LO) tertiles. Data were analyzed in a mixed effects model with experiment as a random effect. The DMI was lower ($P < 0.01$) postpartum for LO than for IN or UP, and was lower for LO than for UP on d −7 ($P < 0.05$). Milk yield was greatest for UP, lowest for LO, and intermediate for IN ($P < 0.05$). Health disorders increased with decreasing LAI (21.7%, 42.9%, and 56.5% for UP, IN, and LO). Liver total lipid and triglyceride concentrations increased modestly postpartum and on d 28 were greater for LO and UP than for IN ($P < 0.05$). Serum glucose was greater prepartum and lower postpartum ($P < 0.05$) for UP than for LO. Serum BHB was greater ($P < 0.05$) for LO than for UP on d 1 and 7, whereas NEFA were greater on d −14, −7, 1, and 7 for LO than for IN ($P < 0.05$). Serum Ca and Mg were lower for LO than for UP postpartum ($P < 0.05$). Globulin concentration was lower prepartum for UP than for IN ($P < 0.05$) and lower postpartum for UP than IN or LO ($P < 0.05$). Haptoglobin postpartum was greatest for LO, lowest for UP, and intermediate for IN ($P < 0.05$). Paraoxonase postpartum was greatest for UP, lowest for LO, and intermediate for IN ($P < 0.05$). Aspartate aminotransferase was elevated in LO on d 7 ($P < 0.05$). Total bilirubin was greater for LO than for IN or UP on d −7, 1, and 7. Tocopherol and β-carotene were greatest pre- and postpartum for UP ($P < 0.05$). Classifying cows by LAI reflected activation of the immune system associated with compromised health and production. The highest LAI occurred in cows with short-lived inflammation responses postpartum, associated with the greatest DMI and more favorable metabolic status.

Key Words: immune activation, acute phase response, transition period

P47 The effects of rumen-protected glucose supplementation in transition of high-yielding Holstein dairy cows.

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Need for vastly growing amounts of glucose due to demands of milk production and immune system is one of the driving forces of the transition period challenge of dairy cows. Feeding of rumen-protected glucose (RPG) is proposed to help cope with the period. The objective of the study was to evaluate the effect of peripartum supplementation of RPG on mobilization of body reserves, inflammatory biomarkers, and milk production. Multiparous Estonian Holstein cows ($n = 19$) housed in a tiestall cubicles were randomly divided into 2 groups (control, C; trial, T). Both groups were fed clover-grass silage based diets tailored to meet their metabolizable energy and protein requirements: prepartum 9.1 MJ/kg DM and 77 g/kg DM and postpartum 11 MJ/kg DM and 95 g/kg DM, respectively. Group T ($n = 9$) received an additional 200 g/d of RPG ("GluNergy," Proficio/Centurion). The trial spanned from 7 d preparturition to 21 d postpartum. Milk samples were taken twice weekly from 4 to 21 d in milk (DIM) and analyzed for protein, fat, and lactose. Blood samples were collected at −4, 5, 12, and 19 DIM; albumin (ALB), nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHB) were analyzed with a clinical chemistry analyzer (Randox Monaco) with Randox kits; and serum amyloid A was analyzed with the ELISA microplate reader (BioTek Epoch 2). Mixed modeling was performed with R statistical software (R Core Team). Lipid metabolism describing biomarker NEFA was higher on group T on d 12 (0.711 vs. 1.196 mmol/L; $P = 0.01$), but this did not bring along differences on BHB concentration as its values were numerically similar throughout the trial period. Likewise, acute phase protein concentrations remained similar between groups and no differences were recorded. Total energy-corrected milk yield between 4 to 21 DIM was 75 kg higher in group T (743 vs. 818 kg), but without a significant difference ($P = 0.14$). Our findings suggest that supplementing RPG in the transition period might increase the production of dairy cow without negative impacts on metabolism and inflammation.

Key Words: acute phase protein, metabolism

P48 Characterizing ruminal resident immune cells in lactating dairy cattle before and after subacute ruminal acidosis induction.

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Enhancing gut health of ruminants is a growing area of investigation across species. We characterized whether there is a distinct ruminal immune cell population and whether this population is altered by diet. We enrolled 2 cohorts (3 and 6 cows, respectively) into an experiment. From d 1–14, multiparous lactating cows were fed a high forage diet (CON; 31% aNDFom, 27% starch) then from d 15–21 they were fed a subacute ruminal acidosis diet (SARA; 28% aNDFom, 32% starch) where 1/3 of the forage was replaced with a 50% wheat/50% barley grain mix. Feed intake and milk yield were measured daily and milk components were collected on d 12, 13, 20, and 21. Rumen and fecal pH were measured every 3 h on d 8 and 18. Rumen evacuations were conducted on d 9 and 19 for extraction of rumen papillae samples. Rumen papillae were used for flow cytometric analysis to investigate ruminal immune cells. Tissue was rinsed 3 times in HBSS with 1% antibiotics, then tissue was minced and incubated in digestion buffer for 1 h. The suspensions resulting from the cell isolation procedure were stained for CD45 (leukocyte marker), CD3 (T-cell marker) and CD172a (marker for myeloid cells such as macrophages and dendritic cells). Milk, DMI, rumen pH, fecal pH, and ruminal immune cells were analyzed with linear mixed models as repeated measures. The SARA diet increased DMI and tended to increase milk yield compared with CON ($P \leq 0.06$). The SARA diet reduced milk fat % and increased milk protein % ($P \leq 0.04$). The SARA diet reduced rumen pH ($P = 0.01$) but not fecal pH ($P = 0.24$). For CON and SARA, 85.9% ± 2.57% and 84.3% ± 2.57% of live $CD45^+$ cells were $CD3^+$ and 5.7% ± 1.54% and 7.5% ± 1.54% were

CD172a$^+$, respectively. The remaining, undefined cells constituted 8.4% ± 1.78% and 8.3% ± 1.78% of live CD45$^+$ cells. There was not an effect of treatment on any ruminal immune cell population ($P \geq 0.38$). Our results suggest there is a ruminal immune cell population which is dominated by T lymphocytes. Further investigations are necessary to determine the lineage and function of these immune cells.

Key Words: acidosis, gut health, immunology

P49 Impact of selecting beef heifers with divergent digestible fiber intake when fed 4 different diets.

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This study evaluated the factors influencing intake and fiber digestion efficiency of beef heifers previously selected for high or low digestible neutral detergent fiber (NDF) intake (DFI, g/kg $BW^{0.75}$) when fed a high forage-based diet (70% barley silage:30% pelleted concentrate; DM basis), using 4 diets differing in forage type and forage inclusion. Sixteen Angus × Hereford cross heifers (584 ± 32.2 kg) classified as either high or low DFI (n = 8/treatment) were individually housed for 4 consecutive 28-d periods. The diets fed in each period (P) were (1) 100% grass hay, (2) 100% mature alfalfa hay, (3) 90% dry rolled barley + 10% mature alfalfa hay, and (4) 90% dry rolled barley + 10% grass hay. All diets were fed ad libitum with the first 14 d of each period used for diet adaptation. Heifers were weighed every 7 d throughout the study. Individual feed intake was recorded daily. Chewing activities were analyzed from d 19 to 22 of each period using a scan-sampling method with 5 min intervals. Apparent total-tract digestibility of nutrients was analyzed from d 24 to 28. Average daily gain (ADG, kg/d) was greater for high DFI heifers in P1 and P2 (0.61 vs. 0.13, $P = 0.04$; 0.44 vs. −0.09, $P = 0.02$), with a tendency for higher digestibility of DM and NDF in P2 (56.38 vs. 54.58, $P = 0.07$; 52.58 vs. 48.15, $P = 0.06$). High DFI heifers tended to eat faster (min/kg DM and NDF) in P1 ($P = 0.08$) and P3 ($P = 0.06$) and ate faster in P4 ($P = 0.04$); furthermore, they had a tendency to spend more time ruminating (min/kg NDF) during P3 and P4 (154 vs. 128, $P = 0.06$; 165 vs. 136, $P = 0.07$). Ruminating:eating ratio was greater for high DFI heifers in P1, P3, and P4 (1.61 vs. 1.30, $P = 0.04$; 2.71 vs. 1.96, $P < 0.01$; 2.5 vs. 1.76, $P < 0.01$). This study indicates that selection of heifers for greater DFI may promote greater ADG, which may be in part explained by the differences in chewing behavior.

Key Words: beef cattle, feeding behavior, fiber digestion

P50 Effects of systemic or uterine lipopolysaccharide challenge at 5 or 40 days postpartum on systemic and uterine inflammation in dairy cows.

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Our objective was to investigate the effects of intravenous (IV) or intrauterine (IU) lipopolysaccharide (LPS) challenge at 5 or 40 d postpartum (DPP) on markers of systemic and uterine inflammation in Holstein cows. Cows (n = 48) at either 5 or 40 DIM, blocked by parity, were randomly assigned to receive IV-LPS (0.0625 mg/kg BW [5 DPP] or 0.1 mg/kg BW [40 DPP] over 1 h), IU-LPS (100 mg [5 DPP] or 300 mg [40 DPP] in 20 mL of saline), or 20 mL of saline IU (CON). Cows were examined and sampled for 12 h after treatment and daily until d 7. Haptoglobin, serum amyloid A (SAA), and LPS-binding protein (LBP) were measured in serum, and uterine inflammation was assessed by the proportion of polymorphonuclear (PMN) in endometrial cytology. Multivariable linear regression models with repeated measures were used to analyze the data for each DDP group separately. Haptoglobin did not change following treatment at 5 DPP, but it was greater from 24 to 72 h in IV-LPS (peak 0.56 ± 0.03 g/L) than IU-LPS or CON (0.19 and 0.12 ± 0.04 g/L) at 40 DPP. Serum amyloid A was greater from 12 to 24 h in IV-LPS (peak 131 ± 16 mg/mL) than IU-LPS or CON (116 and 108 ± 16 mg/mL) at 5 DPP, and greater from 6 to 48 h in IV-LPS (peak 134 ± 10 mg/mL) than IU-LPS or CON (72 and 78 ± 11 mg/mL) at 40 DPP. Likewise, LBP was greater in the IV-LPS than IU-LPS or CON from 8 to 24 h at 5 DPP (peak 10.0 ± 0.5 vs. 6.7 and 8.14 ± 0.5 ng/mL) and 40 DPP (9.1 ± 0.6 vs. 5.5 and 5.2 ± 0.7 ng/mL). Endometrial PMN increased similarly in both IU-LPS and CON at 5 DPP, but it was greater at d 1 in IU-LPS than CON (37 vs. 15 ± 4%) at 40 DPP. Dry matter intake was unaffected, but milk yield was lesser at 12 and 24 h in IV-LPS than IU-LPS or CON at both DPP. Systemic endotoxin challenge resulted in pronounced changes in markers of systemic inflammation at 40 DPP, which were more subtle or inconsistent at 5 DPP. Uterine endotoxin challenge caused transient uterine inflammation at 40 DPP as expected, but not at 5 DPP, and it did not alter markers of systemic inflammation

Key Words: endometritis, endotoxin, transition

P51 Associations of animal, dietary, and physiological factors with vitamin B_{12} concentration of cow milk.

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Table 1 (Abstr. P51). Regression results for milk vitamin B_{12} (ng/g)[1]

Independent variables	Intercept	SE	Slope_1	SE	Slope_2	SE	Slope_3	SE	R^2
DIM (X_1)	2.36	0.37	0.007	0.002					0.382
Milk protein (%) (X_1)	−0.963	1.39	1.14	0.37					0.330
Milk fat (%) (X_1)	0.528	0.820	0.599	0.170					0.296
Plasma insulin (µIU/mL) (X_1)	2.02	0.46	0.058	0.023					0.351
DIM (X_1), milk protein (X_2)	−0.702	1.304	0.005	0.002	0.883	0.362			0.411
DIM (X_1), milk fat (X_2)	0.197	0.788	0.006	0.002	0.494	0.166			0.445
DIM (X_1), plasma insulin (X_2)	1.30	0.46	0.007	0.002	0.049	0.021			0.531
DIM (X_1), plasma insulin (X_2), milk fat (X_3)	−0.951	0.915	0.006	0.002	0.048	0.015	0.518	0.188	0.597

[1] All slopes significant ($P < 0.05$); n = 97, except for models including insulin, n = 64.

Milk is an important source of vitamin B_{12}, but factors affecting its concentration are still not well understood. We studied factors influencing the vitamin B_{12} concentration of milk using correlation and single and multiple linear regression analyses. The data consisted of results from 3 physiological dairy cow experiments (n = 64–97) studying the effects of substituting rapeseed meal and grass silage with legume feeds. We tested 166 different parameters, including parity (1–6), DIM (24–290), DMI, digestibility of nutrients, plasma energy metabolites and insulin, milk yield (19–47 kg/d) and composition, including fatty acids. Cow within an experiment was included as random effect in all regression models. If the model did not converge, covariance between random effects was removed. Variance inflation factor was used to check multicollinearity. Average concentration of milk vitamin B_{12} was 3.34 ng/g. Based on correlation analysis, DIM, milk fat and protein, and plasma insulin concentrations were the best predictors of milk vitamin B_{12}. Milk vitamin B_{12} was positively linearly related to DIM; therefore, DIM was included in all multiple regression models. A regression with DIM, plasma insulin, and milk fat explained around 60% of variation in milk vitamin B_{12} concentration (Table 1). The experiment was part of Leg4Life project funded by the Strategic Research Council established within the Academy of Finland.

Key Words: vitamin B_{12}, milk, cow

P52 Biochar as a feed additive in the diet of dairy sheep: Impact on methane production and productive performance.

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The inclusion of biochar in the diet of dairy ruminants has been proposed as a promising strategy to mitigate enteric methane emissions. However, conflicting results were reported regarding its effectiveness. In the current study, 24 multiparous Latxa ewes were used to examine the effect of including biochar in a dairy sheep diet on ewe productive performance and CH_4 production. All sheep received a daily ration based on a vetch hay ad libitum and 800 g of a concentrate containing 0 (CTR, n = 12) or 50 (BIO, n = 12) g of biochar/kg of concentrate dry matter. The biochar was made from wood chips and inoculated with probiotics (*Lactobacillus casei*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*). The experimental period lasted for 45 d, of which the first 7 d were for covariate determination, the following 13 d served for adaptation to diets, and the last 25 d were used for measurements and samplings. Offered and refused forage and concentrate were recorded daily to determine dry matter intake (DMI). Methane production (g/d) was measured using a nondispersive infrared methane detector. Milk production was recorded daily, and composition was analyzed on d 29, 36, and 43. The inclusion of 5% of biochar did not affect the DMI of concentrate or hay ($P > 0.1$) and, as a result, total daily DMI ($P = 0.976$). Daily milk yield and feed conversion efficiency (kg of DM intake/L milk) were not affected by BIO supply. Milk fat content increased in response to biochar feeding ($P < 0.001$), but the contents of crude protein ($P = 0.108$) and lactose ($P = 0.741$) remained unchanged among treatments. Regarding CH_4, feeding biochar to sheep increased daily CH_4 production by 11% ($P = 0.049$) and CH_4 production expressed in g/kg of DMI by 10% ($P = 0.015$) compared with CTR. Methane production expressed in g/mL of produced milk was 21% greater in biochar-fed animals than in the CTR group ($P = 0.012$). In conclusion, the administration of biochar to dairy sheep failed to mitigate enteric CH_4 production, indeed the opposite effect was observed. However, it did not impair animal performance and increased milk fat content.

Key Words: efficiency, methane, milk

P53 Effect of biochar on diet digestibility and methane production in dairy sheep.

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The ability of biochar to reduce enteric methane emissions is a recent finding that has attracted interest among ruminant nutritionists. However, there is still controversy surrounding this anti-methanogenic effect. This study was conducted to assess the impact of biochar on feed intake, CH_4 production and nutrient apparent digestibility in sheep. Six dry Latxa sheep, with an initial body weight of 66 ± 5.5 kg, were used in a cross over design. All sheep were fed a vetch hay ad libitum and 400 g of a concentrate containing 0 (CTR, n = 3) or 50 (BIO, n = 3) g of biochar/kg of concentrate dry matter. The biochar was made from wood chips and inoculated with probiotics (*Lactobacillus casei*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*). The trial consisted of two 24-d periods, of which the first 14 d served for adaptation and the last 10 d for measurement of feed intake, nutrient apparent digestibility and CH_4 production. During the adaptation period sheep were housed in individual pens. In the 10 d of measurements, the first 7 d animals were allocated in metabolic cages and in the last 3 d animals were introduced in respiration chambers. Feeding biochar did not affect daily intake of dry matter (DM; $P = 0.154$), organic matter (OM; $P = 0.141$), neutral detergent fiber (NDF; $P = 0.246$), acid detergent fiber (ADF; $P = 0.525$), and crude protein (CP; $P = 0.571$) compared with CTR. Apparent digestibility of DM ($P = 0.018$) and OM ($P = 0.014$) increased in BIO-fed sheep as a consequence of increased digestibility of NDF ($P = 0.003$). On the contrary, the supply of biochar did not modify ADF ($P = 0.055$) and CP ($P = 0.947$) digestibilities. Feeding biochar increased daily CH_4 production ($P = 0.003$) without affecting CH_4 production per kilogram of ingested DM ($P = 0.862$) compared with CTR. However, a reduction on CH_4 production per kilogram of digested DM was observed on BIO-fed sheep ($P = 0.001$). In conclusion, biochar increased net CH_4 production by increasing the apparent digestibility of NDF. However, biochar had the potential to mitigate enteric CH_4 production per kilogram of digested DM.

Key Words: biochar, digestibility, methane

P54 Hepatic mitochondrial oxygen consumption rate, but not oxidative gene expression, may differ in mid-lactation Holstein dairy cows of divergent feed efficiency.

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Identifying individual animal sources of variance that contributes to residual feed intake (RFI) can contribute to both the understanding of, and further selection for, feed efficiency (FE). The objective of this study was to determine if hepatic mitochondrial function differed between divergent FE groups, by analyzing mitochondrial oxygen consumption rate (OCR; pmol O_2/min per µg of protein) in Complexes I, II, and IV, and by quantifying genes related to complexes or uncoupling. Multiparous mid-lactation Holstein cows (n = 64; 93 ± 22 DIM) were enrolled on an 8-wk FE study and fed a diet to meet nutrient requirements. In wk 6, RFI status was calculated for the cohort. In wk 8, liver tissue was collected from the top and bottom 20% RFI cows. Final whole-study RFI was calculated post-study to identify the most divergent low and high FE cows for tissue analysis (n = 4 highRFI [lowFE]; n = 5 lowRFI [highFE]). Mitochondria were isolated from fresh liver tissue to measure OCR using 2 injection schemes (IS) capturing either Complex I and IV (IS1) or Complex II and IV (IS2) capacity over time. Data were analyzed (PROC MIXED, SAS, 9.4) for gene expression with fixed effect of RFI and random effect of sampling day and for IS with RFI, time, interaction between RFI × time, and random effect of plate. Means were separated by Tukey adjustment. Expression of genes related to complexes or uncoupling were not affected by RFI status ($P \geq 0.11$). An interaction between IS × time was observed, indicating that OCR was greater ($P < 0.0001$) for Complex I and IV (IS1: 10.1 vs. 7.2 ± 0.5) and Complex II and IV (IS2: 15.2 vs. 10.9 ± 0.8) in highRFI cows compared with lowRFI cows. Differences in OCR suggest this may be a source of variance in high and lowRFI cows. This difference may not be reflected in gene expression and the disconnect between complex capacity and gene expression suggests that these genes may not be rate-limiting or indicate OCR. Further work is needed to determine how mitochondrial function influences energy use and whole-animal efficiency.

Key Words: feed efficiency, mitochondrial respiration, electron transport chain

P55 Associations between insulin resistance and inflammation during the transition period in dairy cows.

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The time of development of transition cow insulin resistance (IR), its cause, and role on different pathologies are still unclear. The aims of this study are to compare the results of glucose tolerance tests before and after parturition and to associate them with markers of inflammation. From November 2022 to March 2023, intravenous glucose tolerance test (GTT) was performed a week before (on average −10 DIM) and after parturition (6 DIM) on Estonian Holstein dairy cows (n = 10) that were housed in tiestall cubicles and received a similar diet based on clover-grass silage. For GTT, 150 mg of glucose per kg of BW was infused into jugular vein as 40% solution and blood was sampled at −5, 5, 10, 20, 30, 40, 50, and 60 min relative to the start of the infusion. Insulin was quantified with a microplate reader (BioTek Epoch 2) using ELISA commercial kits (Thermo Fisher) and total insulin response

Table 1 (Abstr. P55). Correlations (r) of insulin area under the curve values (AUC) with acute phase proteins

	DIM −4				DIM 5			
	Serum amyloid A		Albumin		Serum amyloid A		Albumin	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Insulin AUC −10 DIM	0.69	0.04	−0.44	0.24	0.80	0.01	−0.30	0.40
Insulin AUC 6 DIM	0.17	0.67	0.66	0.05	−0.43	0.21	0.47	0.17

above the basal (−5 min) concentration was calculated as the area under the curve (AUC) between 5 to 60 min as IR marker. Additional blood samples were taken from the tail vein on −4 DIM and on 5 DIM. From those samples, serum amyloid A was analyzed with the ELISA kit (Tridelta Development) and albumin with the clinical chemistry analyzer (Randox Monaco) with Randox kits. Pearson correlation coefficients were calculated with a function "cor" of the program "R" (R Core Team). No association of IR marker (insulin AUC) between pre- and postpartum GTT was recorded (r = −0.11; *P* = 0.76). In addition, the inflammation markers were poorly correlated with postpartum insulin AUC (Table 1). Insulin AUC prepartum was strongly correlated with positive acute phase protein serum amyloid A on −4 DIM and 5 DIM. This suggests an interplay between IR and inflammation, and that IR develops prepartum, which supports our previous results.

Key Words: acute phase protein, glucose tolerance test

P56 Effect of feeding sodium butyrate to beef cows during the transition period on glucagon-like peptide 1 and 2 concentrations in colostrum and transition milk.

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Secretion of glucagon-like peptide 1 (GLP-1) and 2 (GLP-2), a gut-derived peptide secreted from intestinal L-cells, is potently stimulated by dietary butyrate provision. Since the hormones contained in milk are transferred from the maternal blood circulation, an increase in circulating levels of GLP-1 and 2 due to dietary butyrate could result in increased levels in colostrum and transition milk. This study evaluated the effect of feeding beef cows with sodium butyrate (SB) during the late pregnancy and early postpartum periods on concentrations of GLP-1 and GLP-2 in plasma, colostrum, and transition milk. Twelve Japanese Black female cows were fed hay and concentrate according to Japanese feeding standard for beef cows (2008) with (BUTY) or without (CON; n = 6 for each treatment) SB supplementation at 1.5% of the dietary dry matter from 60 d before the expected calving date (−60 d) to 4 d after calving. Blood samples were collected 1 h before feeding on −60, −30, and −7 d, and 0 (calving date), 1, 2, and 3 d after calving. Milk samples were collected immediately after blood sampling postpartum. Data were analyzed by ANOVA of JMP® 14 using fit model procedure. Plasma total cholesterol concentration was higher (*P* = 0.04) for the BUTY (151 ± 6.27 mg/dL) than for the CON (131 ± 6.27 mg/dL). In addition, plasma GLP-1 concentration was higher for the BUTY (0.82 ± 0.092 ng/mL) than for the CON (0.50 ± 0.092) at 3 d after calving (*P* < 0.05). In contrast, plasma GLP-2 concentration was not affected by treatment. This study showed for the first time that GLP-1 and GLP-2 were present in bovine colostrum at higher concentrations as compared with in plasma at −7 and 0 d (*P* < 0.01). In contrast, treatment did not affect metabolite and hormone concentrations in colostrum and transition milk. In summary, feeding beef cows with SB during the calving transition period likely increases plasma GLP-1 concentrations postpartum without affecting the components of colostrum and transition milk.

