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**0217 Follicle-stimulating hormone regulation of proenkephalin in granulosa cells.** A. D. Gullic<sup>\*1</sup>, B. I. Gomez<sup>1</sup>, B. Couger<sup>1</sup>, C. A. Gifford<sup>2</sup>, and J. A. Hernandez Gifford<sup>1</sup>, <sup>1</sup>Oklahoma State University, Stillwater, <sup>2</sup>Department of Animal Science, Oklahoma State University, Stillwater.

Estradiol biosynthesis by ovarian granulosa cells (GC) is crucial for normal female reproductive function and is mediated primarily by FSH. Binding of FSH elicits a multitude of signaling cascades to enhance or inhibit the expression of target genes to regulate estradiol production. The objective of the current experiment was to utilize global expression analysis to identify genes significantly regulated by FSH. Primary rat GC were cultured in the presence or absence of FSH (100 ng/mL) for 24 h and gene expression was analyzed via microarray. Of the 1104 FSH-regulated genes, the opioid precursor proenkephalin (*Penk*) was downregulated ( $P < 0.001$ ) with FSH treatment. Endogenous opioid peptides originate from three protein precursors *Penk*, proopiomelanocortin (*Pomc*), and prodynorphin (*Pdyn*). Stimulation of the  $\mu$ ,  $\delta$ , and  $\kappa$  opioid receptors in GC downregulate basal estradiol concentrations in cultured GC. Therefore, we hypothesized that *Penk* blocks FSH-induced estradiol production. Real-time PCR was used to confirm *Penk* regulation in response to FSH treatment. Rat GC were treated with and without FSH for 24 h ( $n = 3$ ) for quantification of *Penk*. Relative fold change values were evaluated using the GLM procedure of SAS and means were separated using the PDIFF function when the model was significant. In agreement with microarray data, FSH downregulated *Penk* 12.81-fold ( $P < 0.01$ ) when compared with control. To determine if the opioid pathway disrupts FSH target genes, KGN GC, a human granulosa tumor cell line, were pre-treated with vehicle or  $\beta$ -endorphin (100 nM), a ligand for the  $\mu$  opioid receptor, for 5 h followed by treatment with or without 5  $\mu$ M forskolin (FSK) for 24 h ( $n = 3$ ). Steady state mRNA levels for aromatase (*Cyp19a1*) were quantified via real-time PCR. As expected, FSK increased ( $P < 0.01$ ) *Cyp19a1* 33.5-fold compared with vehicle control whereas treatment with  $\beta$ -endorphin was similar to controls ( $P = 0.97$ ). Co-stimulation of KGN GC with  $\beta$ -endorphin and FSK did not decrease *Cyp19a1* ( $P < 0.01$ ). Results from these experiments indicate that FSH regulates opioid peptides in granulosa cells and work in the literature suggests that opioids regulate steroidogenesis. In the current experiment, stimulation of the  $\mu$  receptor and subsequent opioid signaling pathway did not inhibit the ability of FSK to increase *Cyp19a1* indicating that opioid regulation of steroidogenesis could be through mechanisms other than disruption of the cAMP signaling pathway or through other opioid receptors.

**Key Words:** FSH, opioid, aromatase

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**0218 Optimization of probes and PCR conditions for the correlation between 4 genes and production of high citrate in milk.** V. A. Smith<sup>\*1</sup>, R. Manjarin<sup>1</sup>, and R. Jimenez-Flores<sup>2</sup>, <sup>1</sup>California Polytechnic State University, San Luis Obispo, <sup>2</sup>Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.

We have previously demonstrated the existence of 4 gene products associated to key metabolic pathways that are necessary for the production of citrate in milk, namely isocitrate dehydrogenase 1 (*IDH1*), pyruvate dehydrogenase  $\beta$  (*PDHB*), pyruvate kinase (*PKM2*), and solute carrier family 25 member 1 (*SLC25A1*). Following sequencing of genome of a small sample of cows in the Cal Poly herd, it was shown several single nucleotides (SNP) within these genes that were significantly associated with increased milk citrate content, and therefore that could be potentially selected on to influence the outcome of citrate in milk. We now aimed to design primers for identifying the SNPs in the various genes involved in citrate production, and to optimize the conditions for PCR amplification. Primers for *IDH1*, *PDHB*, *PKM2* and *SLC25A1* were designed based on publicly available bovine cDNA and on expressed sequence tag (EST) sequences deposited in the National Center for Biotechnology database using Primer Express software with default settings. Primer pairs were optimized for concentration using a primer optimization matrix and a relative standard curve was used to determine the efficiency. The standard curve was constructed using cDNA synthesized from a RNA pool made of all samples using the following amounts of cDNA (in duplicate): 40 ng, 4 ng, 0.4 ng, 0.04 ng and 0.004 ng. Specific hybridization of the primers was validated by agarose gel electrophoresis of the PCR products. Non-template controls were included to validate that primers were not amplifying contaminating DNA. Our work demonstrates that each set of primers has singular characteristics that deeply influence the efficiency of PCR conditions. We have also developed accurate PCR conditions for the 4 genes of interest. These results are fundamental for our future studies where SNPs identification will be correlated with citrate levels in milk.