Key Words: glucagon-like peptide, beef cow, colostrum

P57 Impact of ruminally pulse-dosed calcium acetate, butyrate, their combination, or $CaCO_3$ on milk composition.

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Unlike other carbohydrates, increasing sugar in dairy cattle diets often increases milkfat yield. Sugar ferments rapidly in the rumen, essentially pulse-dosing acetate (C2), propionate, and butyrate (C4). Both C2 and C4 are lipogenic. To explore the basis for sugar's milkfat effect, we ruminally dosed calcium (Ca) salts of C2 and C4 commensurate with what may be produced from sugar in cows with high dry matter intakes (DMI). In a 4 × 4 Latin square design with 4 treatments (TRT) in four 1-wk periods, ruminally cannulated lactating Holstein cows (12; averaging 48.8 kg milk, 3.51% fat, 2.75% protein, 4.63% lactose) on a common diet were dosed ruminally with Ca C2 (240 g), Ca C4 (Bu; 160 g), Ca C2 + Ca C4 (Ac+Bu; 240 + 160 g), or ground $CaCO_3$ (112 g; CON) at 2 and 6 h post-0700 h feeding; doses were made iso-Ca with $CaCO_3$. Milk samples from each cow taken at each milking (0330, 1030, 1830 h) the day before and the day of dosing were analyzed for fat, protein, and lactose (Table 1). Component yield (g) for summed 1030 + 1830 h milkings were compared by TRT by subtracting values for day of

Table 1 (Abstr. P57). Difference in milk and components between dosing day and previous day for sum of 1030 + 1830 h milkings[1]

						P-value			
TRT	Ac	But	Ac+But	Con	SE	TRT	Ac*	Bu*	Ac+Bu*
Fat, g	−20.9	−28.6	76.9	−111.4	58.7	0.16	0.28	0.31	0.03
Protein, g	−49.4	34.6	−23.0	−35.2	20.3	0.02	0.62	0.02	0.68
Lactose, g	−76.4	43.7	−46.8	−56.2	36.2	0.07	0.69	0.06	0.86
Milk, kg	−2.0	0.09	−1.0	−1.9	0.66	0.09	0.87	0.04	0.38

[1]Ac = Ca acetate; Ac+Bu = Ca acetate + Ca butyrate; Bu = Ca butyrate; CON = $CaCO_3$ control; TRT = treatment; SE = standard error.

*A priori contrasts of TRT with Con.

dosing minus day before dosing. Data were analyzed with the MIXED procedure of SAS with TRT and period as fixed variables, cow as a random variable, and parity, days in milk (<60 d, >60 d), and 3-d average DMI as covariates. A priori contrasts compared TRT with CON. Significance was declared at $P \leq 0.05$. Relative to CON, fat yield increased with Ac+Bu; protein and milk yield were greater with Bu. For increasing milkfat, we hypothesize that C4 and C2 in excess of basal requirements were both essential for starting and elongating de novo fatty acids.

Key Words: acetate, butyrate, milk fat

P58 Changes in blood metabolites during the transition period of dairy cattle supplemented with live *Saccharomyces cerevisiae boulardii* yeast.

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The transition period in dairy cows is characterized by both nutritional and physiological changes potentially affecting immune function and disease resistance. Dietary supplementation with probiotic products has been tested in ruminants, with varying results. *Saccharomyces cerevisiae* is one of the most used yeast strains in ruminant nutrition, with reported beneficial effects on feed intake, rumen fermentation, and milk production. In a trial performed at the ILVO dairy cattle barn, 40 Holstein Friesian cows in transition received a control diet (CON) or a diet supplemented with *S. cerevisiae boulardii* CNCM I-1079 (Lallemand SAS) from d −28 until +35 from calving (LEV). The live yeast supplementation had beneficial effects on milk production (35.1 vs. 37.3 kg/d, $P < 0.05$), specifically for dairy cows in their first transition period (parity = 2). Improved milk production in early lactation may be associated with an increased risk on negative energy balance and metabolic transition disorders. This was evaluated using blood markers, analyzed at d −21, −1, +1, +3, +7, and +21 from calving. The effect of treatment on blood metabolites was analyzed with a linear mixed model in R with treatment, sampling day, and parity as fixed effects and cow as random effect. Concentrations of NEFA and BHBA were in general higher in parity >2 cows than in parity = 2 cows and followed the expected curves around transition. Concentrations of NEFA were significantly higher in plasma of LEV cows at start of lactation (d +3, $P = 0.023$), but stayed below the reported reference values. Concentrations of BHBA tended to be higher in LEV at d +7 in lactation ($P = 0.062$). Blood glucose and insulin were highest in the days before calving and decreased at start of lactation, whereas blood cholesterol was lowest around calving. Parity, treatment, and the interaction between parity and treatment did not affect glucose, insulin, cholesterol, or the inflammation marker haptoglobin. In conclusion, by promoting fat mobilization *S. cerevisiae boulardii* can increase milk production in early lactation without affecting the risk of metabolic transition problems.

Key Words: transition, yeast, metabolism

P59 Effects of feeding heated lactic acid bacteria on immune response, lactational, and reproductive performances during the transition period in dairy cows.

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Heated lactic acid bacteria (HLB) are lactic acid bacteria stabilized by heat treatment and can be stored for a long time at room temperature. The objective of this study was to evaluate the effect of feeding dairy cows with HLB during pre- and postpartum period on blood concentrations of metabolite and immune substances, performances of lactation and reproduction. Twelve Holstein female cows were blocked by body weight (BW) and parity, and assigned to 2 treatments: CON and HLB groups (n = 6 for each treatment). In both treatments, cows were fed total mixed ration (TMR) ad libitum, but HLB additive was supplemented to TMR at 400 mg/d in the HLB group. Blood samples were collected every week from 4 wk before the expected calving to 4 wk after calving. Milk samples were collected at 6, 8, and 12 wk after calving. Date of first ovulation, first estrus, and conception, and number of artificial insemination (AI) were recorded as reproductive performance indices. Data were analyzed by ANOVA of JMP® 17 Pro using the fit model procedure.

Feeding HLB did not affect BW, colostral Brix value, IgA concentration, or birth weight of offspring. No treatment effect was observed for milk yield and milk component levels. Plasma concentrations of most metabolites and immune substances were not affected by treatment, but treatment by time interaction was observed for plasma alkaline phosphatase concentration, which decreased ($P < 0.01$) at 2 and 4 wk after calving compared with 4 wk before calving in the HLB but did not change in the CON. Number of days to first ovulation was lower ($P < 0.01$) for the HLB (20.5 d) than for the CON (31.6 d). Further, number of AI performed until pregnancy diagnosed tended to be lower ($P = 0.07$) for the HLB (1.62) than for the CON (3.13). In summary, feeding HLB during pre- and postpartum period likely accelerates first ovulation and improves fertility without affecting immune response and lactational performance.

Key Words: lactic acid bacteria, reproductive performance

P60 Effects of maternal vitamin A supplementation on blood parameters of Korean native cows and newborn calves.

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This study aimed to investigate the effects of maternal vitamin A (VA) supplementation on the blood parameters of Korean native cows and their newborn calves, as well as the impact on the birth weight of the calves. Sixty pregnant cows at 3 mo of gestation (96 ± 12 d) were randomly assigned to diets with varying VA concentrations and maintained until parturition: a control group (7,800 IU/kg as fed) and a treatment group (22,800 IU/kg as fed). Blood samples were collected from each pregnant cow at the start of the experiment, at the sixth month of gestation, and after parturition, as well as from the newborn calves at 3 d of age. The birth weights of the calves were also measured. Data were analyzed using Student's *t*-test for pregnant cows and a MIXED procedure with Tukey's test for calves, utilizing SAS 9.4 software. As a result, the cows in the VA treatment group exhibited significantly higher ($P < 0.05$) concentrations of serum VA and total protein after parturition, whereas the albumin concentration was lower ($P < 0.05$) than that in the control group. Regarding the calves, the offspring of the VA treatment group showed significantly higher ($P < 0.01$) concentrations of serum VA and elevated ($P < 0.05$) levels of red blood cell (RBC) parameters, including RBC, hemoglobin (HGB), hematocrit (HCT), and mean corpuscular volume (MCV). Additionally, male calves had significantly lower ($P < 0.05$) serum glutamic pyruvic transaminase (GPT) levels and greater ($P < 0.05$) birth weights compared with female calves. Interestingly, female calves that received maternal VA supplementation showed a higher ($P < 0.05$) birth weight compared with those without. In summary, maternal VA supplementation enhanced postpartum and neonatal serum VA levels, improved RBC parameters in offspring, and appeared to be particularly beneficial for the birth weight of female calves.

Key Words: Korean native cow, newborn calf, vitamin A

P61 Exploring dairy cow metabolism through breathomics: Implementation strategy and findings.

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Frequent eructation in ruminant animals results in a blend of exhaled ruminal and breath volatile organic compounds (VOC). This physiological distinction might limit the applicability of breathomics in describing the metabolic phenotype in cows. The primary focus of this study was to differentiate the origin of exhaled VOC, with the aim of utilizing breathomics for the assessment of dairy cow metabolism. Eighteen multiparous lactating Holstein cows (203 ± 60 DIM), producing 32.7 ± 4.95 kg/d, were enrolled in the study. Cows were fed a similar diet 2×/d at 0800 and 1700 h. Exhaled VOC in breath (Br) and exhalome (Ex; a mixture of ruminal eructation and breath) were separately sampled using a head chamber (GreenFeed System®; GF) 8× to represent every 3 h of a day. Methane (CH_4), originated solely from ruminal fermentation, was used as the marker to differentiate breathing from eructation events. Using a previously established method by our laboratory, GF real-time readings were used to collect eructation CH_4 peak events as Ex samples. A threshold of <100 ppm CH_4 was set to sample Br. Both samples were analyzed using secondary electrospray ionization high-resolution mass spectrometry. Data were analyzed using a mixed model with a random effect of cow, and origin × time interactions. A volcano plot was constructed with Br over Ex mean ratio and fold change to quantify the difference in the metabolite's origin. Putative metabolites annotation and most enriched pathways identification were conducted using MetaboAnalyst 6.0. A total of 1,700 features were detected and classified as Br or Ex (1,173 and 527, respectively). The highest enrichment pathway in the breath of cows was the TCA cycle ($P < 0.01$), detecting metabolites such as fumarate, malate, and succinate. For Ex, pyruvate ($P < 0.01$), propionate ($P < 0.05$), and butyrate ($P < 0.05$) were listed as highly enriched pathways, identifying ruminal VFA such as acetate, propionate, and butyrate. Our study not only established a method but also provided compelling evidence of the potential to implement breathomics as a noninvasive tool for conducting metabolic assessment in ruminant research.

Key Words: dairy cow, metabolomics, breath

P62 Health status and mineral metabolism of dairy cattle fed adjusted mineral supplementation.

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The objective was to assess the effect of Cu, Zn, and Mn supplementation adjusted to actual mineral content of forages on health status and mineral metabolism of dairy cows. Seven dairy herds were selected in the South East of France, with an average of 89 dairy cows/herd. Forages and all ingredients of the basal diets were collected monthly and analyzed by ICP-AES for Zn, Cu, Mn, S, Mo, and Fe content. For 6 mo, cows were fed new mineral feeds, formulated by farm, to provide 80 and 70 mg/kg DM for Zn and Mn, respectively, based on the total diet (+11% and −20% of Zn and Mn supplemented on average). The target Cu supply was between 9.5 and 11 mg total/kg DM (−50% on average of Cu supplemented) depending on the level of Cu antagonists in the basal diet (S, Mo, and Fe). In each farm, blood samples were collected 3 times on 10% of the animals (n = 47; T0 = start, T1 = 3 mo and T2 = 6 mo after starting the trial) and analyzed for Cu, Zn, and Mn concentrations, as well as the activity of SODe and ceruloplasmin. The effects of the new mineral feeds were explored through ANOVA integrating in the statistical model the treatment (T0 vs. T1 vs. T2), the farm as fixed effect, and the cow nested within the farm as a random effect. Before feeding the new mineral feeds, there was a global tendency to over supplement the diets in Cu (on average +182% of the requirements). The Cu in plasma was maintained with the new mineral feeds ($P = 0.079$; 13.65 µmol/L on average). The Zn in plasma significantly increased ($P = 0.000$; T2 > T1 > T0; +21%). No statistical differences were observed regarding Mn in plasma for T0 and T2; however, T1 was significantly lower (2.92, 2.82, and 1.96 µg/L, respectively). Ceruloplasmin activity significantly increased in T2 compared with T0 ($P = 0.001$; 7,628 vs. 5,708 mU/mL). No statistical differences were observed regarding the activity of SODe ($P = 0.135$). These results showed that Cu, Zn, and Mn requirements of dairy cattle can be covered with a customized mineralization without inducing any deficiencies or metabolic problems. Last but not least, environmental and economic aspects should be taken into account in such studies.

Key Words: sustainability, precision, livestock

P63 Effects of nutrient restriction and a serine palmitoyltransferase inhibitor on milk production and metabolism in lactating ewes.

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During the transition to lactation, glucose is spared for the synthesis of milk, characterized by reduced insulin-stimulated glucose use by muscle and enhanced adipose lipolysis. In ruminants, fatty acids (FA) and somatotropin (ST) promote accrual of ceramide, an antagonist of insulin action. We conducted 2 experiments (EXP) to study the effects of nutrient restriction (NR) in periparturient ewes (EXP1) and a de novo ceramide synthesis inhibitor (myriocin [MYR], a serine palmitoyltransferase (SPT) inhibitor) in lactating ewes (EXP2). For EXP1, 20 ewes were given one of 2 treatments (trt; n = 10): (i) fed ad libitum (AL) or (ii) 50% NR based on metabolic energy requirements for 5-d periods pre- and postpartum. A glucose tolerance test (GTT; 0.25 g/kg BW 50% dextrose) pre- and post-trt periods was used to assess changes in glucose tolerance. For EXP2, 12 ewes 3 to 5 wk in milk were assigned one of 4 trt (n = 3): (i) control, (ii) MYR (0.075 mg/kg 2×/d IV), (iii) recombinant bovine ST (rBST; 3 mg/kg 1× SQ), or (iv) MYR+rBST. Statistical analyses were conducted in SAS 9.4 using a linear mixed model. For EXP1, NR ewes improved efficiency 2× (milk yield [MY]/intake; trt $P = 0.01$). Despite decreased MY, milk protein yield (MP), and milk lactose yield (ML) (all trt $P < 0.05$), NR ewes had no change in milk fat yield (MF). Elevated daily FA levels in NR ewes (trt $P < 0.01$) suggest enhanced lipolysis. The GTT glucose area under the curve (AUC) increased post NR (trt × time $P < 0.05$) and FA AUC rose to 2× AL. The NR ewes' response to GTT indicates altered glucose and lipid metabolism associated with insulin resistance. For EXP2, we observed severe loss of appetite (trt × time, $P < 0.05$) and decreased ML and MP yields by d 3 in MYR/MYR+rBST ewes while MF increased 2% and 3%, respectively (trt × time, $P < 0.01$). These outcomes developed with a 10 and 20× rise in plasma FA levels by d 5 (trt $P < 0.01$; trt × time, $P < 0.01$) in MYR and MYR+rBST ewes, respectively. We infer from our data that insulin resistance develops in periparturient ewes to maintain milk production efficiency and MYR at this dose is a potent appetite suppressant in sheep.

Key Words: lactation, metabolism, ceramide

P64 Guanidinoacetic acid supplementation enhances placental vascularity in pregnant beef cows during late gestation.

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Our objective was to evaluate whether guanidinoacetic acid (GAA) affects placental vascularity, liver enzyme activity, serum metabolites, and plasma amino acid concentrations in pregnant beef cows during late gestation. Twenty-eight pregnant Brahman cows, averaging 532 ± 15.1 kg and carrying male (n = 15) and female (n = 13) fetuses, were used. On d 170 of gestation, the cows were housed in individual pens for an adaptation period of 10 d. The basal diet consisted of 688 g/kg corn silage, 147 g/kg sugarcane bagasse, 47.7 g/kg corn, 89.6 g/kg soybean meal, 6.86 g/kg urea, and 21.2 g/kg mineral mixture (DM basis). The intake was adjusted daily, allowing up to 5% in orts. Maternal treatments started on d 180 and extended until d 270 of gestation. The following treatments were evaluated: control (no addition of GAA) or addition of 0.2% GAA to the total diet (DM basis). On d 227 of gestation, blood samples were collected via jugular venipuncture. Placental vascularity was evaluated by using color doppler ultrasound. On d 229 of gestation, liver sampling was performed via needle biopsy. The enzymatic activity of arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) was assessed using immunoassay techniques. The data were analyzed considering diet and fetal sex as fixed effects using the MIXED procedure of SAS 9.4. Dietary GAA addition enhanced ($P < 0.001$) placental vascularity when compared with control. Indeed, serum nitric oxide increased with the addition of GAA to the diet ($P < 0.05$). Similarly, the addition of GAA to the diet improved ($P < 0.028$) AGAT activity in the liver but had no effect ($P > 0.68$) on GAMT activity. A higher concentration of both arginine ($P < 0.007$) and ornithine ($P < 0.011$) was observed for cows that received GAA in the diet. Conversely, dietary GAA led to a decrease in plasma methionine concentration ($P < 0.017$). Moreover, cows receiving GAA exhibited a lower serum homocysteine concentration than control ($P < 0.001$). Guanidinoacetic acid enhances placental vascularity in pregnant beef cows during late gestation by exerting an effective arginine-sparing effect.

Key Words: arginine, beef cattle, fetal programming

P65 Abomasal infusion of docosahexaenoic acid to mid-lactation dairy cows dose-dependently enhanced neutrophil phagocytosis in whole-blood in vitro stimulations. H. L. Reisinger*[1], M. L. Miller[1], K. C. Krogstad[1], D. Vocelle[2], G. A. Contreras[3], A. L. Lock[1], and B. J. Bradford[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, [3]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most bioactive fatty acids in the omega-3 family, with known anti-inflammatory effects. However, they are subject to rumen biohydrogenation when fed to cows. The objective of this study was to evaluate effects of low-dose DHA delivered via abomasal infusion on immune cell populations in healthy cows and on neutrophil function following in vitro whole-blood bacterial stimulation. Ruminally cannulated multiparous Holstein cows (n = 8; 97 ± 37 DIM, 49 ± 3 kg/d milk) were enrolled in a 4 × 4 Latin square design. Treatments were 0, 2, 4, or 6 g/d DHA abomasally infused for 11 d (4 infusions/d). A 10-d washout period preceded treatment periods. The DHA was provided via an enriched algal oil (64.5% DHA) and suspended in ethanol (~200 g/d). Blood was collected on d 11 of each period. White blood cell differentials were determined using an automated leukocyte counter. Whole blood (100 µL) was stimulated with lyophilized pHrodo *S. aureus* or *E. coli* Bioparticles and incubated with dihydrorhodamine 123 (DHR) for 30 min (39°C, 5% CO_2). Samples were analyzed on the Attune CytPix flow cytometer to evaluate bacterial uptake and oxidative burst (DHR) by neutrophils. The DHA quadratically decreased circulating eosinophil populations ($P < 0.05$) with a nadir at 4 g/d. No other changes in immune cell populations were apparent. The DHA did not alter the percentage of neutrophils phagocytosing *S. aureus*. In contrast, DHA linearly increased the proportion of neutrophils phagocytosing *E. coli* and cubically increased (peak: 4 g/d) bioparticle median fluorescence intensity (MFI; $P < 0.05$), a proxy for bioparticle uptake per neutrophil. Among neutrophils positive for both DHR and bioparticles, bioparticle MFI increased in a curvilinear manner ($P < 0.05$) for *S. aureus* (peak: 2 g/d) and *E. coli* (peak: 4 g/d). In conclusion, intestinal supply of 2–4 g/d DHA decreased circulating eosinophils but in a whole-blood stimulation improved phagocytic capacity of neutrophils. These findings suggest the reported anti-inflammatory effects of DHA may not impair innate immunity.

Key Words: omega-3, immune function

P66 Connecting prepartal liver glutathione with postpartal performance parameters, blood biomarkers, and gene expression in transition dairy cows. A. F. Souza Lima*[1], G. Goncalves Begalli[1], M. H. de Oliveira[1], R. Chaves Barcellos Grazziotin[1], J. Halfen[1], E. Trevisi[2], and J. S. Osorio[1], *[1]School of Animal Science, Dairy Science, Virginia Tech, Blacksburg, VA, [2]Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of*

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Our objective was to determine the effects of prepartal liver glutathione (GSH) concentration on milk performance parameters and postpartum welfare. Sixty Holstein dairy cows were enrolled at −21 d relative to calving and remained on trial until 30 d in milk (DIM). All cows received the same close-up diet (−21 DIM to calving; 1.59 Mcal/DM and 14.63% CP) and lactation diet (calving to 30 DIM; 1.82 Mcal/DM and 18.39% CP). A liver biopsy was performed in all cows at −10 d (±3.2 d) relative to calving and total GSH was measured. This metric was used to perform retrospective analysis and classified cows by prepartal liver GSH content as high (HGSH; n = 15, 2.40 ± 0.07 m*M*), medium-high (MHGSH; n = 14, 1.63 ± 0.07 m*M*), medium-low (MLGSH; n = 16, 1.13 ± 0.06 m*M*), and low (LGSH; n = 15, 0.47 ± 0.06 m*M*). Blood and liver samples were collected at various time points from −21 to 21 DIM to evaluate metabolism and liver function biomarkers. Target genes related to the GSH metabolism were evaluated via RT-qPCR. Statistical analysis was performed using the MIXED procedure of SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Greater milk yield ($P = 0.04$; +2.5 kg/d) and milk protein yield ($P = 0.05$; +160 g/d) were observed in the HGSH group compared with other groups. A trend ($P = 0.10$) in postpartal BCS indicated that HGSH had a greater ($P < 0.05$) BCS compared with other groups. We observed elevated glucose levels ($P = 0.01$) for HGSH and MHGSH cows compared with MLGSH and LGSH. In contrast, the MLGSH group exhibited a greater insulin concentration ($P = 0.03$) than the MHGSH and HGSH. The mRNA expression ($P \leq 0.04$) of *GGCT*, *GCLC*, *GGT*, and *GSR* had a linear downregulation across HGSH to LGSH groups. Overall, our results suggest a potential association among prepartal liver GSH, improved milk yield and milk protein yield, and postpartal BCS and metabolic parameters. The correlation between liver mRNA expression of GSH-related genes and liver prepartal GSH holds important implications for understanding how GSH may be synthesized, stored, and used during the transition period.

Key Words: peripartum period, oxidative stress, glutathione

P67 Validation of an in vivo dual marker technique used to characterize regional gastrointestinal tract permeability in ruminants.

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This study was conducted to evaluate sampling methods and frequency of sample collection for a dual marker technique to characterize total gastrointestinal tract (GIT) and post-ruminal permeability. Six lactating Holstein cows were blocked by parity (189 DIM ± 25.2) and enrolled in a crossover design. Experimental periods included a 5-d baseline phase, 5-d challenge phase (CHAL), and 2 wk of recovery. During CHAL, cows within parity were randomly assigned to either receive 100% ad libitum feed intake (AL) or 40% of ad libitum feed intake (FR). To assess total-tract and post-ruminal permeability, Cr-EDTA and Co-EDTA were infused into the rumen and abomasum, respectively, to achieve a dose 0.369 mmol/kg BW for each marker. Infusions were conducted on d 3 of CHAL. Following infusions, total urine and feces were collected every 8 h over 96 h, and blood samples were collected at h 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 32, 40, 48, and 64 to facilitate calculation of area under the curve (AUC) for Cr and Co. Total Cr recovery in urine, feces, and milk was 88.5% for AL and 82.2% for FR ($P = 0.03$), and total Co recovery was 84.4% for AL and 78.8% for FR ($P = 0.32$). Correlations between plasma AUC and urine recovery for Cr (r = 0.76; $P < 0.01$) and Co (r = 0.87; $P < 0.01$) were detected. Using a brute force approach, blood samples collected at h 2, 8, 20, 40, and 48 could predict plasma Cr and Co AUC within 1.9% and 6.2%, respectively. The reduced sampling AUC were correlated with urine Cr and Co output (r = 0.75 and 0.92, respectively; $P < 0.01$). These results suggest that when using Cr- and Co-EDTA to evaluate regional GIT permeability, plasma AUC can be used as an acceptable indicator for total urinary recovery of Cr and Co and blood collected at h 2, 8, 20, 40, and 48 can be used, at least when comparing GIT permeability for lactating dairy cows exposed to AL and FR.

Key Words: Cr-EDTA, Co-EDTA, gut permeability

P68 Dietary glutamate and casein affect duodenal taste receptor 1 and SGLT1 but not hepatic PEPCK expression of preruminant calves.

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Holstein bull calves (n = 15) were fed 1 of 3 starch-based milk replacers for either 10 or 35 d to determine response of RNA gene expression in duodenum and liver. Calves were bottle-fed a cornstarch-based milk replacer (control; 727 g of cornstarch daily) alone, or the same milk replacer with added casein (154.7 g/d) or with added glutamate (45.5 g/d) for 10 or 35 d. Before receiving treatments calves were fed a commercial milk replacer for either 12 or 15 wk so that tissues were collected at the same day of age. At 115 d of age, calves were euthanized, tissues were collected and immediately placed on dry ice and frozen at −80°C until analysis. Immediately before tissue collection, preruminant status was confirmed by addition of Co-EDTA to milk replacer; measures of ruminal cobalt were less than 0.08 ± 0.04% of total Co consumed. RT-qPCR was performed for mRNA expression of liver cytosolic and mitochondrial PEPCK, duodenal taste receptor 1 subunits (TAS1R1, TAS1R2, TAS1R3), the principal glucose transporter (SGLT1) in duodenum, and duodenal CCK. The mRNA expression relative to 18S was analyzed using the MIXED procedure of SAS; diet and duration of treatment were the fixed effects. There was no effect of diet or duration of treatment ($P \geq 0.25$) on hepatic PEPCK. There was, however, a significant interaction of diet and duration on expression of TAS1R1 and TAS1R3 ($P < 0.01$). Expression of TAS1R1 did not differ in response to diet after 10 d, but after 35 d expression was greatest in glutamate, intermediate in casein, and least for control. Expression of TAS1R3 and SGLT1 were similar to TAS1R1, but after 35 d glutamate was greater ($P < 0.01$) than casein and control. Expression of TAS1R2 tended to be greater ($P = 0.08$) among calves fed for 10 d compared with 35 d, but diet had no effect on TAS1R2 expression ($P = 0.15$). The CCK tended to be greater ($P = 0.08$) among calves fed control, but duration of treatment did not affect CCK. Data from this study indicate that feeding glutamate for 35 d increased expression of the umami taste receptor (TAS1R1/TAS1R3) and SGLT1 in preruminant calves.

Key Words: ruminant, taste receptor, gene expression

P69 Abomasal infusion of branched-chain amino acids or branched-chain keto acids decreased risk of oxidative stress in fresh cows.

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The objectives of this study were to determine the effect of branched-chain amino acids (BCAA) and branched-chain keto acids (BCKA) on blood and liver biomarkers of liver function, oxidative stress, and inflammation. Thirty 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 = 10); BCAA (n = 10) including 67 g valine, 50 g leucine, and 34 g isoleucine; and BCKA (n = 10) including 77 g ketovaline, 57 g ketoleucine, and 39 g ketoisoleucine. All cows received the same diet. Blood was collected at 3, 7, 14, and 21 d postpartum for profiling of 20 biomarkers. Liver was also harvested on 7, 14, and 21 d postpartum for quantification of GSH and protein carbonylation. Treatment effects were determined using PROC GLIMMIX in SAS. No treatment differences ($P > 0.30$) were observed for liver function biomarkers (bilirubin, cholesterol, GGT, AST, paraoxonase). Cows receiving BCAA had lower blood NO_2^- ($P = 0.05$, 3.79 vs. 4.46 μmol/L, SEM 0.20) and NO_3^- ($P < 0.01$, 21.36 vs. 24.50 μmol/L, SEM 0.83) concentrations compared with CON. A tendency for lower advanced oxidized protein products was also observed in BCAA cows compared with CON ($P = 0.06$, 18.31 vs. 21.61 μmol/L, SEM = 1.21). Additionally, on d 7, BCAA cows also had lower protein carbonylation compared with CON ($P = 0.05$, 6.25 vs. 8.76 nmol/g protein, SEM = 0.89). In contrast, BCKA cows had higher plasma thiol ($P < 0.01$, 271.70 vs. 212.36 μmol/L, SEM 12.28), albumin ($P = 0.02$, 33.85 vs. 32.56 μmol/L, SEM 0.35), liver reduced ($P = 0.03$, 40.23 vs. 19.96 nmol/g protein, SEM 5.37) and total GSH ($P = 0.05$, 42.19 vs. 22.49 nmol/g protein, SEM 5.65) compared with CON cows. Overall, these results suggest that BCAA infusion reduced oxidative damage whereas BCKA infusion increased plasma and liver antioxidant concentrations. Further work is required to determine how these changes contribute to the favorable changes in lactation performance and liver TAG concentration as observed previously.

Key Words: branched-chain amino acid, branched-chain keto acid, oxidative stress

P70 Changes in oxo and hydroxy fatty acids in milk fat during diet-induced milk fat depression.

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Increases in *t*-10 fatty acids (FA) are well characterized during biohydrogenation-induced milk fat depression (MFD), but changes in oxygenated FA have not been well established. Milk FA from 2 experiments with differing degrees of MFD were analyzed using gas chromatography with a 30 m DB-FastFAME cyanopropyl phase column and a mass selector detector. The concentration of 10-hydroxyoctadecanoic (10-O-SA) and 10-oxooctadecanoic acid 1 (0-OH-SA) were quantified using a 10-OH-SA standard and reference libraries (e.g., NIST 11 and Lipid Maps). Data were analyzed in a mixed model with treatments fixed and cow and period as random effects in JMP Pro 17. The relationships among FA were tested in a regression and principal component analyses. In the first experiment, 12 cows in a Latin square design were fed a control diet or a diet designed to cause MFD. The MFD diet reduced milk fat to 2.2% and increased *t*-10 18:1 to 8.16% of FA. The concentration of 10-O-SA was increased from 0.03 to 0.12% of FA in MFD and 10-OH-SA was increased from 0.05% to 0.19% ($P < 0.05$). In addition, milk fat from a crossover design with 48 cows fed a high corn silage basal diet where both treatments experienced moderate MFD was analyzed. There was a negative linear relationship between milk fat concentration and 10-OH-SA in milk fat ($P < 0.01$) and a negative quadratic relationship for 10-O-SA ($P < 0.08$). Milk fat ranged from 1.69% to 5.4%, *t*-10 18:1 ranged from 0.27% to 12.0% of FA, 10-O-SA ranged from 0.003% to 0.23% of FA, and 10-OH-SA ranged from 0.04% to 0.32% of FA. The first 2 components described 70.7% of the total variation. Cluster 1 consisted of total odd and branched chain, 16C, and <16C FA positively correlated with milk fat concentration while cluster 2 included *t*-10 18:1, total *t*-18:1, FA >16C, 18:2 n-6, 10-O-SA, and 10-OH-SA FA (10-O-SA, and 10-OH-SA negatively correlated with milk fat concentration r = −0.56, and −0.59). In conclusion, the oxygenated 18C FA were associated with MFD and were related to *t*-10 intermediates. The bioactivity of the FA and potential role in induction of MFD warrant further investigation.

Key Words: oxygenated fatty acid, milk fat concentration, milk fat depression

P71 Modulation of phagocytosis in cow blood using an aqueous garlic extract. P. Pande*, M. R. Uzzaman, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Neutrophils are white blood cells that contribute to increased somatic cell counts in milk. They are innate immune phagocytes that combat infection and contribute to inflammatory diseases. Dietary feed additives such as garlic can be used as environmentally friendly immune modulators. The objective of this study was to determine if garlic affects phagocytosis, in cow neutrophils. Blood samples were collected from 3 clinically healthy adult lactating cows at North Carolina A&T State University Dairy. Neutrophils were isolated by differential centrifugation and hypotonic lysis of red blood cells. The viability and total neutrophil count were determined using the Trypan blue dye exclusion method on the TC20 (Bio-Rad). Neutrophils (1×10^6 cells/mL) were treated with 10 µg of garlic, 10 µg of *E. coli* lipopolysaccharides (LPS), garlic+LPS, or phosphate-buffered saline (PBS) and incubated (37°C, 5% CO_2, 85% humidity for 30 min). The phagocytic activity of bovine neutrophils was assessed using the Vybrant Phagocytosis Assay (Thermo Fisher Scientific Inc., Waltham, MA) per the manufacturer's instructions. The fluorescence was measured using an Epoch microplate reader (BioTek) at 480/520 nm excitation/emission wavelengths. The average % phagocytosis effect for each treatment was calculated. All treatments stimulated phagocytosis (764% for garlic, 158% for LPS, 493% for garlic+LPS, and 144% for PBS). The highest increase was observed with garlic, with a 430%-fold increase in phagocytosis activity compared with PBS control. Cow variation was observed in phagocytosis and response to modulators. In conclusion, this study demonstrates that garlic modulates phagocytosis by cow neutrophils immunomodulator. Future investigations will explore the mechanisms involved in this immune modulation, using a larger sample size for a more comprehensive understanding of the study's implications.

Key Words: cow, neutrophil, phagocytosis

P72 Effect of palmitic acid supplementation and a milk-fat-depressing diet on milk production, fatty profile, and metabolomics. C. Matamoros*[1,2], N. Boyle[2], F. Hao[2], I. Koo[2], A. Patterson[2], and K. 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). Milk production was measured at the end of each period and samples were taken for fatty acid, metabolomics, and lipidomics analysis. Data were analyzed with a model that included the fixed effect of treatment and random effect of period and cow. When compared with CON, milk fat percent and yield increased by 0.30 units and 160 g/d with the PA supplement, and MFD decreased milk fat by 1.35 units and 520 g/d ($P < 0.001$) concomitant with an increase in milk *trans*-10 18:1 concentration of 7.8 percentage units ($P < 0.001$). The MFD diet altered the concentration of intermediates of the citric acid cycle (TCA) 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, PA supplement had no effect on any TCA cycle metabolites. Compared with the CON in the untargeted lipidomics analysis, 245 lipids were modified by MFD, while only 1 lipid differed in PA. Interestingly, there was a generalized increase in milk sphingolipids during MFD, suggesting possible metabolic dysregulation during MFD. Overall, PA supplement and MFD resulted in the expected changes in milk fat yield and FA profile and the modified milk metabolites and lipids provide further insight into the regulation of lipogenic pathways during changes in milk fat yield.

Key Words: lipid, metabolomics, citrate

P73 Selection for production traits differentially affects beef calves' immune phenotype before weaning. A. R. H. Main*, C. Reddout-Beam, and J. L. Salak-Johnson, *Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK.*

Breeding strategies have focused on intense selection for economic traits emphasizing growth and efficiency, which may alter energy allotment and, ultimately, the immune phenotype of the progeny. This study aimed to determine the effect of genotype on the immune phenotype of beef calves at 3–4 and 6–7 mo of age. Fall-born steer and heifer calves of dams bred to Angus sires were categorized into one of 4 genotypes: high growth, high milk (HH, n = 12); high growth, low milk (HL, n = 11); moderate growth, high milk (MH, n = 17); and moderate growth, low milk (ML, n = 7). Serum and whole-blood samples were collected and analyzed for lymphocyte proliferation, immunoglobulins (Ig), and cytokine profiles. Body weights were also recorded. Data were analyzed using PROC GLM with growth trait, milk trait, and sex as fixed effects for analyzing calf measures at the 2 ages and all possible interactions (SAS 9.4). At 3–4 mo, high-growth calves exhibited increased T-cell proliferation ($P < 0.01$) compared with moderate growth. In addition, low milk steers exhibited greater T-cell proliferation ($P < 0.01$) than their high milk counterparts and low milk heifers at 3–4 mo. Conversely, B-cell proliferation was differentially affected at 6–7 mo with a growth × milk × sex interaction ($P = 0.06$). The HL heifers had a greater proliferation than all other heifer genotype combinations and HL steers. At both 3–4 mo and 6–7 mo, growth × sex interactions were observed in moderate growth steers, which had greater IgG1 and total IgG ($P < 0.01$) than high-growth steers and moderate-growth heifers. High growth influenced several cytokines, such as decreased IL-4 at 3–4 mo and increased IFNy at 6–7 mo ($P < 0.05$). Furthermore, HH heifers weighed more than all other genotype combinations at 3–4 mo and 6–7 mo ($P < 0.05$, respectively), suggesting the milk trait may affect female weight. These findings may imply that selection for extreme economic traits does differentially affect the immune phenotype of calves at 3–4 mo and 6–7 mo, in turn influencing animal health and well-being.

Key Words: immune, genetic selection, growth

P74 Blood transcriptome and its microbial changes in young calves with feed-induced ruminal acidosis. A. Larsen[1,2] and W. Li*[1], [1]*United States Dairy Forage Research Center, Madison, WI,* [2]*Animal and Dairy Science, University of Wisconsin–Madison, Madison, WI.*

In cattle, diet-induced subclinical ruminal acidosis (SARA) models reported ruminal pH depression, ruminal epithelium (RE) damage, and increased concentration of bacterial lipopolysaccharides (LPS) in the blood. However, besides measuring ruminal pH, early diagnostic tools for SARA are lacking. Here, with our established feed-induced SARA model, we investigated the blood transcriptome (BT) and its associated microbial communities using RNaseq in young calves. Ten Holstein bull calves were randomly assigned to either acidosis-inducing (AC; 43% starch and 15% neutral detergent fiber [NDF]) or blunting diet (Control; 35% starch and 25% NDF) (n = 5) at birth until 17 wk of age. Blood samples were collected at 8 and 16 wk. Differentially expressed genes between the treatments ($P \leq 0.05$, fold change ≥1.5, read count [RC] ≥ 5) were identified using DEseq2. Gene ontology enrichment (GOE) analysis ($P < 0.01$) was done with DAVID. Microbial abundance was quantified with Kraken2 using rRNA sequencing reads. At 8 wk, 143 upregulated genes (UG) and 194 downregulated genes (DG) were found in AC. Upregulated genes were enriched in molecular pathways related to glycoprotein synthesis and disulfide bond formation, both of which have reported roles in cellular repair and inflammatory response. Downregulated genes showed a GOE in lyase activity, whose deficiency was linked to metabolic acidosis in both human and mouse. At 16 wk, 144 UG and 124 DG in AC were identified. For UG, GOE in innate immunity was identified. The DG in AC at both 8 and 16 wk showed GOE in extracellular structures and functions. Five differentially abundant microbial genera (average RC > 10, $P < 0.01$) were identified at 16 wk. Among these, *Bacteroides*, an LPS-producing genus, was the most abundant and showed a more than 10-fold increase in the AC group. Collectively, our study suggested that the impact of feed-induced SARA can be captured by the BT as early as 8 wk. Additionally, changes in molecular mechanisms related to blood cellular structure and immune responses might be part of the effect SARA elicits beyond traditionally investigated RE.

Key Words: ruminal acidosis, blood transcriptome, young calf

P75 Prolactin and oxytocin concentrations at different lactation phases as influenced by breeds of goat and levels of turmeric powder inclusion. O. A. Oderinwale*[1,2], B. O. Oluwatosin[2], S. D. Amosu[2], D. P. Toviesi[2], O. O. O. Adewumi[2], J. O. Alabi[1,2], O. O. Adelusi[1], C. C. Anotaenwere[1], J. M. Enikuomehin[1], and U. Y. Anele[1], [1]*North Carolina Agricultural and Technical State University, Greensboro, NC,* [2]*Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria.*

The study investigated effects of breeds of goat and turmeric powder (TP) inclusion on prolactin and oxytocin concentrations at different lactation phases. Forty-five lactating does comprising of 15 each of Kalahari Red (KR), KalaWAD (KW), and West African Dwarf (WAD) goats were used. The does were allotted on weight equalization to 3 dietary treatments (fed at 5% body weight during pregnancy and lactation) comprising 5 does/breed/treatment. The diets were concentrate diet (CD) as T1; CD + 2 g/kg TP as T2; and CD + 5 g/kg TP as T3. Blood samples were collected into plain bottles via the jugular vein puncture per goat within 24 h post-kidding (PK) and at the 12th week of lactation (12WL) for serum extraction and laboratory analyses. Data obtained were arranged in a 3 × 3 factorial layout in a randomized block design. Analysis of variance and Duncan multiple range test for mean separation were done using SPSS (v29.0) at 5% probability. Breed effect revealed that KW had the highest ($P < 0.05$) values for prolactin and oxytocin at PK, while the respective parameters were highest for WAD; and KR and KW at 12WL. The variations in values at 12WL less that of PK indicated that KW had an increase in oxytocin by 1.42 pg/mL. The effect of TP indicated that T1 recorded the highest value for prolactin at PK, while T3 had the highest values for prolactin and oxytocin at 12WL. Both T2 (3.43 ng/mL) and T3 (0.86 ng/mL) had the least reductions in prolactin, while T3 had increase in oxytocin value by 1.0 pg/mL. For interaction effect, KW fed T1 (19.3 ng/mL) and KR fed T3 (17.7 ng/mL) had the highest prolactin values at PK and 12WL, respectively. Conversely, KW fed T2 (38.9 pg/mL) and KR fed T3 (37.1 pg/mL) had the highest oxytocin values at PK and 12WL. The WAD and KR fed T3 had the highest increase in prolactin and oxytocin values by 3.7 ng/mL and 7.7 pg/mL, respectively. The study concluded that breed and TP influenced prolactin and oxytocin concentrations. Overall, feeding of diets with TP to lactating goats generally improved the prolactin and oxytocin concentrations, which are essential for improved milk production and let-down.

Key Words: goat, turmeric, hormone

P76 Does high-starch diet early in life affect metabolic response to high-starch diet later in life?

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The aim of the study was to determine the long-term effect of a high-starch diet in the first weeks of life on selected blood parameters once exposed to such a diet later in life. Twenty-four lambs (12 rams and 12 ewes; 7–14 d of age; 2 blocks) were randomly divided into 2 groups (i.e., control [C] and starch [S]), and kept in 4 group pens (2 pens/treatment). Animals were fed ad libitum milk replacer (MR), and either a concentrate mixture (77% barley, 17.5% soybean meal, 5% chopped hay; S; n = 12) or meadow hay with a small inclusion of concentrate mixture (up to 150 g/day/animal; C; n = 12) ad libitum. Milk replacer was fed for 6 wk, whereas experimental solid feeds were continued for 2 additional weeks after weaning. From wk 9 onward both groups were transitioned to the same hay- and pasture-based diet (fed ad libitum). This common diet continued until the age of 8 mo for rams and 9.5 mo for ewes. Thereafter, all animals were placed in individual pens, and challenged with a high-starch diet by replacing 50% of voluntary hay intake with barley (on a dry matter basis). After 7 d of the challenge, blood samples were taken 3 h after feeding and analyzed for plasma glucose, cholesterol and urea, and serum β-hydroxybutyric acid (BHBA). Blood parameters were analyzed separately for rams and ewes with a mixed model procedure of SAS (version 9.4). Statistical model included fixed effect of treatment and random effect of block of animals. Blood parameters did not differ between treatments, both for rams (Table 1) and ewes (Table 2). In conclusion, sheep fed the high-starch diet and sheep fed a hay based diet early in life did not differ in terms of investigated blood parameters once exposed to the high-starch diet later in life.

Table 1 (Abstr. P76). Selected blood parameters in rams after a high-starch diet

Item	C	S	SE	*P*
Glucose (mg/dL)	66.7	64.2	2.44	0.50
Cholesterol (mg/dL)	39.6	44.8	2.33	0.16
Urea (mg/dL)	28.6	30.2	2.07	0.52
BHBA (mmol/L)	0.46	0.51	0.067	0.57

Table 2 (Abstr. P76). Selected blood parameters in ewes after a high-starch diet

Item	C	S	SE	*P*
Glucose (mg/dL)	67.5	65.8	3.66	0.75
Cholesterol (mg/dL)	47.8	51.5	3.83	0.52
Urea (mg/dL)	26.2	26.2	2.00	0.99
BHBA (mmol/L)	0.47	0.41	0.046	0.36

Key Words: ruminant, blood, high starch

P77 The profiles of rumen small peptides and their potential roles in nitrogen utilization efficiency in dairy cows.

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In the gastrointestinal tract of dairy cows, small peptides (SP) are vital for enhancing nitrogen utilization efficiency (NUE) by aiding microbial metabolism in the rumen and milk synthesis in the mammary gland. However, limited knowledge about SP in the rumen prevents NUE improvement. Two experiments (Exp1 and Exp2) were conducted. In Exp1, rumen contents of 30 mid-lactation Holstein cows were collected under the same dietary and management conditions. The SP were identified based on chemical isotope labeling LC-MS by targeting the amine/phenol submetabolome. In Exp2, 8 cows in each group with high and low NUE were selected and analyzed for differential SP and potential functions. In Exp1, a total of 331 SP were identified including 2 tripeptides and

328 dipeptides. Among these, the number of dipeptides containing only nonessential amino acids (NEAA) exceeded those containing only essential AA (EAA; 90 vs. 69). However, when evaluating peptide abundance based on signal intensity, EAA-EAA dipeptides demonstrated higher abundance compared with NEAA-NEAA dipeptides ($P < 0.01$). Particularly, Gly-Met and Trp-His exhibited significantly higher abundance, each containing at least one EAA. Furthermore, 186 dipeptides were annotated as dipeptidyl peptidase IV (DPP-IV) inhibitors, known to enhance NUE. In Exp2, despite similar DMI ($P = 0.22$), high NUE cows produced 6.8 kg/d more milk ($P < 0.01$). High NUE cows showed a higher milk protein to metabolic protein ratio ($P < 0.05$), suggesting superior mammary gland SP or AA uptake, affecting NUE. The 2 groups exhibited clear distinctions in the OPLS-DA plot. Totally 25 different dipeptides ($P < 0.05$, VIP > 1) were identified, with 22 upregulated and 3 downregulated in high NUE cows, including Asp-Gln and Tyr-Phe. Notably, 4 upregulated dipeptides (e.g., Phe-Lys) are linked to milk synthesis. Additionally, 14 upregulated dipeptides and 2 downregulated dipeptides exhibited DPP-IV inhibitory activity. This study provides the first comprehensive insight into rumen SP composition and associated biological activities, identifying potential peptide targets for enhancing NUE in dairy cows.