**Key Words:** milk, citrate, SNPs

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## ASAS/ASN JOINT SYMPOSIUM: GUT MICROBIOTA, DIET AND HEALTH

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**0219 Modulation of the gut microbiota: An ecological perspective.** J. Walter<sup>\*</sup>, University of Alberta, Edmonton, Canada.

Diverse strategies have been used for several decades to improve human and animal health through the modulation of

the gut microbiota, spanning from the administration of defined probiotic strains (or Live Biotherapeutics), whole microbial consortia (e.g., fecal bacteriotherapy), to the provision of bacterial growth substrates (prebiotics and dietary fiber). However, we still lack a conceptual understanding on how the gut microbiota can be modulated. In this presentation I will summarize how ecological theory can provide a framework by which to understand characteristics of the human gut microbiota and the impact of microbiome-modulating strategies. I will present some of our own studies that investigated basic ecological questions regarding how the temporal, spatial, and global patterns of the human microbiome, the factors that shape these patterns, and the ecological constraints within the human microbiome can be manipulated by diet and probiotics. The methodological toolset that is now available (e.g., through next-generation sequencing) provides an unprecedented opportunity to obtain phylogenetic, compositional, and functional information of microbial communities. When analyzed in the light of ecological theory, this has the potential to elucidate the factors and ecological processes that determine and potentially predict the response of the gut microbiota to therapeutic modulations.

**Key Words:** gut microbiota, probiotic, ecological theory

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**0220 Effects of early antibiotic exposure on host**

**metabolism.** L. M. Cox\*, *Harvard Medical School and Brigham and Women's Hospital, Boston, MA; New York University Langone Medical Center, New York.*

The intestinal microbiota, consisting of trillions of bacterial, viral, and fungal cells, can influence growth and development. From infancy through early childhood, the microbial community develops with a succession of key organisms that likely have important roles in shaping metabolic health. Disrupting these ancient patterns of microbe-host maturation may have lasting metabolic consequences. Low-dose antibiotics, especially administered during infancy, can increase weight gain and feed efficiency in a wide variety of host species. To identify key members of the microbiota that may participate, we administered low-dose penicillin (LDP) to mice, measured changes in body composition, and characterized changes in the microbiota by high-throughput sequencing. We found that LDP administered only during the first 4 wk of life increased fat mass later in adulthood, despite the fact that the microbiota recovered, indicating microbiota interactions in infancy may be critical determinants of long-term host metabolic effects. In addition, LDP enhanced the effect of high-fat diet induced obesity. The growth promotion phenotype was transferrable to germ-free hosts by LDP-selected microbiota, showing that the altered microbiota, not antibiotics per se, play a causal role. Four different bacteria were consistently suppressed during infancy in multiple independent experiments, suggesting that they may have beneficial roles in metabolic development.

These studies characterize important variables in early-life microbe-host metabolic interaction and identify several bacteria consistently linked with metabolic alterations.

**Key Words:** antibiotics, microbiome, weight gain

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**0221 Impact of gut microbiota on brain and behavior.**

J. F. Cryan\*, *University College Cork, Ireland.*

The concept of the gut influencing brain and behavior has existed for almost two centuries. However, a new player has emerged in the past decade: the gut microbiota, which is now seen as a key regulator of the gut-brain axis. The gut is home to a diverse array of trillions of microbes which significantly outnumber human cells. Advances in sequencing technologies show that the microbiota influences almost all aspects of human biology. Bacterial colonization of the gut plays a major role in postnatal development and maturation of key systems that have the capacity to influence central nervous system (CNS) programming and signaling, including the immune and endocrine systems. Individually, these systems have been implicated in the neuropathology of many CNS disorders and collectively they form an important bidirectional pathway of communication between the microbiota and the brain in health and disease. Evidence of a crucial role for the microbiota in regulating stress-related changes in physiology, behavior, and brain function has emerged mostly from animal studies. Mice that grow up devoid of a microbiome (in a germ-free environment) have an exaggerated hypothalamic-pituitary axis response to stress and altered anxiety-related behaviors. Converse findings have shown that stress (either early in life or in adulthood) changes microbiota composition. Moreover, the concept that bacteria were required for normal brain development has emerged and that the microbiota regulates many key processes in the adult brain, such as neurogenesis, blood brain barrier function and microglia activation. Thus, the ability to target the brain via the microbiome is viewed as a paradigm shift in neuroscience and psychiatry and has led to the concept of psychobiotics (bacteria with positive effects on mental health) being put forward. Moreover, microbiota is essential for both social cognition and visceral pain. Finally, there are critical time-windows early in life when the effects of microbiota on brain and behavior appear to be more potent. Our data also demonstrates that these effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. Such data offer the enticing proposition that specific modulation of the enteric microbiota by dietary means may be a useful "psychobiotic"-based strategy for brain disorders.

**Key Words:** stress, neurodevelopment, probiotic

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**0222 The human milk microbiome and oligosaccharides: What's normal and so what?**

M. K. McGuire<sup>\*1</sup> and M. A. McGuire<sup>2</sup>, <sup>1</sup>*Washington State University, Pullman*, <sup>2</sup>*University of Idaho, Moscow*.