Key Words: small peptide, nitrogen utilization efficiency

P78 Nutrient extraction ratio and specific transporter gene expression in the mammary gland of peripartum dairy cows.

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The composition of colostrum differs significantly from that of regular milk, characterized by low lactose and high milk fat content. Blood glucose and β-hydroxybutyrate (BHBA) are typical precursors for lactose and milk fat, respectively, and are taken up by the mammary gland. In this study, we hypothesized that the activation of BHBA uptake precedes the activation of glucose uptake in the prepartum period for colostrum synthesis. The objective was to investigate changes in the nutrient extraction ratio and specific transporter genes expression in the mammary gland of peripartum dairy cows. Ten Holstein cows (parity 2.5 ± 0.4) were examined from 8 wk before calving to 6 wk postpartum. Mammary gland tissue samples were collected by biopsy at 8 and 1 wk before calving, immediately after calving, and 6 wk after calving. Q-RT-PCR measured the expression levels of specific transporter mRNA. Blood samples from the coccygeal artery and milk vein of 5 out of 10 cows were collected simultaneously to calculate the mammary arterial-venous differences (AVD) and extraction ratio of precursors. The glucose extraction ratio in 1–2 wk before parturition was significantly lower than that after parturition ($P < 0.05$). The BHBA extraction ratio in 1–2 wk before calving was significantly higher than that in 8 wk before calving ($P < 0.05$) and comparable to that after calving. Monocarboxylate transporter-1 mRNA expression was significantly upregulated 1–2 wk before parturition ($P < 0.05$). Triglyceride (TG) accumulation in mammary tissue was significantly higher ($P < 0.05$) in 1–2 wk before parturition than 8 wk before parturition, and TG accumulation tended to decrease after parturition when colostrum was secreted. These findings suggest that the uptake of milk fat precursors is activated before lactose precursors in the pre-parturition period. Milk fat synthesis and temporary accumulation are promoted in the mammary gland, leading to colostrum synthesis with low lactose content and high milk fat content.

Key Words: colostrum synthesis, lactation physiology, mammary biopsy

P79 Litter male ratio in multifetal pregnancies affects cortisol, testosterone, and placental gene expression in ewes.

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Late pregnancy partial litter loss (PLL) occurs in 28% of lambings in the Volcani experimental sheep flock by an unknown mechanism. Our previous study 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 MR, dams' plasma cortisol and testosterone, and placenta gene expression. Blood samples were collected at 80, 138, and 143 d in pregnancy (DIP) for analysis of cortisol and testosterone, and placenta cotyledons were collected from 12 ewes at lambing and 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 a high male ratio (HMR; >50% males) or a low male ratio (LMR; ≤50% males). Data were analyzed with PROC MIXED of SAS. The average cortisol concentration at 80 and 143 DIP were not different but were higher in the LMR than in the HMR at 138 DIP (68.3 and 44.2 ng/mL, respectively; $P = 0.03$). The average testosterone concentration at 80 and 138 DIP were higher in the HMR than in the LMR ewes (1.37 and 1.17 ng/

mL, respectively; $P < 0.001$). Relative gene expression of SLC2A1, which encodes the glucose membrane transporter, was higher in HMR compared with LMR ($P = 0.02$), and the 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 influencing fetus growth was higher in LMR than in HMR ($P = 0.02$). The higher cortisol concentration at 138 DIP in the LMR may indicate higher stress in the LMR, as indicated in our previous study for glucose and BHBA concentration. The higher testosterone in the HMR is probably related to the higher male ratio in this group. The results of this study may be associated with the higher PLL in HMR pregnancies. However, further research is required to establish these findings and to discover the underlying mechanism for the effect of PLL and MR in ewes.

Key Words: sheep, partial litter loss, male ratio

P80 Bovine neutrophils and mononuclear cells differentially express hydroxycarboxylic acid receptor 2 at early and mid lactation. P. O. McDonald*[1], L. K. Mamedova[1], K. C. Krogstad[2], and B. J. Bradford[1], [1]*Michigan State University, East Lansing, MI,* [2]*The Ohio State University, Wooster, OH.*

Immune cells express a variety of receptors to modulate downstream pathways. Transition cows often experience dysregulated immune responses after calving, in some cases associated with ketosis. The ketone β-hydroxybutyric acid is a potent ligand for hydroxycarboxylic acid receptor 2 (HCAR2) activation. HCAR2 is implicated in several processes linked to immune responses in transition cows, like neutrophil apoptosis and lipolysis. The objective of this study was to characterize expression of HCAR2 on immune cells from fresh and mid-lactation cows using flow cytometry. Whole-blood samples from 6 healthy early (DIM <10 d) and 6 healthy mid-lactation (115 < DIM < 200) cows (parity 1–4, median 2) at the Michigan State University Dairy Farm were prepared for flow cytometry. Cells were stained for viability (DAPI), for general cell populations (CD172a for monocytes, CD3 for T cells, and CD21 for B cells), and for HCAR2 before data collection on an Attune CytPix benchtop brightfield-imaging capable flow cytometer. Data were analyzed by ANOVA to assess the fixed effect of stage of lactation. Early-lactation cows tended to have fewer HCAR2-positive leukocytes than mid-lactation cows (1.71 vs. 3.53 ± 0.85% of live cells; $P = 0.09$). Lymphocyte populations exhibited differences in HCAR2 expression by stage of lactation. Early-lactation cows tended to have reduced proportions of HCAR2+ B cells ($P < 0.01$) and T cells ($P = 0.09$). No differences were observed across lactation stages in monocytes or granulocytes. Our data demonstrate that HCAR2 is present in all studied populations, but HCAR2$^+$ cells consistently comprised less than 10% of each population. Data from other species suggest that inflammatory conditions may enhance HCAR2 expression on immune cells, so it is possible that unhealthy cows may have greater abundance of this receptor. These data suggest that there are changes in immune cell HCAR2 expression across lactation, thus providing insight into potential ketone-mediated impacts on immune responses in dairy cows.

Key Words: ketosis, flow cytometry, immunometabolism

P81 New data on the interaction between bone and energy metabolism in transition cows. S. U. Kononov[1], J. Meyer[2], J. Frahm[2], U. Meyer[2], K. Huber[3], S. Daenicke[2], J. Rehage[4], and M. R. Wilkens*[1], [1]*Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany,* [2]*Institute of Animal Nutrition, Federal Research Institute for Animal Health, Brunswick, Germany,* [3]*Institute of Animal Science, University of Hohenheim, Stuttgart, Germany,* [4]*Clinic for Cattle, University of Veterinary Medicine Hannover Foundation, Hanover, Germany.*

In a pilot study, we observed that dairy cows could be divided into 3 distinct groups based on their ante partum (ap) serum concentrations of undercarboxylated osteocalcin (ucOC), which differ from each other in terms of peripartum insulin sensitivity. The aim of the present study was to confirm these results in a larger population and to gain further insights into potential interactions. The study included datasets from 40 multiparous German Holstein cows. DMI had been recorded and blood samples had been collected between d 42 ap and d 100 postpartum (pp) as part of a former joint project on supplementation with L-carnitine (MitoCow). Blood was analyzed for NEFA, BHB, insulin, and glucose. RQUICKI as a measure for insulin sensitivity was calculated. For the present study, blood samples collected on d −42, −7, 3, 7, 100, and 6 h pp relative to calving were additionally analyzed for ucOC. Relations between DMI and ucOC in serum were analyzed by nonlinear regression analysis. After exclusion of a potential effect of L-carnitine, cows were reassigned to a low (n = 9), medium (n = 24) and high ucOC group (n = 6) based on the serum concentration of ucOC on d −7. Statistical analysis was performed using a generalized linear model, considering time, group, lactation number and respective interactions as main factors. DMI during the first week pp, but not ap, was positively associated with serum concentration of ucOC determined on d −7. In contrast to our former results, insulin sensitivity did not differ between groups. Glucose was only affected by time, but NEFA and BHB concentrations were affected by time and group: Highest concentrations were found in the low ucOC group. Results indicate an association of ucOC and energy metabolism in dairy cows. So far, it is not possible to distinguish between cause, consequence, and coincidence: A higher DMI might result in higher concentrations of ucOC due to higher energy supply. However, no association between ap DMI and ucOC could be found which might indicate that the effects of ucOC or other osteokines on energy and liver metabolism that have been described in rodents could also play a role in metabolic stability and appetite of the dairy cow.

P82 Induced hindgut acidosis in sheep affected ruminal fermentation and gut permeability. H. F. Linder, B. R. Loman, R. C. Fries, S. D. Gutierrez-Nibeyro, E. F. Garrett, and J. C. McCann*, *University of Illinois, Urbana-Champaign, Urbana, IL.*

The objective was to determine the effects of induced hindgut acidosis in sheep on cecal pH, ruminal fermentation, and gut permeability. Eleven ruminally and cecally cannulated ewes (49 ± 4 kg) were assigned to one of 2 treatments: control (CON) or induced hindgut acidosis (HGA). To induce hindgut acidosis, 3 g of wheat starch/kg BW per 24 h was continuously infused via the cecal cannula for 4 d. Control ewes received a constant infusion of deionized water. The diet contained 40% grass hay pellets, 35% whole shelled corn, 15% dried distillers grains, and 10% alfalfa cubes. Chromium EDTA was dosed once daily via the cecal cannula as a marker of gut permeability. Rumen fluid was collected for pH, volatile fatty acids (VFA), and ammonia analysis. Feces and cecal contents were sampled for pH. After euthanasia, tissue samples from the ileum, cecum, and colon were mounted in Ussing chambers to measure transepithelial electrical resistance (TEER). There was a treatment × time effect ($P = 0.05$) for cecal pH with HGA ewes having lesser cecal pH after d 1. By d 4, cecal pH had dropped to 5.07 for HGA ewes compared with CON ewes which remained above 6.40 throughout the experiment. A treatment × time interaction was also observed ($P < 0.01$) for fecal pH and followed the same trend as cecal pH. Rumen pH was not affected ($P = 0.87$) by the interaction of treatment and time. However, treatment affected ($P < 0.01$) rumen pH as ewes on the HGA treatment had a lesser rumen pH than CON ewes, 6.08 versus 6.28, respectively. Control ewes also had lesser ruminal VFA and ammonia concentrations than HGA ewes ($P < 0.01$). Urinary Cr recovery was not affected by the interaction of treatment and time, or treatment ($P \geq 0.13$). In cecal tissue, TEER tended ($P = 0.09$) to be greater in CON ewes than HGA ewes (78.2 ohms cm^2 vs. 53.7 ohms cm^2, respectively) indicating decreased permeability. In contrast, TEER was not different ($P \geq 0.83$) in ileal or colonic tissues between treatments. Induced hindgut acidosis in sheep altered rumen fermentation and tended to increase ex vivo measures of cecal permeability.

Key Words: hindgut acidosis, cecum, gut barrier function

P83 Effects of biochar, monensin, and nitrate on in vitro digestibility in beef cattle diets. J. Souza*[1,2], E. Batista[1], J. McCann[2], I. G. Favero[1], A. X. Mucida[1], L. K. Huang[1], and R. A. Lino[1], *[1]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, [2]University of Illinois, Urbana-Champaign, IL.*

The objective of this study was to evaluate and compare the in vitro digestibility of biochar with monensin and nitrate. The study was conducted using 2 dietary conditions: high-forage (Study I) and high-concentrate diet (Study II), to determine the level of biochar inclusion. In Study I, a substrate consisting of 80% low-quality hay and 20% concentrate was utilized, while in Study II, a substrate composed of 20% corn silage and 80% concentrate was employed. Treatments were applied as follows: control (without additive); biochar (40 g/kg DM and 20 g/kg DM for Study I and II, respectively). Both studies followed a Latin square design 4 × 4, with each of the 4 treatments being applied to one of the jars in each battery, totaling 4 repetitions per treatment. The substrates of each treatment were individually weighed (500 mg) into heat-sealed nonwoven textile filter bags (100 g/m^2) measuring 4 × 4.5 cm. Each jar received 24 filter bags (18 containing substrate and 6 blank filter bags) and 400 mL of ruminal inoculum and 1,600 mL of buffer solution. After 96 h for Study I and 48 h for Study II, the filter bags were washed with hot water, dried in an oven, and subjected to analysis of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and hemicellulose (HEM) to evaluate in vitro digestibility (IVD). Statistical analysis was conducted using the PROC MIXED package implemented in SAS (Statistical Analysis System, version 9.4). The results indicated that monensin had higher IVDDM ($P < 0.05$) in Study I, while nitrate showed better IVDOM ($P < 0.01$) and lower IVDCP ($P < 0.001$). In Study II, biochar and nitrate increased IVDDM ($P < 0.001$), with nitrate also improving IVDOM in relation to the control, and no difference observed between monensin and biochar. Biochar showed an increase in IVDCP, while monensin had the opposite effect ($P < 0.02$). The addition of biochar and nitrate improved IVDNDF and IVDHEM ($P < 0.01$). The comparable effectiveness demonstrated by these additives across various IVD parameters underscores their potential utility for a common purpose.

Key Words: additives, ruminants, sustainability

P84 Effects of dietary protein level and urea source on ruminal ammonia nitrogen and urea nitrogen in plasma of Nellore heifers. J. Souza*[1,2], E. Batista[1], J. McCann[2], I. G. Favero[1], A. X. Mucida[1], L. K. Huang[1], and R. A. Lino[1], *[1]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, [2]University of Illinois, Urbana-Champaign, IL.*

The objective was to determine the interactions between different dietary crude protein (CP) and 2 urea sources on rumen ammonia nitrogen and blood metabolites were evaluated. Eight ruminally cannulated Nellore heifers (initial body weight = 525 ± 70 kg) were randomly assigned to 1 of 4 treatment diets in a replicated 4 × 4 Latin square design balanced for residual effects with a 2 × 2 factorial arrangement of treatments to test interactions between 2 urea sources (feed grade urea [FGU] vs. postruminal release urea [PRU]) and 2 levels of CP (11% vs. 14%). The diet composition comprised 24% sorghum silage, 34% ground corn, 34% rehydrated and ensiled corn, 3% vitamin-mineral mix, and, for diet 11% CP + U contained 3.4% soyhulls and 1% U, 11% CP + PRU contained 3.4% soyhulls and 1.2% PRU, 14% CP + U contained 5.4% soybean meal and 1.3% U, 14% CP + PRU contained 5.4% soybean meal and 1.6% PRU. The replacement of soyhulls with soybean meal was carried out to maintain the same source of protein. Four 27-d periods were conducted with 14 d for adaptation to the diet, d 15 to 27 for sample collection, and d 24 to collect blood and rumen samples every 2 h over 24 h. Blood samples were analyzed for urea nitrogen with quantitative colorimetric kits. Rumen fluid samples were analyzed for nitrogen ammonia ruminal with colorimetric analysis. Data were analyzed using the MIXED procedure of SAS 9.4. The results revealed no effects of treatment or interactions between treatment and time on ruminal ammonia nitrogen ($P = 0.20$ and $P = 0.47$, respectively). However, ruminal ammonia nitrogen was affected by time ($P = 0.04$), with the highest nitrogen ammonia ruminal concentration observed at 22 h postfeeding and the lowest at 8 h postfeeding. The blood urea nitrogen levels demonstrated an effect of treatment ($P < 0.001$) and time ($P = 0.08$). Animals receiving the highest level of crude protein (14% + ULP and 14% + UC) exhibited the highest concentrations, measuring 28.4 mg/dL and 28.0 mg/dL, respectively. Higher protein levels increase the level of urea nitrogen in plasma regardless of the type of urea.

Key Words: cattle, feedlot diet, nitrogen metabolism

P85 Evaluating the effects of acidosis and bacterial concentrations on the development of liver abscesses in beef steers.

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We evaluated the association of diet type, ruminal acidosis bout frequency, and intraruminal bacterial inoculum concentration on liver abscess (LA) development in steers. Beef × dairy steers (n = 40, initial BW = 107 ± 11 kg) were assigned randomly to 1 of 5 treatments that included either a high-dose (HD; 1×10^9 and 1×10^9 cfu/mL) or a low-dose (LD; 1×10^6 and 1×10^6 cfu/mL) of *Fusobacterium necrophorum* ssp. *necrophorum* and *Salmonella enterica* serovar Lubbock, respectively. Treatments included: NCON = high-forage diet with no intraruminal inoculation; CON+HD = high-forage diet with a single, HD inoculation; 2AD+HD = 2 acidotic diet cycles with a single, HD inoculation; 4AD+3LD = 4 acidotic diet cycles with a series of 3 LD inoculations; and 4AD+HD = 4 acidotic diet cycles with a single, HD inoculation. Individual animal was the experimental unit. Continuous data were analyzed using mixed models, and categorical data were analyzed as binomial proportions with treatment, time, and their interactions as the fixed effects. Hematology was evaluated before harvest on d 21 to evaluate indications of systemic disease. Although no difference was observed in rumenitis among treatments ($P = 0.79$), steers with LA presented with 8 percentage points greater rumenitis than steers without LA. Steers consuming the high-forage diet also presented with rumenitis. Prevalence of LA was 0%, 37.5%, 37.5%, 50%, and 12.5% for NCON, CON+HD, 2AD+HD, 4AD+3LD, and 4AD+HD, respectively ($P = 0.68$). A key observation of this study was that steers fed a high-forage diet presented with LA when dosed with of *F. necrophorum* and *S. enterica*, even in the absence of the acidotic diet.

Key Words: disease challenge model, *Fusobacterium necrophorum*, *Salmonella enterica*

P86 Lipidomic profiling of *Acremonium terrestris* culture identifies glycerophospholipids as modulators of milk production in Holstein dairy cows.

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The study evaluated the effects of supplementing the diet of lactating cows with *Acremonium terrestris culture* (ATC) on milk production, serum antioxidant and inflammation indices, and serum lipid metabolomics. Over 90 d, 24 multiparous Chinese Holstein cows in mid-lactation (108 ± 10.4 d in milk, 637 ± 25 kg body weight, 30.23 ± 3.7 kg/d milk yield) were divided into either a control diet (CON) or a diet supplemented with 30 g of ATC daily. All the data were analyzed using the Student's *t*-test by SPSS20.0 software. Results showed that feeding ATC significantly increased milk yield, antioxidant capacity, and immune function compared with the CON group. A lipidome screening identified 143 lipid metabolites that differed between the 2 groups. Further analysis using "random forest" machine learning revealed 3 serum metabolites from glycerophospholipids that could serve as lipid markers with a predictive accuracy of 91.67%. The study suggests that ATC can be a useful dietary supplement to improve lactational performance in dairy cows and provides valuable insights into developing nutritional strategies to maintain metabolic homeostasis in ruminants.

Key Words: *Acremonium terrestris culture,* glycerophospholipids, sphingolipids

P87 Clinical cure failure of metritis is not associated with differences in the uterine metabolome of dairy cows.

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The objective was to characterize differences in uterine metabolome associated with clinical cure failure of metritis in lactating Holstein cows. This prospective cohort study was conducted from February to November 2018 in 2 dairy herds in Florida. Vaginal discharge was evaluated within 15 DIM using the Metricheck device and metritis was characterized by the presence of fetid, watery, reddish-brownish vaginal discharge (d 0). Cows with metritis (n = 24) were paired with cows without metritis of similar DIM and parity (NoMET; n = 24). On d 0, the uterine lumen of all cows was flushed with 15 mL of saline solution using a plastic infusion pipette connected to a syringe and cows with metritis received antimicrobial therapy. On d 5, clinical cure failure of metritis, characterized by the persistence of fetid, watery, reddish-brownish vaginal discharge (Cure; n = 8 and NoCure; n = 16), was assessed before the performance of a second uterine flush. The uterine metabolome was evaluated using untargeted gas chromatography time-of-flight mass spectrometry. Autoscaled and normalized data were analyzed in Metaboanalyst using ANOVA and Principal Component Analysis. Enriched pathway analysis was performed whenever differences in uterine metabolome composition between Cure and NoCure cows was observed. False discovery rate (FDR) was used to adjust P-values for multiple comparisons. A total of 188 annotated primary metabolites, characterized by different peak intensities and charge, composed the dataset in the study. On d 0, the abundance of 107 metabolites differed (FDR ≤ 0.05) between the uterus of cows with and without metritis. Most compounds were associated with the metabolism of amino acids, carbohydrates, and fatty acids. No differences in the uterine metabolome were observed between Cure and NoCure cows on d 0. No differences in the uterine metabolome were observed among any of the groups on d 5. This demonstrates that although metritis is associated with differences in the uterine metabolome on the day of diagnosis, clinical cure failure of metritis is not associated with differences in the uterine metabolome.

Key Words: uterine health, disease, cure

P88 The effect of lavender *officinalis* extract on some immunological, hematological characteristics and performance of lambs under the influence of heat stress.

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Heat stress (HS) affecting ruminants is a new concern in the climate change scenario. Among its most important effects, we can mention the reduction of growth, production, and natural immunity, and reducing metabolism. The hydroalcoholic extract of lavender has anti-inflammatory properties. The 40 Sangsari male sheep with an average live weight of 36 ± 2 kg and an average age of 6 ± 1 mo were randomly selected. Lavender hydroalcoholic extract (LC) at 4 levels (0, 100, 200, 400 mg of extraction per kg of feed) was encapsulated in 2 replicates. The sheep were under HS (36 ± 0.5°C) for 4 h/d in a 35-d experimental period. At the end of the course, cholesterol (C), triglyceride (T), HDL, LDL, IgG, TNF-α, AST, ALT, and ALP were measured, and the amount of dry matter intake and weight gain were evaluated. The amount of C increased significantly compared with the control. While with the increase of LC level, C decreased. HDL and LDL showed an increasing and decreasing trend with the increase of LC level compared with the control sample, respectively. Also, the value of T was not significantly different with the increase of LC level compared with the control sample, which indicates the positive effect of LC on the increase of T. The use of LC led to a significant change in ALT and ALP, while no significant difference was observed in AST. The amount of ALP and ALT were decreased compared with control. The amount of TNF-α in the test samples decreased with increasing LC level. Also, with the increase of LC level, IgG increased. Finally, an increase in the consumption of dry matter and the final weight of the lambs was observed. The results of this research indicated the applicability of lavender extract in HS on small ruminants such as sheep. The best result was related to the dose of 400 mg/kg LC in terms of immunological and hematological characteristics.

Key Words: heat stress, lavender extract, lamb

P89 Impact of adding water to a barley-based finishing feedlot diet on cattle feeding behavior and ruminal fermentation.

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Table 1 (Abstr. 90). Association of metabolites (low or high levels) and haptoglobin (Hp) interaction on performance of Holstein cows

	Low Hp		High Hp			*P*-value		
Metabolite	Low	High	Low	High	SEM	Hp	Metabolite	Interaction
NEFA							NEFA	Hp × NEFA
Milk yield, kg/d	42.8	45.5	41.6	41.6	1.05	<0.01	0.12	0.13
DMI, kg/d	22.6^{a}	22.6^{a}	22.3^{a}	20.8^{b}	0.58	<0.01	0.03	0.05
BHB							BHB	Hp × BHB
Milk yield, kg/d	44.0	44.9	42.0	40.9	1.1	<0.01	0.88	0.25
DMI, kg/d	23.2^{a}	22.0^{a}	22.8^{a}	20.3^{b}	0.41	<0.01	<0.0001	0.04
TAG							TAG	Hp × TAG
Milk yield, kg/d	44.0	45.8	42.4	39.9	1.48	<0.01	0.81	0.12
DMI, kg/d	22.9	21.6	22.3	19.7	0.52	0.02	<0.001	0.21

Finishing feedlot diets usually have high grain and low forage (≤10% diet dry matter [DM]) contents, resulting in a dry and easy to sort diet. Despite "slick-bunk" management, competition among cattle pen-mates may result in variability in the diet consumed. The objective of this study was to evaluate the effects of water addition to a finishing total mixed ration (TMR) as a strategy to bind the ingredients together and reduce sorting potential of the diet. Eight ruminally cannulated Hereford crossbred growing beef steers were used in a replicated 4 × 4 Latin square study with 4 dietary treatments. The diet consisted of 88.0% dry-rolled barley, 7.7% barley silage, 4.1% vitamin/mineral premix, and 0.2% titanium dioxide (DM basis). Aggressively dry-rolled barley grain with a processing index of 62.2% (±2.1%) and 3.2% fines (±1.0%) was used, containing sufficient fines to detect treatment differences. The treatment diets incorporated water at 0%, 10%, 20%, and 30% relative to the barley grain weight on a DM basis. The 21-d experimental periods were divided into 16 d of adaptation and 5 d of data collection, where ingredients, refusals, and ruminal digesta samples were collected. Data were analyzed using the MIXED procedure of SAS to evaluate linear and quadratic effects of increasing water inclusion. As water inclusion increased, both dry matter intake (DMI) and water intake linearly increased ($P < 0.01$ and $P = 0.04$, respectively). As water inclusion increased, sorting against small particles ($P < 0.01$), mean ruminal pH ($P < 0.01$), and maximum ruminal pH decreased linearly ($P = 0.02$). However, increasing the water addition linearly increased the time ruminal pH was below 5.5 ($P = 0.02$). Total short-chain fatty acid and rumen ammonia-nitrogen concentrations were not affected ($P \geq 0.46$ and $P \geq 0.22$, respectively) by the water addition. Rumen lipopolysaccharide (LPS) concentration increased linearly as the water inclusion increased ($P < 0.01$). These data suggest that adding water to the TMR may enhance DMI and water intake and reduce dietary sorting. These outcomes were confirmed with a greater extent of ruminal pH depression and increased concentration of LPS.

Key Words: feedlot, barley

P90 Interplay between energetic metabolites and inflammation and postpartum cow performance.

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We hypothesized that negative associations between postpartum cow performance and concentrations of nonesterified fatty acid (NEFA) and β-hydroxybutyric acid (BHB) in serum and hepatic triglyceride (TAG) were influenced by the severity of inflammation at parturition. The objective of the experiment was to test the influence of inflammation as measured and indicated by serum haptoglobin on the associations of NEFA and BHB in serum and hepatic TAGon lactation performance. A retrospective observational cohort study was conducted utilizing data from the previous 3 experiments (n = 252 multiparous). Cows were classified according to their blood concentrations of NEFA and BHB, as well as hepatic TAG levels on a wet basis, using thresholds of 0.46m*M*, 0.80m*M*, and 7.50% respectively, at d 7 postpartum. Likewise, cows were categorized as low haptoglobin or high haptoglobin around the median value of serum haptoglobin at parturition. Data were analyzed using linear mixed-effects models through the MIXED procedure of SAS by incorporating the fixed effects of Hp, metabolite (NEFA, BHB, and TAG), the interaction between Hp and metabolite and calf sex. Treatments of experiments were included as a random effect. Results are presented in Table 1. The severity of postpartum inflammation is the primary factor contributing to decreased milk yield and DMI.

Key Words: nonesterified fatty acid (NEFA), β-hydroxybutyric acid (BHB), inflammation

Poster Session 4: Integration of Environment, Physiology, and Well-Being

P91 Impact of colostrum lactose concentration in relation to metabolomic profiles on kid growth performance of Black Bengal goats in Thailand.

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Colostrum contains nutrients, growth factors, and passive immunity, which is essential for kid growth, while the composition of nutrients and metabolites in relation to kid's growth performance of Black Bengal (BB) goats has not yet been elucidated. This study aims to investigate the importance of lactose in relation to metabolites in colostrum on kid's growth performance of BB goats. Macronutrients from MilkoScan and metabolomic profiles measured by ^{1}H-NMR technique were determined in colostrum from 37 dams and the relationship with kid's growth performances including birth weight, average daily gain at 0–4 weeks (ADG0–4W), 4–8 weeks (ADG4–8W), and weaning weight were investigated. Factors affecting growth performances were investigated using the general linear model (PROC GLM) for univariate analysis. The generalized linear mixed model procedure (PROC MIXED) was used to investigate the effect of metabolites (high and low concentration based upon average mean) on kid growth. The results showed 51 metabolites in colostrum of BB goats. There were positive correlations between ADG0–4W and lactose ($P < 0.001$), betaine ($P < 0.05$), glycine ($P < 0.05$), and triox ($P < 0.01$), whereas most of metabolites showed negative relationships. Both litter size and parity number had effects on kid growth. Kids that consumed high lactose (>2.89%) had higher ADG0–4W ($P < 0.001$) and weaning weight ($P < 0.05$) than those that consumed low lactose (≤2.89%) (92.11 ± 3.42 vs. 72.22 ± 3.91 g/d). However, kids that consumed colostrum with high protein and metabolites (fumarate, myoinositol, vitamin B2, and vitamin B7) had lower ADG0–4W ($P < 0.05$) than those that consumed colostrum of low concentrations. The results suggested that lactose is essential for neonatal growth, especially 1 mo postpartum, and may reflect colostrum yield of the dam due to an osmotic effect.

Key Words: growth, lactose, metabolome

P92 Prediction milk yield responses in dairy cows exposed to heat stress using machine learning techniques and artificial neural networks.

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A prediction model is key for improving production, cost efficiency, risk management, and operational effectiveness in the dairy cattle industry, but research on a heat stress (HS) prediction model affecting productivity and physiological aspects is limited. Therefore, we aimed to develop a HS prediction model based on analysis results using a database accumulated from HS research based on the dairy cow temperature-humidity index (THI) chart. For the prediction model development, we collected dairy cow data (n = 364) from previous HS research in chamber. We removed outliers and normalized the data through preprocessing. For variable selection, we employed a knowledge-based approach, Pearson correlation matrix, and variance inflation factor score analysis. We used machine learning regression (MLR) and artificial neural networks (ANN) for prediction modeling. K-fold cross-validation, mean absolute percentage error (MAPE), and root mean square error (RMSE) were used for evaluating model performance. We evaluated the accuracy of HS prediction model by using the MAPE and compared dairy cows performance with data from ambient environments (n = 7621). The research results, 7 models each through MLR and ANN. The most accurate formula in MLR is milk yield = 34.10 − 0.15 × THI − 4.23 × lactation stage − 7.59 × rectal temperature (accuracy: 95.65, RMSE: 1.44, relative RMSE: 4.78). Meanwhile, the highest accuracy in ANN was observed when using THI, lactating stage, and rectal temperature (accuracy: 96.73, RMSE: 1.41, relative RMSE: 4.68). By validating the developed prediction model through MLR with data collected from dairy cows in ambient environments, we obtained MAPE values of 0.18, 0.18, 0.33, 0.16, 0.17, 0.17, and 0.20. Based on the MAPE values, the fourth model demonstrated the best results. In conclusion, the developed prediction model is demonstrated to facilitate the establishment of a management system that reduces HS in dairy cows, thereby supporting the implementation of animal welfare.

Key Words: heat stress, dairy cow, milk yield prediction model

P93 Effects of processing grains on ruminal and intestinal digestibility of starch, rumen health, and performance of dairy cows.

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The study aims to investigate effects of mechanical and chemical treatment of grains on ruminal degradability, intestinal digestibility, rumen health, and performance in dairy cows. Average particle size (APS) was calculated from dry sieving of ground corn, crushed wheat, wheat-NaOH, ground barley, crushed barley, and rolled barley on 8 sieves. Nonprocessed wheat-NaOH (DM of 783 g/kg) was treated with 3% of NaOH and stored on a roofed concrete slab. Ruminal degradation rate (k_dST) and bypass starch (%BST) were derived from in situ nylon-bag incubations of the treated grains (Centraal Veevoeder Bureau, Protocol bepaling in situ pensafbreekbaarheid van voedermiddelen, 2004). Intestinal digestibility of starch (%TDBST) was measured using mobile nylon-bag technique. In a randomized block design, 72 mid-lactating cows were allocated into 1 of 6 groups supplied with rations of 60% grass and 20% corn silage, 5% soybean meal, 15% of one of the treated grains. Individual feed intake, milk performance, apparent total-tract digestibility (ATTD) of starch, and reticular pH values were measured over 6 weeks. Cow-week data were analyzed by one-way ANOVA and Tukey post hoc test. The greatest APS and least k_dST were observed for wheat-NaOH. In contrast, the greatest k_dST was found in ground barley and crushed wheat. Ground corn and wheat-NaOH were characterized by the greatest %BST, whereas %TDBST was higher for crushed wheat and rolled barley. Feed intake, milk yield, and reticular pH were not affected by grain processing. Starch ATTD was greater for crushed wheat than ground corn, wheat-NaOH, and rolled barley ($P < 0.001$), but not different when compared with ground and crushed barley. In conclusion, grain processing significantly influences starch degradation and digestibility, but did not affect performance and rumen health.

Key Words: digestibility, starch, grain processing

P94 Evaluation of milk replacer program on physiological changes in calves to 7 months of age.

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This study aims to investigate how different milk programs affect the growth and physiological changes in calves. We hypothesized that low nutrient milk programs could adequately meet nutritional needs without compromising long-term growth. Sixteen crossbred (Holstein × Japanese Black) calves were assigned randomly to 2 milk replacer (MR) groups: high protein, high fat (HPF; CP 27.5%, fat 20%) and low protein, low fat (LPF; CP 23%, fat 11.5%). Starter and bermudagrass hay were provided ad libitum until weaning, after weaning all calves were fed the same diet. Growth performance, feed intake, blood metabolites, rumen fermentation, and microbiota were monitored weekly from 1 to 9 weeks of age (preweaning), then at 12 weeks (postweaning), and again at 7 mo of age. Data were analyzed using a 2-way repeated measures design, focusing on MR treatment, week, and their interaction. Results showed consistent growth performance across both groups during all experimental periods. The HPF group exhibited temporary increases in blood urea nitrogen, total cholesterol, nonesterified fatty acids, and phospholipids ($P < 0.05$), but these levels normalized to those of the LPF group after weaning. Conversely, the LPF group's increased hay consumption ($P < 0.001$) to meet energy needs resulted in shorter weaning periods ($P < 0.001$) compared with HPF. This adaptation led to higher ruminal protein fermentation indicators, such as ammonia and isobutyric acid, at 12 weeks ($P < 0.05$), indicating an increase in bacterial diversity at 7 mo ($P < 0.05$), which is crucial for effective protein digestion. Correlation analysis showed positive associations between body weight and blood calcium concentration, as well as the presence of rumen bacteria such as *Acidaminococcus* and *Clostridia_UCG-014* at 12 weeks ($P < 0.05$), and negative correlations with creatine kinase at 12 and 28 weeks ($P < 0.05$). Despite its lower nutritional value, the LPF-MR did not negatively impact calf growth, and most physiological changes

Table 1 (Abstr. 93). Average particle size, ruminal degradation rate of starch, bypass starch fraction, and total digestible bypass starch of treated grains

Treatment	APS (µm)	Starch (g/kg)	k_dST (%/h)	%BST	%TDBST
Corn ground	477	615	10.0	27.1	94.7
Wheat crushed	2,662	550	38.9	6.8	97.5
Wheat-NaOH	4,195	482	8.3	19.2	94.0
Barley ground	597	491	41.3	8.6	94.7
Barley crushed	2,086	485	18.2	13.0	94.6
Barley rolled	2,435	500	26.0	11.5	96.3

observed during MR feeding were temporary. Moreover, the LPF-MR program may offer a cost-effective feeding strategy due to its lower cost.

Key Words: calf nutrition, milk replacer, physiological changes

P95 Economic impact of the enteric methane emissions in small scale dairy systems in the highlands of Mexico.

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Small scale dairy systems (SSDS) are key to combat poverty and hunger. However, the lower milk price, the family labor dependence, and the feeding cost are the main limitations that result in a low income. In addition, enteric methane emissions represent an energy loss to the animal and, therefore, an economic loss for the farmer. The aim of this work was to estimate the enteric methane emissions of dairy cows and the economic impact of this loss of energy. Data from 294 SSDS from the highlands of Mexico, recollected in 2011, 2019, and 2023, were used. Farms were classified by their level of production: low (<10 kg milk/cow), medium (between 10 and 16 kg milk/cow) and high (>16 kg milk/cow). Enteric methane emissions were estimated using the equation of Congio et al. (2020; Prediction of enteric methane production and yield in dairy cattle using a Latin America and Caribbean database; Sci. Total Environ. 825:153982): CH_4 (g/cow/d) = 30.6 + 16.3 × DMI (kg) + 2.04 × protein and energy-corrected milk (kg). A general linear model was used to analyze the data. Results showed statistical differences between methane emissions: 273 g CH4/cow daily for Low, 295 g CH4/cow daily for Medium and 319 g CH4/cow daily for high. In relation to the feeding cost, these emissions represent a cost of \$0.42/cow/d on average for the 3 levels of production. The estimated gross energy intake transformed into methane was 13%, 12%, and 10% for Low, Medium and High, respectively, which is in the range of 2% to 12%. In terms of milk loss, methane emissions accounted for 3.04, 3.29 and 3.55 kg milk/cow/d for Low, Medium and High, respectively. The above represents a loss of \$2,714, \$3,555, and \$4,412 per farm per year for Low, Medium, and High, respectively. Increasing feed efficiency would reduce the emission of methane, using that energy to increase the MY and therefore, this translates into greater economic performance for the producer. Reducing methane emissions would not only generate an increase in profits via an increase in milk yield, but would also increase feed conversion and therefore decrease feed costs.

Key Words: methane, milk, cost

P96 Developing models for enteric methane emissions using animal performance, feeding behavior, and gas emissions from Angus crossbred heifers in late gestation.

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Reducing CH_4 emissions is a global goal for the agriculture community. This study aimed to investigate the relationship between enteric gas emissions, animal performance, and feeding behavior, and to develop empirical models to predict CH_4 emissions. Angus crossbred heifers (n = 70) blocked by expected calving date had ad libitum access to a haylage-based diet formulated to meet nutrient requirements for the last 8 weeks of gestation. Heifers were weighed and body condition scored every 28 d. Individual feed intake and behavior data were recorded daily using Insentec feed stations (Insentec B.V.). Heifers had access to GreenFeed trailers (C-Lock Inc.) to measure CH_4 and CO_2 emissions, and consumption of O_2. Data were analyzed using PROC GLIMMIX in SAS, with CH_4 emission (g/d) quartile (Q) group as the fixed effect and pen as a random effect, comparisons between Q groups were assessed with a Tukey test. Low CH_4 heifers (Q1) had lower ($P \leq 0.03$) BW (582 vs. 628 ± 18.9 kg), DMI (8.8 vs. 10.2 ± 0.25 kg/d), RFI (−0.41 vs. 0.53 ± 0.229), spent less time (149 vs. 180 ± 7.3 min/d) at the Insentec, consumed less feed per meal (1.1 vs. 1.4 ± 0.11 kg DM/meal), and produced less CH_4 (202.2 vs. 261.6 ± 3.99 g/d) and CO_2 (7,578 vs. 8,263 ± 150.2 g/d) compared with high CH_4 (Q4) heifers. Data were used to develop models to predict CH_4 production (g/d), yield (g/kg of DMI), and intensity (g/kg of BW) using principal component analysis (PCA), principal component regression (PCR), and partial least squares (PLS) to remove collinearity. Model evaluations were based on mean square prediction error (MSPE) and concordance correlation coefficient (CCC). Root MSPE (rMSPE) was lowest and CCC was greatest for PLS-derived equations for CH_4 production (4.75%, 0.84), yield (6.01%, 0.86), and intensity (6.77%, 0.73), respectively. Developed models used time at Insentec, number of visits per day, time spent per visit, and eating rate as factors which reduced CH_4 emissions. In addition to feed intake, feeding behavior may be an important factor that influenced CH_4 production in forage fed beef heifers.

Key Words: enteric methane, late gestation, empirical model

P97 Early life inoculation: Steering calf rumen microbiome toward low methane emission.

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The objective was to steer the calf rumen microbiome toward a composition that is associated with low CH_4 emission using early life inoculation with rumen fluid from low CH_4 emitting donor cows. Sixty female calves were randomly assigned to 1 of 3 inoculation treatments: rumen fluid from low CH_4 emitting donor cows (LOW), rumen fluid from high CH_4 emitting donor cows (HIGH), or water (CON). Donor cows, 6 low and 6 high CH_4 emitters (17.2 and 24.0 g/kg DMI, respectively), were selected from a screening trial in which CH_4 emission was measured from 192 lactating dairy cows over 3 weeks. Calves were inoculated 5 times with 100 mL inoculant: 24 h after birth and subsequently twice weekly for 2 weeks. Rearing conditions were similar for all calves. Feed intake, BW, and CH_4 emission were measured from 12 to 52 weeks of age. Rumen fluid was collected from donor cows (inoculant) and from calves at 26 and 52 weeks of age, using the oral stomach tube technique, to determine the microbiota composition. Preliminary results show no effect of inoculation treatment on feed intake, BW, weekly BW gain, and feed efficiency of the calves. Calves of the LOW treatment had a lower ($P = 0.05$) CH_4 intensity compared with calves of the HIGH treatment (25.0 vs. 25.7 g/kg BW, respectively), with both treatments not differing from CON. The calf rumen microbiome appeared to be distinct from that of the donor cows (RDA $P < 0.01$; PERMANOVA on Bray-Curtis $P < 0.05$), but the rumen microbiome of the donor cows did explain 2.4% of the variation in the calf rumen microbiome ($P < 0.01$). Furthermore, the calf rumen microbiome was affected by day of sampling. Adjusted for this effect, the inoculation treatments accounted for 3.3% of the variation in the calf rumen microbiome ($P < 0.01$), highlighting the significant impact of early life inoculation on calf microbiota. In conclusion, these preliminary results show that early life inoculation with rumen fluid from donor cows varying in CH_4 emission induces changes in the rumen microbiome and level of CH_4 emission of calves, without negatively affecting growth performance and feed intake.

Key Words: early life inoculation, methane emission, rumen microbiome

P98 Evaluation of bovine colostrum replacer supplementation to reduce intestinal permeability during weaning transition in dairy calves.

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During weaning, dairy calves often experience hunger, gastrointestinal challenges, and reduced weight gain. Decreased growth is associated with lower future production, so ways to mitigate the negative effects of weaning are needed. The objective of this study was to assess the efficacy of supplementing bovine colostrum replacer (BCR) during weaning as a method to reduce intestinal permeability and improve growth. Female Holstein calves (n = 65) were housed individually until 70 d and fed milk replacer (MR; 150 g/L) 3 times daily, plateauing at 12 L/d from d 22–56. Calves were weaned over 8 d from d 57–64. At d 57, calves were blocked by birthweight and randomly assigned to 1 of 2 treatments, equal in metabolizable energy, that were fed once daily during weaning: control (CON; n = 31): 3.8 L MR (150 g/L) fed by nipple bottle, or BCR supplementation (COL; n = 34): mixture of 1 L BCR (125 g/L) and 3 L MR (150 g/L) with 3.8 L of the mixture fed by nipple bottle. Body weight (BW) was recorded at d 0, 57, 60, 64, 70, 77, and 84. To assess intestinal permeability, calves were orally dosed with Cr-EDTA (0.1 g/kg BW), lactulose (0.4 g/kg BW), and D-mannitol (0.12 g/kg BW) at d 56 and 65. Marker recovery from plasma was analyzed using mass spectrometry, with lactulose and D-mannitol samples run in duplicate and the mean value used for analysis. At d 57, there was no difference in BW ($P = 0.97$) between CON (90.8 ± 0.7 kg) and COL (90.8 ± 0.6 kg); however, COL were 2.79 kg (95% CI: 0.90–4.68; $P = 0.004$) and 2.76 kg (95% CI: 0.86–4.65; $P = 0.004$) heavier than CON at d 77 and 84, respectively. Also, COL tended to gain 100 g/d more than CON from d 57–84 (95% CI: −10.41–207.13; $P = 0.08$). There was no difference in recovery of Cr-EDTA ($P = 0.58$), lactulose ($P = 0.94$), or D-mannitol ($P = 0.80$), nor the lactulose to D-mannitol ratio ($P = 0.72$). Calves supplemented with BCR once daily during the 8-d weaning had improved growth 2 and 3 wk after weaning, but there were no detected effects of BCR on intestinal permeability. These results suggest that BCR supplementation during weaning may support growth, but the mechanism is unclear.

Key Words: growth, wean, supplement

P99 Projection of climate change impacts for lactating performance of Holstein cows in summer season in Japan.

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World average air temperature is expected to continue to rise at least until the middle of this century (IPCC AR6). In Japan, annual average air temperature at the end of the 21st century is expected to rise nationwide (0.5–5.4°C), and higher latitudes are predicted to experience greater increases than lower latitudes (Ministry of the Environment Japan, Synthesis Report on Observations, Projections and Impact Assessments of Climate Change, 2018). About 60% of cows are raised in high-latitude regions in Japan (Ministry of Agriculture, Forestry and Fisheries, Statistical data for number of adult animals for dairy cow, 2023), so it is important to consider the regional impact of future climate change on milk production. Therefore, this study was made to clarify the impact of climate warming on milk production in summer based on an original milk production model, latest climate models and warming scenarios considering the region on a 1km × 1km area mesh analysis. According to 9 feeding trials in a temperature- and humidity-controlled room, each combining different conditions of ambient temperatures of 18, 23, and 28°C and percentages of relative humidity (RH; 60%, 70%, and 80%), the following model was estimated ($y = - 0.0637 x2 + 6.744x - 71.21$ where x: temperature-humidity index (THI), y: the percentage decrease in 3.5% fat-corrected milk (FCM) as compared with at 18°C and 60% RH). Spatial distribution of the impact of warming on summer milk production was analyzed by using a 1 km × 1 km area mesh data (Ishizaki, N. N., Bias corrected climate scenarios over Japan based on CDFDM method using CMIP6, Ver. 1, NIES, 2021). The data analyzed were the daily average air temperature and RH for July, August, and September from each target year 1900–1910, 1995–2005, 2025–2035, 2045–2055, and 2090–2100 from CMIP6 5 climate models; MIROC6, MRI-ESM2–0, ACCESS-CM2, IPSL-CM6A-LR, and MPI-ESM1–2-HR for each warming scenarios; SSP1–2.6, SSP2–4.5, and SSP5–8.5. Reduction rates of FCM were estimated for all locations, and a map image was created. Except for the prediction by SSP5–8.5 at 2090, there was less widespread distribution of areas where FCM decreased by 10% or more in the highest latitude regions in any scenario. Based on the predictive climate change models used, model predicted milk production in high-latitude regions may be maintained at a level close to the current situation unless global warming follows the worst-case scenario.

Key Words: dairy cow, global warming, area mesh data

P100 Long-term impact of a high-starch diet early in life on selected blood parameters. M. Jamrogiewicz*[1], M. Przybylo[1], J. Kanski[1], J. Kowal[2], A. Wyrobisz-Papiewska[2], J. Flaga[1], D. Wojtysiak[3], R. Militko[4], M. Clauss[5], and P. Górka[1], [1]*Department of Animal Nutrition and Biotechnology, and Fisheries, Faculty of Animal Sciences, University of Agriculture in Kraków, Kraków, Poland,* [2]*Department of Zoology and Animal Welfare, Faculty of Animal Sciences, University of Agriculture in Krakow, Kraków, Poland,* [3]*Department of Animal Genetics, Breeding and Ethology, Faculty of Animal Sciences, University of Agriculture in Krakow, Kraków, Poland,* [4]*The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna, Poland,* [5]*Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zürich, Switzerland.*

We aimed to determine the long-term effect of a high-starch diet in the first weeks of life on selected blood parameters in sheep. Fifty-four male lambs (5–15 d of age; collected in 3 batches) were randomly divided into 2 experimental groups: control (C) and starch (S), and kept in 8 group pens (6–7 animals/pen; 4 pens/treatment). Animals were fed ad libitum milk replacer (MR), and either a mixture of meadow hay and alfalfa hay (1: 1; C; n = 27) or a concentrate mixture (70% barley, 15% wheat bran, 10% soybean meal, 5% chopped hay; S; n = 27) ad libitum. MR was fed for 7 wk whereas experimental solid feeds were fed for additional 2 wk after weaning. From wk 10 onwards both groups were transitioned to the same hay-based diet (fed ad libitum). This common diet continued till the age of 7 mo. Blood samples were collected once a month from 3 randomly selected animals per pen (12 animals/treatment) and analyzed for plasma glucose, cholesterol, urea, serum β-hydroxybutyric acid (BHBA) and free fatty acids (FFA); they were analyzed with a mixed model procedure with a fixed effect of treatment, sampling point and interaction between those, and random effect of batch of animals and pen within a batch. Plasma glucose was higher ($P < 0.05$) whereas plasma cholesterol and serum FFA lower for S compared with C in the second month of study (i.e., during weaning transition). Plasma urea was higher ($P < 0.05$) for C compared with S in second, fifth, and sixth month of study. Serum BHBA was higher ($P < 0.05$) for C compared with S in the third month of study. In conclusion, a high-starch diet early in life resulted in higher plasma glucose during the weaning transition, whereas a hay-based diet increased blood BHBA and urea at its further stages. These observations suggest a long-term impact of the diet consumed early in life on metabolic processes and rumen functioning in sheep. This research was funded by the National Science Centre, Poland under the OPUS call in the Weave program (project No. 2021/43/I/NZ9/02222).

Key Words: ruminants, concentrates, metabolism

P101 High-starch diet early in life affects body weight, feed intake, and forestomach morphometry later in life. M. Jamrogiewicz[1], M. Przybylo*[1], J. Kanski[1], J. Kowal[2], A. Wyrobisz-Papiewska[2], J. Flaga[1], D. Wojtysiak[3], R. Miltko[4], M. Clauss[5], and P. Górka[1], [1]*Department of Animal Nutrition and Biotechnology, and Fisheries, Faculty of Animal Sciences, University of Agriculture in Kraków, Kraków, Poland,* [2]*Department of*

Zoology and Animal Welfare, Faculty of Animal Sciences, University of Agriculture in Krakow, Kraków, Poland, [3]Department of Animal Genetics, Breeding and Ethology, Faculty of Animal Sciences, University of Agriculture in Krakow, Kraków, Poland, [4]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna, Poland, [5]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Zürich, Switzerland.

We aimed to determine the long-term effect of a high-starch diet in the first weeks of life on body weight, feed intake, and forestomach morphometry. Fifty-four male lambs (5–15 d of age; collected in 3 batches) were randomly divided into a control (C) and a starch (S) group, kept in 8 group pens (6–7 animals/pen; 4 pens/treatment) and fed ad libitum milk replacer (MR), and either a mixture of meadow hay and alfalfa hay (1: 1; C; n = 27) or a concentrate mixture (70% barley, 15% wheat bran, 10% soybean meal, 5% chopped hay; S; n = 27) ad libitum. Milk replacer was fed for 7 weeks and experimental solid feeds were fed for 2 additional weeks after weaning. From wk 10 all animals were transitioned to the same hay-based diet (fed ad libitum) that was continued until the age of 7 mo. One week after weaning (Phase 1; P1) and at the age of 7 mo (Phase 2; P2), 7 animals (P1) and 8 animals (P2) were randomly selected/treatment (1–2 animals/pen) and placed in individual pens for voluntary dry matter intake (DMI) and body weight (BW) control, and forestomach development screening. Data were analyzed with a mixed model procedure and included fixed effect of treatment, and random effects of animal batch and pen within a batch. Body weight of animals did not differ between treatments in P1 but in P2 was higher in S than C group ($P < 0.01$). In P1, DMI (%BW) was higher in S than C group but in P2 was higher in C group ($P \leq 0.02$). In P1, full reticulorumen (RR), RR digesta, reticulum (RT) tissue, full omasum (OM), and OM digesta weight (g/kg BW) was higher in C than S, but RR and rumen tissue was higher in S group ($P \leq 0.05$). In P2, full OM and OM digesta weight was higher in C than S group ($P \leq 0.04$). Moreover, OM tissue tended to be higher in C than S group ($P = 0.06$), but there were no other morphological differences. In conclusion, high-starch diet early in life resulted in higher BW but lower DMI later in life; however, no substantial impact on RR morphometry was found. This research was funded by the National Science Centre, Poland under the OPUS call in the Weave program (project No. 2021/43/I/NZ9/02222).

Key Words: rumen, reticulum, omasum

P102 Physiological effects of vitamin E supplementation on growth performance, physiological indicators, and blood hematology in Holstein calves under long-term heat stress.

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This study was performed to investigate the effects of vitamin E supplementation on growth performance, physiological indicators, and blood hematology in Holstein calves under long-term heat stress (HS). Twelve Holstein calves (5.5 ± 0.35 mo, 196.1 ± 18.1 kg body weight) were blocked by body weight and randomly assigned to 2 groups, and housed in environmentally controlled chambers with the temperature-humidity index (THI) set at 84. The environments inside the chambers were adjusted for one week of adaptation at ambient temperature. After adaptation, the calves were subjected to HS for 3 weeks. The control group was given a basal diet consisting of 4.8 kg of concentrate and 2 kg of tall fescue (basal diet), whereas the treatment group received the basal diet supplemented with vitamin E (1,000 IU/day; α-tocopherol acetate). Daily monitoring was conducted for dry matter intake (DMI), water consumption, respiration rate (RR), and rectal temperature (RT). Additionally, blood samples were collected weekly for hematological analysis. All data were analyzed using the Student's *t*-test of SAS. Compared with the control group, calves supplemented with vitamin E showed a decrease in DMI in the first week ($P = 0.020$) and an increase in RR in the second week ($P = 0.042$). Additionally, there was a tendency for increased RR in the third week ($P = 0.073$) and throughout the entire experimental period ($P = 0.070$). Blood hematology results showed that vitamin E supplementation in calves led to an increase in monocyte counts ($P = 0.040$), a decrease in lymphocyte percentage in the first week ($P = 0.08$), and a tendency for decreased monocyte percentage in the third week ($P = 0.07$). However, vitamin E did not affect average daily gain (ADG), feed efficiency, and RT during this study. In conclusion, our study demonstrates that supplementing the basal diet of Holstein calves with vitamin E led to a decrease in DMI and respiration rate, but did not affect ADG, feed efficiency, and rectal temperature.

Key Words: heat stress, Holstein calves, alpha-tocopherol

P103 Effects of prepartum feeding strategy on health, reproductive success, and performance of Holstein cows.

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A total of 408 cows (354 multiparous and 54 primiparous) from 7 different experiments were analyzed. The net energy for lactation (NEL) intake (NELI) was calculated from each cow's respective dietary NEL density and average DMI. Treatments consisted of controlled-energy (CE; median NELI = 13.7 Mcal/d) or high-energy (HE; median NELI = 22.1 Mcal/d) diets fed during the far-off or close-up (CU) dry periods. Milk yield, milk components, displacement of abomasum (DA), and ketosis (KET) were the variables used to assess performance and health status. The association between physiological factors and reproductive performance (days to conception; DTC) were analyzed. Principal component (PC) analysis was conducted on 8 variables: glucose wk 3, glucose wk 4, β-hydroxybutyrate wk 1, insulin wk 2, NEFA wk −1, energy-corrected milk wk 4, fat-corrected milk wk 4, and milk urea nitrogen wk 5. Before analysis, the multinormality of the variables was assessed. PC with eigenvalues ≥0.9 were extracted. The effect of PC was investigated using linear and logistic (LR) regressions. For LR analysis, animals were classified in 2 groups based on first and fourth quartile values of DTC as high (S, ≥174 d) or low (F, <87 d). All analyses were carried out using SAS (version 9.4, SAS Institute Inc.). There was no difference for milk yield in the first 4 wk ($P > 0.05$). Cows fed HE during CU had greater milk fat concentration in wk 2, 3, and 4; and greater milk protein concentration in wk 3 and 4 than cows fed CE ($P \leq 0.05$). Cows in HE had greater odds of experiencing DA and KET than cows in CE ($P \leq 0.05$). Four PC were extracted from the analysis, accounting for 79.6% of total variability. PC in a logistic model revealed that cows with higher values of PC2 were associated with greater odds of being classified as S (greater DTC) when compared with cows classified as F (odds ratio [OR] = 2.26, 95%CI = 0.98 to 7.76; $P = 0.054$). In conclusion, energy intake prepartum did not affect milk yield, but cows in HE had higher milk components. Additionally, PC explained and predicted reproductive success in dairy cows in this dataset.

Key Words: prepartum energy intake, transition period, health

P104 Assessing the impact of disease symptoms on rumen temperature and environment in Holstein cows using bolus sensors.

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The objective of this study was to assess the impact of disease symptoms on rumen temperature and activity in cows using a bolus sensor. Twenty-four Holstein cows were randomly selected to receive bolus sensors for monitoring rumen temperature and environment. The temperature and activity within the rumen were assessed using a wireless bolus sensor in cows exhibiting symptoms of metabolic disorder (n = 3), low calcium (n = 3), dyspepsia (n = 3), and lumpy skin disease vaccination (LSDV; n = 15). A statistical comparison between the normal and disease symptom groups was performed by using JMP software (SAS Institute Inc.). Significance was set as $P < 0.05$, and the tendency was declared at $0.05 < P \leq 0.1$. The metabolic disorder groups exhibited an increased level of zero rumen activity compared with the normal group ($P = 0.04$). Rumen temperatures were significantly higher in the low-calcium cows and lower in the dyspeptic cows compared with average temperatures ($P < 0.05$). Bolus sensors were used to measure rumen temperature and activity in cows, but there were no changes in mean rumen temperature and activity. Additionally, when cows were vaccinated against LSD, the frequency of above-average rumen temperatures decreased for 3 d and then returned to normal by d 4 ($P < 0.05$). In conclusion, our study provides basic biometric data related to LSDV in lactating Holstein cows by monitoring rumen temperature and activity. These data may be useful in the development of an effective smart disease symptom detection device. However, it is important to note that more data are needed to provide sufficient biometric information related to disease symptoms.

Key Words: bolus wireless sensor, disease symptom, rumen temperature

P105 Lactational performance and plasma amino acids in dairy cows fed a red clover-based silage with or without faba bean and rumen-protected methionine.

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Methionine is often a limiting AA in dairy cow diets, and legume protein has a low Met. We studied supplementing forage legume (red clover, *Trifolium pratense*) – cereal-based diet either with grain legume (faba bean, FB, *Vicia faba*) or rumen-protected Met (RPMet), or both. Eight Nordic Red cows averaging 75 DIM were used in a 4 × 4 Latin square trial with 4-wk periods. The treatments were as follows: (1) without FB and RPMet (NF-), (2) without FB and with RPMet (NF+), (3) with FB and without RPMet (FB-), and (4) with FB and RPMet (FB+). The NF diets consisted of (% of DM) red clover silage (42%; 36.2% NDF, 18.8% CP), grass silage (21%; *Phleum pratense*, *Festuca pratensis*; 53.5% NDF, 15.5% CP), cereals (36%) and minerals. In the FB diets, cereals were partially (15% of diet DM) substituted with FB meal. The NF diets had 16.0% and the FB diets 18.5% CP of DM. Cows were given 1 kg/d molassed sugar beet pulp either with (NF+ and FB+) or without (NF- and FB-) 24 g/d Smartamine RPMet. Data were analyzed with diet, square, and period (square) as fixed and cow(square) as a random effect. DMI and nutrient digestibilities were unaffected by diets, but CP intake was greater ($P < 0.001$) and fat and starch intakes lower ($P < 0.001$) in FB vs. NF diets. Milk and component yields were unaffected, except for lactose yield, which tended ($P = 0.06$) to be greater in FB+ vs. other treatments. Further, MUN, N-efficiency, rumen NH_3, microbial protein production, and plasma Cit were greater ($P < 0.05$) and plasma Cys, Glu, Gly, and Tau were lower ($P < 0.05$) in FB vs. NF diets. Plasma Gly and Ser were lower ($P < 0.05$) and concentrations of all sulfur containing AA greater ($P < 0.01$) in RPMet fed diets. Plasma His tended ($P < 0.10$) to be greater in FB vs. NF diets and lower in RPMet fed diets. Based on the estimated MP supply, the most limiting AA was His in NF diets but Met in FB diets. Despite this and low plasma Met on diets without RPMet, supplementing RPMet had no positive effects on milk production. Supplementing grass-clover diet with FB did not affect lactation performance. It is evident that Met was not a limiting factor in this study.

P106 The first extensive biomonitoring mycotoxin exposure survey. A. Vidal, C. Bandyk*, and C. Gougoulias, *Innovad, Antwerpen, Belgium.*

Mycotoxin exposure is a daily reality for livestock, jeopardizing animal well-being and performance. Ruminants face unique mycotoxin challenges due to the volume and diversity of their diets, including exposure via forages and byproducts. Changing climatic conditions can contribute to increased mycotoxin prevalence. There is a clear need for more accurate methods to quantify actual animal exposure to mycotoxins. As such, biomarker-driven analytics have been proven a successful method for assessment of animal exposure to xenobiotics by determining concentrations of biomarkers in biological matrices like blood. However, due primarily to sample collection difficulties, mycotoxin biomarker analysis has not yet been widely adopted. Recently a UHPLC-MS/MS method targeting 36 mycotoxin biomarkers via dried blood spots was validated (Lauwers et al., 2019). Here the methodology enabled analyses of mycotoxin biomarkers at an industrial scale using minimal blood volumes. More than 1,000 ruminant animals were tested from 14 different countries. To establish a more complete mycotoxin risk assessment, TMR consumed the day of blood collection was also analyzed. Performance and health status were recorded to establish possible correlations with mycotoxin exposure. The results detected mycotoxin exposure in all studied farms and demonstrated a persistent exposure to multi-mycotoxins; 97% of the studied cases were exposed to 2 or more mycotoxins simultaneously, and, notably, 57% of farms were exposed to 5 or more. Of note, the emerging mycotoxins produced by *Fusarium* (enniatins and beauvericin) and *Alternaria* (e.g., tenuazonic acid) were the most prevalent mycotoxins indicated. Tendencies between mycotoxin exposure and health status were identified, including a positive relationship between reproductive disorders and exposure to specific toxins. In conclusion, the first of its kind extensive mycotoxin exposure survey elucidated that ruminants under real farming conditions are regularly exposed to multi-mycotoxins and that biomarker analysis can be key in optimizing animal health and performance.

Key Words: mycotoxins, biomarkers, exposure

P107 Extruded linseed as methane mitigating feed ingredient in a grass and a maize silage-based basal diet. J. Van Mullem*[1,2], N. Peiren[1], B. Ampe[1], V. Fievez[2], and L. Vandaele[1], [1]*Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium,* [2]*Ghent University, Ghent, Belgium.*

To reduce enteric methane (CH_4) emissions from ruminants, feed strategies appear to be the most promising short-term solution. Extruded linseed (EL), rich in polyunsaturated fatty acids (PUFA), shows CH_4 mitigating properties. However, the effectiveness of PUFA could depend on the overall diet composition. Given the large diversity in dairy cattle diets, it is crucial to investigate the interaction between mitigation strategies and diet composition. This study aims to evaluate the potential of EL in 2 different diets: a grass silage (GS) based diet and a maize silage (MS) based diet. A 2x2 crossover experiment was conducted with 6 rumen-fistulated Holstein dairy cows (137 ± 38 d in milk and 38.1 ± 5.9 kg milk day^{-1}). Two basal diets were formulated: a MS-based diet (MS:GS of 75:25) and a GS-based diet (MS:GS of 25:75). Each diet was supplemented with concentrates that did not or did include 1.9 kg DM of an EL product (400 g crude fat day^{-1}) resulting in 4 dietary treatments: (1) MS-based diet (MS-CTRL), (2) MS-based diet supplemented with EL (MS-EL), (3) GS-based diet (GS-CTRL), and (4) GS-based diet supplemented with EL (GS-EL). For each animal, the experiment consisted of 4 experimental periods of 28 d: 21 d adaptation followed by 7 d measurement with in sacco rumen incubations and collection of rumen fluid, rumen solids, and feces and CH_4 emissions measured in open-circuit chambers. Animal performance was not affected by the interaction between diet and treatment. Absolute CH_4 production (449 g and 471 g for CTRL and 397 g and 418 g for EL in the GS diet and MS diet resp.) was not affected by an interaction between diet and treatment. However, a trend ($P = 0.084$) for an interaction was observed when CH_4 was expressed as g per kg dry matter intake (25.7 and 28.0 for CTRL and 24.9 and 22.6 for EL of GS and MS diets resp.). These results imply that the effectiveness of EL may vary in relation to the basal diet depending on the measurement unit, whether it be CH_4 emission or CH_4 yield. Given the limited number of animals, further analysis is needed to support this.

Key Words: methane, dairy cattle, extruded linseed

P108 Do beef heifers previously classified as high– or low–enteric methane emitters respond the same to a canola oil methane mitigation strategy?

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The objective of this research is to determine if heifers previously classified as high or low enteric methane emitters respond similarly to a lipid supplementation mitigation strategy. Canola oil can lower enteric methane emissions in cattle by scavenging hydrogen ions for unsaturated fatty acids, instead of allowing them to contribute to microbial methane production. However, it is not known if animals previously identified as high or low emitters respond the same to a dietary methane mitigation strategy. Therefore 64 Angus crossbred heifers from the Ontario Beef Research Centre were first classified as either high (average 31.77 ± 1.68 g CH_4/kg DMI) or low (26.14 ± 2.46 g CH_4/kg DMI; $P < 0.001$) CH_4 emitting animals based on 2 7-d measurement periods using a C-Lock Greenfeed trailer. In a 2 × 2 factorial arrangement of treatments, high and low classified heifers either remained on the same straw/haylage control ration (Nem = 1.43 Mcal/kg DM, NDF = 53.12% DMB, CP = 0.85% DMB) or received 6% (DM basis) inclusion of canola oil. Heifers were adapted to the diets for 21 d, then each pen of 8 heifers had GHG emissions measured over two 7-d periods using the Greenfeed. The data were analyzed using the GLIMMIX procedure in SAS to evaluate the effects of diet and classification on methane emissions. Oil supplementation significantly ($P = 0.003$) reduced methane emissions. The high and low emitting groups were still significantly different from one another ($P < 0.001$). Though not statistically significant ($P = 0.165$), the classification x diet interaction caused a 10.22% decrease in g CH_4/kg DMI in high emitters and a 4.50% decrease in g CH_4/kg DMI in low emitters showing that the magnitude high emitters can be lowered by is more than twice that of the low emitters. Canola oil successfully reduced methane emissions in high-emitting beef heifers to levels observed in low emitters. The findings indicate that greater reduction in emissions can be achieved when target-feeding animals that are high emitters.

Key Words: beef heifer, GHG emissions, dietary methane mitigation, canola oil methane reduction

P109 Investigating dairy calf hunger after an electrolyte meal using a taste aversion test.

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Preweaning dairy calves are commonly fed electrolytes in lieu of milk during periods of sickness to reduce dehydration. While feeding electrolytes may benefit health, it is unclear how replacing a milk meal with electrolytes affects calf welfare, specifically hunger. The objective of this study was to investigate preweaning dairy calf hunger after being fed electrolytes using a taste aversion test. Preweaning dairy calves (n = 100) were enrolled onto a Latin square design study at 23 ± 1 d of age. The treatment of feeding milk or electrolytes was applied at the morning feeding (0500 h) and the taste aversion test was applied at the evening feeding (1700 h). A taste aversion test was applied by feeding calves milk with a bitter additive (0.35 g/L quinine) or unaltered milk. All meals were 2.7 L. Daily combinations for the morning treatment and evening test, respectively, included (1) milk and unaltered milk, (2) milk and bitter milk, (3) electrolytes and unaltered milk, and (4) electrolytes and bitter milk. Treatment and test combination order received by the calf was balanced. Testing occurred twice per week over a 2-week period. Fed milk and refusals were weighed (g). Calves had ad libitum access to water and starter. On test days, starter was weighed when it was provided after the morning treatment application and before the evening test (g). Calves fed unaltered milk at the evening test consumed more of their meal than calves fed bitter milk (98.4% ± 3.1% vs. 51.4% ± 3.0%, respectively; $P < 0.001$). There was no impact of treatment on the amount of milk consumed at the evening test (74.1% ± 4.0% electrolytes vs. 75.7% ± 3.2% milk, respectively; $P = 0.77$). Interestingly, calves fed electrolytes in the morning ate more grain between the morning and evening feeding than calves fed milk in the morning (135.4 ± 24.8 g vs. 82.4 ± 23.2 g, respectively; $P = 0.004$). The difference in grain consumption may suggest that calves fed electrolytes were hungrier than calves fed milk. A lack of difference in bitter milk consumption between the electrolyte and milk treatment groups could be due to a myriad of reasons, including the greater grain consumption.

Key Words: hunger, electrolytes, dairy calf

P110 Effects of milk replacer fat levels on feeding behavior and performance of male dairy calves.

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The effects of fat ratio in milk replacer (MR) on feeding behavior and performance of dairy calves are still unclear. This study aimed to determine the effects of fat ratio in MR on feeding behavior and performance of dairy calves. Holstein male calves (n = 126) were housed individually and enrolled in a 91-d trial divided in 3 phases: preweaning (1–42 d), weaning (43–63 d), and postweaning (64–91 d). Calves were randomly assigned to 3 treatments consisting of a MR with a palm and coconut fat blend with low fat ratio in relation to the crude protein level (17%; low-fat [LF] %; n = 42), moderate fat (21%; mod-fat [MF] %; n = 43), and high fat (31%; high-fat [HF] %; n = 41). The MR was fed twice daily (Mapleview Agri Ltd., ON, Canada; 26% CP; 130 g/L) following the step-up, step-down program: from d 0 to 6, 520 g/d; from d 7 to 13, 650 g/d; from d 14 to 20, 910 g/d; from d 21 to 41, 1040 g/d; from d 42 to 48, 910 g/d; from d 49 to 63, 650 g/d. Statistical analyses were performed using PROC MIXED procedure of SAS. Overall, average daily gain was greater for LF dairy calves during preweaning (LF = 0.89 ± 0.02, MF = 0.76 ± 0.02, HF = 0.74 ± 0.02 kg/d; $P < 0.01$) but did not differ among treatments across weaning and postweaning phases ($P = 0.17$). Final body weight was greater for LF dairy calves across all phases (LF = 119.60 ± 1.04, ME = 110.49 ± 1.20, HF = 110.30 ± 1.44 kg; $P < 0.01$). Total DMI (MR + calf starter) was greater for LF calves across the weaning and postweaning phases ($P < 0.001$). Grain intake was greater for LF calves across the weaning and postweaning phases ($P < 0.001$). Fat ratio in the MR affected feed efficiency (FE) during preweaning phase as calves in LF were the most and calves in HF were the least efficient (LF = 0.78 ± 0.01, MF = 0.72 ± 0.02, HF = 0.67 ± 0.02; $P < 0.001$). No difference was observed for FE between treatments during the weaning phase ($P = 0.48$). MF calves were more efficient during postweaning ($P < 0.05$). We hypothesize that MF and HF were associated with greater calf satiety negatively affecting calf starter intake and reducing calf performance. Future research should investigate the effects of fat ratio in the feeding behavior and weight gain composition.

Key Words: calf, nutrient intake, fat ratio

P111 Effects of casein presence in colostrum replacer on IgG absorption in neonatal calves. J. M. V. Pereira[1], J. V. R. Lovatti[1], E. V. Lopez-Bondarchuk[1], G. Mazon[1], A. J. Geiger[2], and J. H. C. Costa*[1], [1]*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT,* [2]*Zinpro Corporation, Eden Prairie, MN.*

This study aimed to assess the impact of casein presence in colostrum on serum total protein (STP), immunoglobulin (IgG) serum concentration, and apparent efficiency of absorption (AEA) in neonatal dairy-beef calves. Holstein-Angus calves (n = 24/treatment; 45.3 ± 5.9 kg of birth weight) were assigned randomly to one of 3 colostrum treatments at birth: Colostrum Replacer: CR-C (85 g/L of IgG; Premolac Plus, Zinpro), Colostrum Replacer with casein reconstituted: CR+C (85 g/L of IgG; Premolac Plus, Zinpro; added 95 g/L of casein), and Maternal Colostrum: MC (85 g/L of IgG). Calves received 3.7 L of solution (total 300 g IgG) via tube feeding at 3.1 ± 0.2 h postbirth; casein addition was calculated to match the MC treatment. Blood samples were collected immediately before colostrum feeding (baseline time - 0h) and at 6, 12, 18, 24, 36, 48, and 72 h post colostrum feeding. Serum was analyzed for STP, IgG concentration, and AEA analysis. Mixed linear regression models were used to evaluate the effect of colostrum treatments on STP, total IgG and AEA, with fixed effects of time, calving score, calf sex, body weight and treatment by time interaction. Time was included as a repeated measure. Treatment affected STP levels and serum IgG concentration ($P < 0.001$) depending on time, post colostrum feeding (6 to 72h). Serum IgG was lower for CR+C compared with CR-C treated calves (from 6 to 72h; $P = 0.03$). No difference was observed for serum IgG between CR+C and MC treated calves, regardless of time of blood collection ($P = 0.13$). Additionally, treatment impacted AEA, with CR+C treated calves exhibiting reduced AEA compared with CR-C calves (CR-C = 36.7 ± 2.0^{a}, CR+C = 27.4 ± 2.1^{b}, MC = 31.3 ± 2.1^{ab} %; $P = 0.01$). For STP, no differences were observed between CR+C and CR-C treated calves (from 6 to 72h; $P = 0.26$). However, calves fed CR+C displayed lower STP levels compared with MC treated calves (from 12 to 24h; $P < 0.001$). These findings suggest that casein presence in CR influences calf STP levels, serum IgG concentration, and AEA in neonatal calves. Future research should investigate the role of casein in the release of IgG from the abomasum to the small intestine.

Key Words: colostrum replacer, apparent efficiency of absorption (AEA), casein

P112 Effect of measurement timepoints on methane concentration of growing steers fed corn or corn-soybean silage at different crude protein levels. T. A. Aloba*[1,2], E. E. Corea[3], M. Mendoza[3], M. Chagunda[1], U. Dickhoefer[4], and J. Castro-Montoya[3], [1]*Institute of Animal Nutrition and Rangeland Management in the Tropics and the Subtropics, University of Hohenheim, Stuttgart, Baden Württemberg, Germany,* [2]*Dr Eckel Animal Nutrition GmbH, Niederzissen, Rhineland Palatinate, Germany,* [3]*Faculty of Agricultural Sciences, University of El Salvador, San Salvador, El Salvador,* [4]*Institute of Animal Nutrition and Physiology, Christian-Albrechts-Universität zu Kiel, Kiel, Schleswig-Holstein, Germany.*

Methane (CH_4) emissions from ruminants may follow a daily pattern, tightly linked to digestive processes and typically increasing after meals. Monitoring CH_4 levels at different time intervals can reveal variations linked to the animals' daily routines. Notably, measuring immediately after feeding can capture the peak CH_4 concentrations. However, these peaks are subject to influence from factors such as diet composition and timing. In an experimental setting, the impact of crude protein (CP) levels, silage type, measurement time points, and their interactions on CH_4 concentration in growing steers was assessed. Sixteen tropical crossbred steers were assigned to a 4 × 3 incomplete Latin square design over 17-d periods. Dietary treatments followed a 2 × 2 factorial design, involving 2 main silages (corn silage [CS] and corn-soybean silage [CSS]) offered at 2 CP levels each (115 and 135 g/kg of DM). Using a hand-held laser methane detector, CH_4 concentrations in ppm were measured for 4 min at 0500, 1100, 1400, and 1700 h on each sampling period's first, third, and fifth day. Analysis using the mixed model of the SAS procedure (PROC MIXED) indicated a significant influence of measurement time points on CH_4 concentration in growing steers ($P < 0.01$), with the highest concentration observed at 1100 h. Furthermore, there was a significant interaction effect between silage type and CP level on CH_4 concentration ($P < 0.05$). While CH_4 concentration increased in CS diets with high CP levels, it decreased in CSS diets with high CP levels. The peak CH_4 concentration at 1100 h aligns with the morning feeding time during the experiment, underscoring the impact of feeding activity on CH_4 concentration and the role of diet composition.

Key Words: methane, silage, crude protein

P113 Relationships between residual feed intake, nutrient digestibility, and meat quality traits in Nellore cattle. D. A. Vesga[1], R. N. S. Torres[2], O. R. Machado Neto[1,2], J. A. Torrecilhas[2], L. A. L. Chardulo[1,2], and W. A. Baldassini*[1,2], *[1]UNESP - School of Agricultural and Veterinarian Science, São Paulo State University, Jaboticabal, SP, Brazil, [2]UNESP - School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, SP, Brazil.*

Residual feed intake (RFI) is used for assessing feed efficiency in livestock species. Animals with lower RFI are more efficient due to reduced dry matter intake (DMI) without altering mature size. The literature remains inconclusive regarding the meat quality traits of *Bos indicus* animals classified as low RFI. This meta-analysis aims to investigate the impact of low (more efficient) and medium/high (less efficient) RFI classifications on DMI, nutrient digestibility, and meat quality traits of Nellore cattle. A comprehensive literature search across Web of Science, PubMed, and Science Direct identified 23 peer-reviewed publications meeting inclusion criteria. The Metafor package in R Statistical Software analyzed 37 treatment means for DMI, digestibility (DMd, CPd, EEd, NDFd, and NFCd), and meat quality variables (pH, myofibrillar fragmentation index [MFI], shear force [SF], and color [L^*, a^*, b^*]) using weighted mean difference calculations. Meta-regression analysis was performed to identify the effects of categorical covariates and subgroup analysis. Covariates included: age, sex, experimental period, RFI goups, concentrate levels, and metabolizable energy. Efficient animals (low RFI) exhibited a significant reduction ($P < 0.01$) in DMI (−0.950 kg vs. −0.420 kg). Nutrient digestibility (DMd, CPd, and NDFd) remained similar between RFI groups ($P > 0.05$), except for lower EEd ($P = 0.05$) in more efficient animals (−13.20 g/kg DM). These changes may be attributed to variations in ruminal microbial fatty acid biosynthesis, indicating higher microbial growth in more efficient animals, although not measured. Furthermore, low RFI bulls showed lower final meat pH ($P < 0.01$) and MFI ($P = 0.01$). Meat tenderness slightly decreased in low RFI bulls (SF increased by 0.22 kg; $P < 0.01$) and color parameters such as L^* ($P = 0.917$) and b^* ($P = 0.315$) remained similar. However, lower a^* was found in low RFI animals ($P < 0.01$). Nellore cattle classified as low RFI demonstrated reduced DMI without substantial effects on nutrient digestibility, while meat quality traits were impaired in response to RFI phenotype.

Key Words: *Bos indicus*, feed efficiency, metabolism

P114 Effects of copper, zinc, and manganese IntelliBond versus sulfate on 48-hour in vitro fermentation and methane production. C. B. Peterson*[1], G. M. Boerboom[2], J. S. Heldt[1], K. E. Griswold[1], and J. Johnston[3], *[1]Selko USA, Indianapolis, IN, [2]Selko Feed Additives, Nutreco Nederland BV, Amersfoort, Utrecht, the Netherlands, [3]Fermentrics Technologies Inc., Arnprior, ON, Canada.*

The objective was to understand the effect of copper (Cu), zinc (Zn), and manganese (Mn) sources, either from IntelliBond or sulfate, on 48 h in vitro fermentation. Supplemental trace mineral (STM) levels were calculated to be 125 Cu, 750 Zn, and 500 Mn mg/d, for a lactating dairy cow consuming 25 kg DM with a 120 L rumen. The STM were scaled to fit a 250 mL fermentation vessel containing a mix of KSU buffer (80%) and rumen fluid (20%) with 400 mg of 6mm ground TMR inside 50 ± 10 micron Ankom bags. Vessels were incubated for 48-h in a 39.5°C insulated water bath. Due to restrictions on sampling number, treatments were split into 2 runs with 6 replicates each: Run 1: Control (no trace mineral), Sulfate Cu (SC, 1.04 mg), Sulfate Zn (SZ, 4.40 mg), Sulfate Mn (SM, 3.36 mg), and Sulfate Cu, Zn, Mn (SMix, 1.04 Cu, 4.40 Zn, 3.36 mg Mn). Run 2: Control, IntelliBond Cu (IBC, 0.48 mg), IntelliBond Zn (IBZ, 2.84 mg), IntelliBond Mn (IBM, 2.37 mg), and IntelliBond Cu, Zn, Mn (IBMix, 0.48 Cu, 2.84 Zn, 2.37 mg Mn). Data were analyzed as a complete randomized design with PROC MIXED in SAS (SAS Institute Inc., Cary, NC) and included the mean of the response variable, run, replicate, and treatment which was nested within run. Significance was set at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.20$. For Run 1, the SC ($P < 0.001$) and SMix ($P = 0.014$) resulted in less apparent organic matter disappearance (aOMD) than control. SZ tended to increase aOMD ($P = 0.12$). For CH_4/OMD, SC ($P = 0.10$) and SMix ($P = 0.18$) tended to be higher than control. For the second run, IBM showed a trend for higher aOMD than control ($P = 0.092$), but all other treatments were NSD.

There was a trend for lower CH_4/OMD with IBC (P = 0.01), IBM (P = 0.001) and IBZ (P = 0.008) versus the control whereas the IBMix was NSD. Apparent microbial biomass production (aMBP) was NSD. These results show that a mix of sulfate trace minerals seems to negatively affect rumen fermentation, whereas the IntelliBond mix prevents negative effects on rumen fermentation due to lower reactivity of the minerals.

Key Words: hydroxy trace minerals, fermentation, methane

P115 The impact of a gut health supplement on growth performance, indicators of gastrointestinal health, and carcass characteristics in finishing feedlot steers.

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Gut health is a challenge for feedlot producers which can lead to production-limiting diseases like acidosis. The objectives of this study were to evaluate the effects of adding a gut health supplement on animal performance, indicators of gut health, and carcass quality in finishing feedlot steers. A total of 72 Angus crossbred steers were blocked by weight and then assigned randomly to one of 2 dietary treatments: CON (no additive, n = 36) or GH (10 g/steer daily, n = 36) added to a high-grain finishing diet (56% high moisture corn, 20% corn grain, 14% corn silage, and 10% soybean premix containing 33 ppm monensin, DM basis). The supplement is a proprietary blend of organic acids, and. plant and fermentation extracts. Steers were weighed every 28 d, and individual DMI was recorded daily using Insentec feeding stations. Ruminal fluid samples were collected by oral gavage 2 weeks before slaughter for pH measurement. At the end of the trial, cattle were slaughtered and a dissection of the GI tract, liver abscess scoring, cecal pH, serum samples for measurement of serum amyloid A (SAA), and carcass quality was assessed. All data were analyzed using the GLIMMIX procedure of SAS as a randomized complete block design with dietary treatment as fixed effects and block as a random effect. Treatment means were compared using ESTIMATE and significance was declared at $P < 0.05$. Steers from both treatments performed similarly, as dietary treatment did not affect ADG (P = 0.27), F:G (P = 0.46), and ADI (P = 0.31). As well, gut health indicators such as SAA concentration (P = 0.75), rumen pH (P = 0.76), cecal pH (P = 0.37), and rumen (P = 0.74), and hindgut (P = 0.66) health scores also did not differ between treatments. Similarly, carcass characteristics HCW (P = 0.26), marbling (P = 0.56), and rib eye area (P = 0.97) were similar across treatments. The findings suggest that the inclusion of the GH supplement at this dosage in high-grain finishing feedlot diets did not improve overall animal performance, gut health, and carcass quality over controls.

Key Words: gut health, feedlot performance

P116 Relation between feed efficiency, milk production and enteric methane emissions in small scale dairy systems in Mexico.

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In small scale dairy systems (SSDS), the feeding costs and the milk yield are the pillars to obtain a good revenue. However, milk yield in SSDS is heterogeneous, which implies a different proportion of feed efficiency between SSDS. In addition, methane emissions result in a loss of energy that could be used in milk production. The aim of this work was to estimate the feed efficiency of dairy cows in SSDS. Data from 294 SSDS from the highlands of Mexico, recollected in 2011, 2019, and 2023, were used. Farms were classified by their level of milk yield: low (<10 kg milk/cow; n = 102), medium (10–16 kg milk/cow; n = 116) and high (>16 kg milk/cow; n = 76). Protein and energy-corrected milk (EPCM) was calculated using the equation of Niue et al. (2018). Enteric methane emissions were estimated using the equation of Congio et al. (2022): CH_4 (g/cow/d) = 30.6 + 16.3 × DMI (kg) + 2.04 × EPCM (kg). A general linear model was used to analyze the data. Results showed a difference ($P < 0.001$) of 1.5 kg DM intake between the group High and Low, which results in a difference ($P < 0.001$) of 10.8 kg of EPCM/cow/d, but only 46 g of CH_4 between the groups ($P < 0.001$). Feed efficiency was of 0.6, 0.9 and 1.3 kg of EPCM/kg of DMI ($P < 0.001$) for Low, Medium, and High, respectively. Feed efficiency of SSDS are in the range of 0.5 to 1.4 kg of EPCM/kg of DMI, but lower than the observed in cows with high milk production (average of 32 kg of FPCM; feed efficiency of 1.6 to 2.7 kg of FPCM/kg of DMI). Methane emissions decreased with an higher milk production (34, 23, and 17 g of CH_4/kg of EPCM for Low, Medium, and High, respectively). The above points out the importance of increasing milk yield, not only due to a dilution effect, but also due to a feed efficiency and better use of the gross energy intake.

Key Words: feed efficiency, methane, small-scale dairy systems

P117 Biomass production, crude protein, and fiber variations of forage at pre- and post-rotational sheep grazing pastures.

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Rotational grazing has become a regular pasture management strategy for ruminant production systems. Frequent rotational grazing indicated to benefit ruminant production in several holds, such as more efficient utilization of pasture forage biomass, stimulate regrowth of plants, increase animal weight gain, and particularly disrupt gastrointestinal parasitic infection cycles in animals. The objective of this study is to investigate fescue grass dominated forage biomass (ton/ha), dry matter (DM%), plant height (cm), crude protein (CP%), and fiber content variations in pasture samplings at the pre and post rotational grazing with weaner lambs. Rotational grazing scheduled twice a week on pasture plots in size of one ha sectioned by electric wire fence. Thirty-six weaner lambs with an average body weight of 21.0 kg grazed continuously within the fenced paddock with ad lib water supply. Four paddocks were rotational grazed by each group. Pasture forage biomass was estimated by the clipping method and forage samples were collected at each pre- and postgrazing intervals for an 8-week grazing experiment. Animals were recorded for body weight weekly and daily weight gain was calculated. Forage samples were analyzed for DM, organic matter (OM), CP, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash composition. Data were analyzed by GLM procedure fitting sex and age as fixed effect in model. The results indicated that forage biomass did not appreciably change though it slightly decreased postgrazing; whereas, DM content changed because of precipitation. Likewise, plant height was shorter ($P < 0.05$) at postgrazing. The mean values for OM, CP, CF, NDF, ADF, and ash were 91.3, 10.3, 26.7, 33.9, 60.1, and 8.7%. No significant difference was found in forage compositions although notable fluctuations in weekly measurements were noted pre- and post-rotational grazing pastures. Lamb body weight increased during the weekly measurements and gained 179 g/lamb daily. Rotational grazing in weaned lambs can increase live weight.

Key Words: forage biomass, rotational grazing, crude protein

P118 Estimation of internal body temperature of dairy cows using thermographic images. J. Schulz[1], K. Heinichen[2], and M. R. Wilkens*[1], [1]*Institute of Animal Nutrition, Nutrition Diseases and Dietetics, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany,* [2]*Oberholz Farm for Teaching and Research, Leipzig University, Leipzig, Germany.*

Internal body temperature (IT) is the main indicator used to determine whether or not a dairy cow is experiencing heat stress. The aim of this preliminary study was to test if thermography is a suitable technique for estimating IT. Holstein dairy cows kept in the same barn at the animal facility of Leipzig University were studied from July to September 2022. The temperature-humidity index (THI) was calculated based on data from loggers at 3 different locations according to the German Society of Nutrition (2023). We measured IT of 10 animals and determined surface temperature (ST) using a FLIR E60 thermocamera on Thursday evening and Friday morning for 10 weeks. Four ST locations were defined: the surface of the udder, the left thoracic wall, the left abdominal wall and the left metatarsus. The THI influenced IT and ST at all locations significantly. Internal body temperature varied between 37.5°C and 41.7°C, with a mean of 38.9°C over all measurements. Surface temperature at the metatarsus (ST_MT) showed the widest range (18.0°C to 38.6°C), while ST at the udder (ST_U) showed the narrowest range (28.4°C to 40.0°C). Results of the regression models are shown in Table 1. While ST_U seems to be more suitable to estimate IT on herd level, ST_MT allows a better prediction for the individual animal. Information based on herd level could be used for example to adapt fans in the barn. During milking, ST_UT could be easily detected but it might be confounded by inflammatory diseases of the mammary gland. The correlation between ST_MT and IT depends more strongly on THI and might be more suitable to identify individual animals more vulnerable to heat stress.

Key Words: heat stress, thermography

P119 Effect of dietary inclusion of brown seaweed, tannin, and garlic polysulfides on enteric methane emissions and rumen physiology in dairy cows. D. Kirovski*[1], D. Bošnjakovic[1], I. Vujanac[2], R. Prodanovic[2], S. Nedic[2], S. Arsic[2], S. Dražic[1], M. Stojkovic[1], and L. Jovanovic[1], [1]*University of Belgrade, Faculty of Veterinary Medicine, Department of Physiology and Biochemistry, Belgrade, Serbia,* [2]*University of Belgrade, Faculty of Veterinary Medicine, Department of Ruminant and Swine Diseases, Belgrade, Serbia.*

This preliminary study aimed to investigate the effects of providing brown seaweed *Ascophyllum nodosum,* chestnut tannins and garlic polysulfides on enteric methane emissions (EME), rumen pH and temperature and productivity in dairy cows. Twelve peak lactating and individually fed Holstein-Friesian cows, chosen at 45.4 ± 3.1 d in milk (mean ± standard error) were divided into 4 numerically equal groups: CON (nonsupplemented cows),

Table 1 (Abstr. 118). Regression of body surface temperatures at the udder (ST_U) or the metatarsus (ST_MT) with rectal temperature (RT) as independent variable on animal (AL) and herd level (HL)

Y	Level	Equation	*P*-value coefficient	*P*-value intercept	R^2
ST_U	AL	Y = 1.71 ± 0.177 * x − 31.2 ± 6.90	< 0.001	< 0.001	0.33
	HL	Y = 2.10 ± 0.340 * x − 46.4 ± 13.1	< 0.001	0.002	0.68
ST_MT	AL	Y = 2.83 ± 0.281 * x − 79.0 ± 11.0	< 0.001	< 0.001	0.42
	HL	Y = 3.41 ± 0.612 * x − 101 ± 24	< 0.001	0.001	0.65

BS (cows received 100 mL/d of 10% *A. nodosum*), CNT (cows received 80 g/d of chestnut tannins) and GDP (cows received 1 g/d of garlic dipropyl polysulfides). In each cow, EME was measured using a laser methane detector, while rumen parameters (pH and temperature) were measured using smart rumen bolus. These measurements were performed before supplementation, after 15 and 30 d of supplementation, and the results obtained were tested using mixed design ANOVA with the dietary treatment as a between-subject factor, period as a within-subject factor, and examined parameter as a dependent variable. Inclusion of all 3 supplements significantly reduced EME, estimated by methane production (ppm) and methane intensity (ppm/kg of fat-protein corrected milk) at 15 and 30 d of supplementation, respectively. There was no significant difference in rumen pH and temperature between the examined groups of cows. No difference in dry matter intake (CON = 21.52 ± 0.1 kg/d, BS = 21.47 ± 0.1 kg/d, CNT = 21.23 ± 0.1 kg/d, GDP = 21.39 ± 0.3 kg/d, after 30 d of supplementation) and milk yield (MY) was found between examined groups of cows, with exception of lower MY in GDP than in CON cows after 15 d of supplementation. However, both GDP and CNT cows had significantly higher milk lactose and fat content compared with CON cows after 15 d of supplementation. In conclusion, dietary treatments with *A. nodosum*, chestnut tannins and garlic polysulfides reduces EME with no effect on rumen physiological parameters in dairy cows, but with an improvement in milk composition when providing chestnut tannins and garlic polysulphides.

Key Words: cows, methane, supplements

P120 Feeding frequency affects the circadian cycle of physical activity and ear temperature.

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This study aims to characterize the effect of feeding frequency (FF) on the circadian cycle of physical activity and ear temperature. Six Holstein heifers were fitted with ear monitoring tags (CowManager B. V., the Netherlands). The experimental period consisted of 98 d, divided into 7 periods (14 d). Each period consisted of 7 d of baseline FF, fed ad libitum once a day at 0830 h (BFF), 4 d of high FF, fed 95% of ad libitum divided into 12 meals (HFF), and 3 d of the baseline as recovery phase (BRP). Data were continuously registered and summarized by hour on d 6 and 7 for BFF, 9 and 10 for HFF, and 13 and 14 for BRP. Data were classified as eating, ruminating, not active, active, and highly active using a proprietary algorithm and average ear temperature. Data were analyzed using R software with a linear mixed-effects model, with fixed effects of FF, hour, and period and random effects of heifers with significance of $P < 0.05$. The DMI (kg/d) was lower for HFF than for BFF (10.8 vs. 11.6) due to a 95% intake restriction; however, the cows did not recover intake on BRP (BRP, 11.1 kg/d). Time spent eating (min/d) was similar for BFF (159) and HFF (159) and lower for BRP (146). Heifers on HFF and BRP ruminate less (24 min/d) than BFF. All variables significantly interacted between FF and hour when heifers were evaluated hourly. The HFF spent 5 to 10 min/h eating and 15 to 25 min ruminating continuously over 24 h; BFF and BRP had 2 eating peaks, first around 1200 and second from 1900 to 2200 (over 20 and 10 min/h, respectively), and decreased the rumination time at 1200 (below 5 min/h). HFF had a few activity peaks due to external intervention. Heifers on BFF and BRP had a peak of high activity from 1000 to 1200. Ear temperature dropped from 26°C (HFF) to 18°C (BRP) and 14°C (BFF) from 1000 to 1300; no changes in the environmental temperature were observed during this time. The drop in ear temperature during peak intake may indicate a change in blood flow from extremities to the digestive system. More studies must be performed to evaluate blood flow change in different body tissues (e.g., mammary glands) during intense eating time.

Key Words: eating pattern

P121 Can we improve net food production by feeding high-byproduct diets to lactating cows?

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Cows can produce high-quality food for human consumption by consuming human-inedible byproducts. Our objective was to evaluate the effects of feeding diets high in byproducts on production performance, and net food production. In exp. 1, we fed 30 multiparous Holstein cows (113 ± 28 DIM, 700 kg BW) with diets composed of 20% byproducts (CON1) or 70% byproducts (BYP1). BYP1 diet consisted of 25% corn silage, 8% straw, 15% gluten feed, 15% bakery waste, 12% beet pulp, 8% soyhulls, and 17% supplements. In exp. 2, we fed 31 multiparous Holstein cows (90 ± 23 DIM, 730 kg BW) with diets of 20% byproducts (CON2) or 70% byproducts (BYP2). BYP2 diet consisted of 25% corn silage, 18% corn gluten feed, 15% bakery waste, 10% whole cottonseed, 10% wet beet pulp, 8% wheat straw, and 13% supplements. Corn grain, soybean meal, the grain portion of corn silage, and the leaves of alfalfa haylage were considered human edible. Both experiments were designed as crossover with 2 diets, and their control diets contained mostly corn silage, haylage, and corn grain. Cows in each study fed a 50:50 mix of both diets for 1 wk, then the respective control and byproduct diets for 4 wk, mix for 1 wk, and then opposite diet for 4 wk. The effects of diet and period were considered as fixed and cow as random. In exp. 1, cows fed BYP1 consumed 1 kg more DM ($P = 0.02$) and produced 0.80 kg less milk (*P* = 0.2) with 0.18 percentage units less fat (*P* < 0.01), 0.04 percentage units less protein (*P* = 0.02), and 0.05 percentage units less lactose (*P* < 0.01). Regarding exp. 2, cows fed BYP2 consumed 1 kg less DM (*P* = 0.02) and produced 2.2 kg less milk (*P* < 0.01) with 0.13 percentage units more fat (*P* < 0.01), 0.02 percentage units less protein (*P* = 0.01), and 0.04 percentage units less lactose (*P* < 0.01). When evaluating efficiencies, cows fed either BYP1, or BYP2 were 3-fold more efficient converting human-edible feed energy into milk, and 2-fold more efficient converting human-edible feed protein into milk. Feeding diets up to 70% byproducts in dairy cattle decreased production slightly but improved environmental sustainability by decreasing the land demand for crops to produce high-quality human-edible food.

Key Words: byproducts, environment, sustainability

P122 High-starch diet early in life affects reticulorumen fermentation parameters later in life.

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We aimed to determine the long-term effect of a high-starch diet in the first weeks of life on reticulorumen (RR) fermentation parameters in sheep. Fifty-four male lambs (5–15 d of age; collected in 3 batches) were randomly divided into a control (C) and a starch (S) group, kept in 8 group pens (6–7 animals/pen; 4 pens/treatment) and fed ad libitum milk replacer (MR), and either a mixture of meadow hay and alfalfa hay (1: 1; C; n = 27) or a concentrate mixture (70% barley, 15% wheat bran, 10% soybean meal, 5% chopped hay; S; n = 27) ad libitum. MR was fed for 7 weeks and experimental solid feeds were fed for 2 additional weeks after weaning. From wk 10 all animals were transitioned to the same hay-based diet (fed ad libitum) that was continued till the age of 7 mo. One week after weaning (Phase 1; P1) and at the age of 7 mo (Phase 2; P2), 7 animals (P1) and 8 animals (P2) were randomly selected/treatment (1–2 animals/pen) and placed in individual pens. After 3 weeks, animals were killed 3 h after feeding and RR fluid samples were collected for pH and short-chain fatty acids (SCFA) analysis. Data were analyzed with a mixed model procedure and included fixed effect of treatment, and random effects of animal batch and pen within a batch. DM intake (kg) before killing did not differ between treatments in both phases. In P1, RR pH was lower for S than C group (*P* < 0.01) but total SCFA did not differ between treatments. The molar proportion of acetate (%) in total SCFA was higher for C than S group (*P* < 0.01), butyrate was higher for S (*P* = 0.03) and valerate tended to be higher for S than C group (*P* = 0.07). In P2, RR pH, total SCFA and the molar proportion of particular SCFA did not differ between treatments. In conclusion, high-starch diet early in life had no substantial impact on RR fermentation parameters later in life. This research was funded by the National Science Centre, Poland under the OPUS call in the Weave program (project No. 2021/43/I/NZ9/02222).

Key Words: ruminants, concentrates, fermentation

P123 Relationship between fecal mucin content and fermentation profile in lactating dairy cows.

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High starch intake in lactating cows induces excessive fermentation in the rumen and hindgut, resulting in an increased concentration of lipopolysaccharides and the accumulation of organic acids and the decreased pH. The hindgut is presumed to be more susceptible to excessive fermentation than the rumen. In a study on rats, excessive fermentation in the colon caused damage to the intestinal mucosa and increased mucin excretion in feces. Fecal mucin content may also be related to the intestinal status of lactating cows. It is suggested that the hindgut has a low buffering capacity and the absorption rate of VFA is less variable. Therefore, hindgut fermentation may be highly correlated with fecal pH and organic acids. The aim of this study was to evaluate the relationship between the fecal mucin content and fermentation profiles of lactating cows. Feces were collected from 5 to 8 lactating cows in spring and summer from 4 dairy farms. Fecal pH was measured immediately after sampling. Fecal mucin content was determined using a fluorometric assay. Fecal starch and organic acid content were determined using a colorimetric assay and HPLC, respectively. The correlation analysis was performed. Data were analyzed using the mixed model with the fixed effect of season and the random effect of farm and cow. Fecal pH decreased, and fecal mucin and starch content increased in spring than in summer, respectively (*P* < 0.01). There was a tendency for fecal organic acid content to be increased in spring than in summer (*P* < 0.10). It was presumed that feed intake was lower in summer, resulting in lower starch flow into the hindgut and altering the fermentation profile. Data from all farms (n = 50) showed that fecal mucin content was negatively correlated with pH (r = –0.80, *P* < 0.01) and positively correlated with organic acids (r = +0.67, $P < 0.01$). There was a positive correlation tendency between fecal mucin and starch content (r = +0.24, $P < 0.10$). These results imply that an increased flow of starch into the hindgut induced an accumulation of organic acids and a lower pH, which stimulated the enhanced excretion of mucin into the feces.

Key Words: lactating cow, fecal mucin, fermentation profile

P124 Performance evaluation and enteric methane emission in bovines treated with natural Fator P additive. L. Fernandes*[1], L. Ferreira[1], W. Araújo[1], A. D'Aurea[1], G. Moura[2], V. Fonseca[2], and A. Maia[2], [1]*Premix Company, Ribeirão Preto, São Paulo, Brazil,* [2]*São Paulo State University, Jaboticabal, São Paulo, Brazil.*

Zootechnical additives in cattle diet modulate the ruminal microbiota, enteric fermentation, and are essential for efficiency of production and increase sustainability. Antibiotic additives or ionophore have been implicated in antimicrobial resistance. The use of natural additives, require research that validates the effectiveness in maintaining productivity and reducing enteric methane emissions. Fator P is natural additive of symbiotic, conjugated fatty essential acids. The aim of this work was to evaluate the enteric methane emission of cattle treated with and without the Fator P using respirometric masks in which gases were analyzed by spectrophotometry with infrared. We used 24 Nelore steers, ages between 18 and 24 mo, average weight of 500 kg, conditioned for the experimental routine. Before the initial phase, the methane gas emission from animals was evaluated, and the results were used to randomize the animals in 2 experimental groups, each composed of 12 animals (control and Fator P), with equivalent averages of methane gas emission. After 21 d of adaptation, the animals received 2.5% of body on a dry matter (DM) in diet comprised of 50% of concentrated and 50% of corn silage. The evaluation occurred in 2 phases of 50 min in the morning, afternoon, night, and early morning. The results, expressed in grams (g) of CH_4 per kilogram (kg) of DM intake (DMI), and the conversion rate used was 1kg CH_4 to 23kg CO_2. The average of methane emission of cattle was 15.27g CH_4/kg DMI for control group, and 13.28g CH_4/kg DMI for Fator P. The difference between control group and Fator P showed a reduced in 1.98g CH_4/kg DM ingested (P-value = 0.034*). The statistical analyses were performed according to Test T ($P < 0.05$) in GraphPad PRISM Program 9.00 version. Considering the average dairy gain (ADG) of 1.32 kg and 1.17 kg respectively, the Fator P group and control, the difference of CO_2 eq/kg of ADG was to 851.5 g less for Fator P group, showing that this additive has the potential to improve animal production and sustainability of livestock farming.

Key Words: ruminants, natural additives, methane

P125 Can preweaning supplementation of beef females influence performance and blood characteristics in other growth phases? L. Pistillo[1], E. Detmann[1], M. Duarte[2], P. Paulino[3], and C. Sampaio*[1], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Department of Animal Biosciences, University of Guelph, Ghelph, ON, Canada,* [3]*Cargill Animal Nutrition, Campinas, SP, Brazil.*

The objective was to evaluate different nutritional planes in performance and blood characteristics in crossbreed beef heifers. Thirty-four F1 Red Angus × Nellore heifers averaging 159 kg of BW at 120 d of age were assigned into a 2 × 2 factorial design. Treatments were divided into preweaning phase (130 d): control (CTRL, n = 17), heifers were supplemented with mineral mixture; and supplemented (SUP, n = 17) heifers received an energy-protein supplementation at 0.5% of BW. At the rearing phase, both CTRL and SUP groups were then divided into 2 groups: high protein supplementation (HIGH-PT, 0.5% of BW) and low protein supplementation (LOW-PT, 0.1% of BW). After the rearing phase, all animals were managed as a single herd for 100 d receiving 2% of BW of supplementation to be finished in a grazing system and were slaughtered at 430 d of age. Data were analyzed considering nutritional planes as fixed effects using the mixed procedure of SAS 9.4. Weaning weight, ADG, and milk production of the dams were similar ($P = 0.76$, $P = 0.75$, and $P = 0.59$, respectively) between treatments. Preweaning supplementation increased the weaning weight ($P = 0.07$) and, when associated with HIGH-PT resulted in a greater weight ($P = 0.04$). The HIGH-PT resulted in greater weight and ADG ($P = 0.02$) in the rearing phase. Was not observed interaction ($P < 0.10$) between preweaning, HIGH-PT or LOW-PT supplementation. Preweaning supplementation decreased total cholesterol ($P = 0.01$), HDL ($P = 0.01$), LDL ($P = 0.09$), creatinine ($P = 0.03$), GOT ($P = 0.07$) and GTP ($P = 0.09$) levels, while urea was greater ($P = 0.08$). Rearing supplementation resulted in greater glucose ($P = 0.07$), total cholesterol ($P = 0.01$), HDL ($P = 0.01$), VLDL ($P = 0.01$), triglycerides ($P = 0.01$), urea ($P = 0.03$), albumin ($P = 0.01$), and IGF1 ($P = 0.01$). There was no effect ($P > 0.10$) of the previous phases nor interaction between them at finishing phase, except for slaughter weight which was influenced by supplementation in rearing ($P = 0.04$). Preweaning supplementation resulted in additional gain, as well as greater levels of supplementation in rearing phase indicating better nutrient supply.

Key Words: beef cattle, grazing, protein supplementation

Poster Session 5: Genomics and Epigenetics

P126 Identification of transcriptional regulators and signaling pathways mediating postnatal rumen growth in cattle. B. Pokhrel*, Z. Tan, and H. Jiang, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

The rumen plays an essential role in ruminant physiology and undergoes substantial postnatal changes in size and function. The molecular mechanisms underlying these changes are not clear. This study aimed to identify the transcription factors and signaling pathways mediating these changes in cattle. Six newborn bull calves and 6 adult steers, all Angus crossbred, were used in this study. The ratios of the empty rumen, reticulum, omasum, and abomasum to total body weight in steers were 4.8 ($P < 0.01$), 3.1 ($P < 0.01$), 6.0 ($P < 0.01$), and 0.8 ($P = 0.9$) times those in calves, respectively. Histological examinations showed a 7.4-, 2.0-, 3.0-, 2.9-, and 4.6-fold increase ($P < 0.01$ for all) in the length of rumen papillae and the thickness of rumen epithelium, tunica mucosa and submucosa, tunica muscularis, and tunica serosa, respectively, from calves to steers. RNA sequencing identified 3,350 differentially expressed genes (adjusted $P < 0.05$ and |log2fold change| ≥ 1) in the rumen between calves and steers, of which 1,481 were upregulated and 1,869 downregulated in steers. Bioinformatic analyses indicated fatty acid metabolism, ketogenesis, immunity, PPAR signaling, and Rap1 signaling were among the functional terms enriched in genes upregulated in steers while organ development and morphogenesis, neuronal differentiation, blood vessel development, Ras signaling, and Wnt signaling were among those enriched in genes downregulated in steers. Bioinformatic analyses also indicated NFKB2, ESRRA, and SPI1 were the transcriptional regulators of many genes upregulated in steers while SOX17, SOX18, GATA6, and ELF5 the transcriptional regulators of many genes downregulated in steers. Based on RNA sequencing, NFKB2, ESRRA, and SPI1 were themselves upregulated and SOX17, SOX18, GATA6, and ELF5 downregulated in steers. In summary, the rumen undergoes remarkable growth, functional, and gene expression changes from newborn calves to adult cattle, and these changes may be mediated by the transcription factors NFKB2, ESRRA, SPI1, SOX17, SOX18, GATA6, and ELF5, and the Rap1, PPAR, Ras, and Wnt signaling pathways.

Key Words: cattle, RNA sequencing, rumen

P127 Hepatic gene transcript changes from late gestation to early lactation. J. K. Drackley* and J. J. Loor, *University of Illinois Urbana-Champaign, Urbana, IL.*

During the transition from late pregnancy to early lactation, the liver undergoes remarkable changes in metabolism and physiology. Many adaptations are likely mediated at the transcriptional level. Our objective was to define a “lactation set” of genes that are expressed differently over the transition. Ten multiparous Holstein cows were fed either controlled-energy or high-energy dry cow diets and then switched to the same lactation diet. Liver was biopsied at d −14 and +10 relative to calving, 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.). Differences between sample days were determined in a mixed-effects model with cow as a random effect. A total of 1,009 sequences were affected by time (FDR <0.05), without interaction with dietary treatment. From these we selected all transcripts with fold-change ≤ 1.5. This revealed 261 transcripts more highly expressed at d +10 than at d −14, with 178 unique annotated transcripts. A total of 135 transcripts more highly expressed on d −14 than on d + 10, with 92 unique annotated transcripts. Highly enriched Gene Ontology (GO) biological processes in the comparison of d +10 versus d −14 included glyoxalate cycle, reverse cholesterol transport, regulation of intestinal cholesterol absorption, isocitrate metabolic process, phosphatidylcholine biosynthetic process, regulation of lipoprotein lipase activity, cholesterol efflux, plasma lipoprotein particle remodeling, positive regulation of fatty acid biosynthetic process, regulation of cholesterol metabolic process, and triglyceride homeostasis, among 35 significant processes. GO biological processes greater on d −14 than d +10 included cellular response to heparin, regulation of glycogen biosynthetic process, positive regulation of phagocytosis, complement activation, regulation of ossification, and polysaccharide metabolic process, out of 14 significant processes. Results are consistent with downregulation of aspects of the immune system and upregulation of many metabolic processes as lactation commences.

Key Words: microarray, metabolism, immune response

P128 Methionine and guanidinoacetic acid supplementation of beef heifers during the periconceptual period impacts fetal hepatic transcriptome. K. M. Hauxwell*[1], R. A. Cushman[2], J. S. Caton[1], W. J. S. Diniz[3], A. K. Ward[4], A. K. Lindholm-Perry[2], A. P. Snider[2], H. C. Freetly[2], C. R. Dahlen[1], S. Amat[1], B. W. Neville[2], J. F. Thorson[2], W. T. Oliver[2], J. R. Miles[2], M. S. Crouse[2], [1]*North Dakota State University, Fargo, ND,* [2]*U.S. Meat Animal Research Center, Clay Center, NE,* [3]*Auburn University, Auburn, AL,* [4]*University of Saskatchewan, Saskatoon, SK, Canada.*

Methyl donor availability is important for fetal growth and developmental programming. However, data regarding methyl donor supply to the bovine fetus and subsequent impacts on transcript abundance are limited. Differential gene expression (DEG) analysis was used in the present study to investigate maternal supplementation of methionine (MET), an obligate methyl donor, and guanidinoacetic acid (GAA), a methyl donor consumer, during the periconceptual period on fetal development. Heifers (n = 80; MARC II) were assigned to 1 of 4 treatments totaling 100 g/d of supplement with ground corn carrier: MET (10 g/d), GAA (40 g/d), MET+GAA (10 g/d Met + 40 g/d GAA), or carrier alone (CON; 0 g/d Met + 0 g/d GAA). Supplementation was started 63 d before and ended 63 d after breeding. Heifers pregnant with male fetuses (MET, n = 8; GAA, n = 7; MET+GAA, n = 10; CON, n = 10) were harvested on d 63 of gestation. Fetal liver (n = 35) RNA was extracted and subjected to transcript abundance analysis using RNA-Seq. The DEG analysis revealed 76 upregulated and 123 downregulated genes from MET versus CON, 65 upregulated and 96 downregulated genes from GAA versus CON, and 148 upregulated and 57 downregulated genes from MET+GAA versus CON treated heifers ($P \leq 0.05$). Over-representation analysis of DEGs highlighted the Wnt signaling pathway from the MET group. This pathway is associated with the regulation of cell proliferation, differentiation, and migration during implantation. Processes involving tissue and biomineral development were also over-represented in the MET group. The *SOX30* gene, required for regulation of embryonic development and determination of cell fate, was downregulated in the GAA group. Genes associated with fetal provisioning of nutrients: *SLC13A4*, *SLC29A2*, and *SLC6A3,* were also downregulated in the GAA group. Methyl donor supplementation to the maternal system during the periconceptual period alters fetal hepatic transcriptome, which is characterized by the overexpression of fetal genes affecting fetal cellular development and tissue formation. USDA is an equal opportunity provider and employer.

Key Words: fetal development, hepatic transcriptome, beef

P129 Mammary transcriptome response to sodium acetate and CLA-induced milk fat depression in dairy cows.

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Milk fat yield can be increased by sodium acetate and decreased by biohydrogenation-induced milk fat depression (MFD). *Trans*-10 and *cis*-12 conjugated linoleic acid (CLA) have been shown to suppress lipogenic transcription factors and key enzymes in milk fat synthesis. However, the molecular effects of sodium acetate remain unclear. Ten cows were used in a replicated 4 × 4 Latin square design with a 2 × 2 factorial of sodium acetate and CLA; sodium acetate was administered at 10 mol/d via continuous ruminal infusion, and CLA was provided at 10 g/d for both *trans*-10,*cis*-12, and *cis*-9,*trans*-12 isomers through abomasal infusion. Sodium acetate led to a 3.6% increase in milk fat yield, while CLA reduced milk fat yield by 39.4%, with no interaction of acetate and CLA. On the final day of each period, mammary biopsies were collected and gene expression was determined through RNA sequencing using Illumina NovaSeq platforms with 150 bp paired-end reads. Analysis of differential gene expression using the DESeq2 package in R and pathway analysis with Ingenuity Pathway Analysis revealed a unique mammary transcriptome response to CLA, with 828 genes upregulated and 608 downregulated (FDR < 0.05). Crucial enzymes and pathways related to mammary fat synthesis were extensively downregulated. Top novel pathways, including genes involved in extracellular matrix organization, integrin cell surface interactions, and collagen biosynthesis, were upregulated, whereas those linked to oxidative phosphorylation, neutrophil extracellular trap signaling, and PTEN signaling were downregulated. Upstream regulators such as TCF1, RB1, and SREBF1 were predicted to be inhibited, and TBX3, CEBPB, and TGF-β were predicted to be activated, indicating complex regulatory shifts. Acetate supplementation did not rescue CLA-induced MFD or reverse lipogenic gene downregulation, showing minimal regulatory impact. CLA-induced MFD affects various cellular aspects, including shifts in metabolism, immunity, and cell dynamics, reducing energy production and altering tissue structure. This offers insights into the effects of dietary changes on mammary gland function and lactation.

Key Words: milk fat, sodium acetate, CLA

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