Human milk is inarguably the only food “designed” to be consumed exclusively by humans, providing all the essential nutrients (and other bioactive compounds and constituents) needed for growth and development of the human infant. As such, understanding human milk composition and variation therein is critical to optimizing human health during this vulnerable time, particularly in the most at-risk infants. More complete characterization of human milk composition may also lend important insight as to what constitutes optimal nutrition in other phases of the lifecycle, not only for the human host but also for the myriad commensal, mutualistic, and sometimes pathogenic microbes with which we coexist. However, our understanding of human milk composition and its impact on host and microbial health is far from complete. For instance, until recent advances in instrumentation allowing the detection and identification of difficult-to-culture bacteria, common dogma was that human milk was sterile unless produced by an infected mammary gland or contaminated after expression. As such, focus on the roles played by complex (an indigestible) human milk oligosaccharides (HMO) has been directed exclusively toward those related to the recipient infant and his/her gastrointestinal microbiota- roles that are without a doubt important for infant health. We now know that human milk contains a diverse population of bacteria. As such, we and others postulate that HMO may impact the microbial communities present in milk and the mammary gland producing it. Here we will briefly describe what is currently known about variation in the human milk microbiome and HMO as well as relationships among maternal diet, milk composition (including microbes and HMO), and the infant gastrointestinal microbiome. In addition, we will introduce an ongoing cross-cutting study funded by the Integrated National Science Foundation Support Promoting Interdisciplinary Research and Education (INSPIRE) mechanism designed to help us better understand what is normal in terms of milk microbes, HMO, and infant fecal microbiome in various locations worldwide. The importance of cooperation and interdisciplinary discussion around methods and vocabulary will be discussed, as will some of the challenges faced in terms of sample collection, storage, transport, and data analysis. Finally, selected preliminary data from the INSPIRE study and a framework for considerations for future studies designed to use big (and interdisciplinary) data to understand variation in global milk composition and how this is related to infant health will be presented.

**Key Words:** human milk, microbiome, oligosaccharides, HMO, health, development

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**0223 Dietary fiber and starch, digestive physiology, and metabolic health.**

R. T. Zijlstra\*, J. M. Fohse, T. Vasanthan, and M. G. Ganzle, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.*

In monogastric nutrition, analyses of fiber and starch have focused on assessing quantity. However, both have a wide range of functional properties. Fibers ranging from low to high viscous affect digesta flow and from slowly to rapidly fermentable alter production of volatile fatty acid (VFA) serving as energy for the gut or the whole body. Likewise, starches ranging from low to high amylose change from rapidly digestible in the upper gut to poorly digestible but fermentable in the lower gut, thereby changing from glucose source into VFA source. Poorly digestible or resistant starch thus basically acts as dietary fiber. Functionality of these carbohydrates for nutrition or health was studied in lab and pig models. Our hypothesis is that total extent, kinetics, and site of digestion or fermentation of starch and fiber are important for whole body energy utilization and gut health. To elucidate their effects, we developed in vitro, lab-based methodologies to describe kinetics of digestion and fermentation and linked these with in vivo models including: (1) ileal cannulation to collect digesta, (2) portal-vein catheterization to sequentially sample blood, (3) slaughter method to collect site-specific intestine tissue and digesta, and (4) indirect calorimetry. Using these models, kinetics of absorption of glucose was associated with insulin and incretin release into the portal vein, intestinal microbiota, and gene expression in intestinal tissue and microbiota. These studies confirmed that slowly digestible starch is partially degraded in the large intestine and fermented into VFA including butyrate (10-fold increase in net portal appearance), reducing insulin responses by 60% and reducing whole body energy utilization. Starch entering the distal intestine altered mRNA abundance of nutrient transporters, increased portal release of the incretin glucagon-like peptide-1 (GLP-1), and was bifidogenic in the large intestine. Extreme viscous purified fiber dampened glycemic responses and reduced digesta passage rate by 50%, thereby increasing small intestine digestion of dietary nutrients, whereas increased fiber in feed grains reduced nutrient digestibility. Fermentable fiber increased butyrate and insulin production. In whole grains with ranging content of amylose and fermentable fiber, effects of similar direction but less extreme were observed. In summary, fiber and starch characteristics influence digestive physiology and thereby gut health, metabolic health, and whole body nutrient utilization. Functional characteristics of fiber and starch should also be considered is diet formulation.

**Key Words:** digestive physiology, fiber, starch

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**0224 Methane matters: From blue tinged moos, to boozy roos, and for the health of humans too.**

E. C. Hoedt<sup>1,2</sup>, P. OCuiv<sup>3</sup>, and M. Morrison<sup>4</sup>,

<sup>1</sup>University of Queensland, School of Chemistry and Molecular Biosciences, St Lucia, Australia,

<sup>2</sup>University of Queensland Diamantina Institute, Woolloongabba, Australia, <sup>3</sup>University of Queensland Diamantina Institute, Woolloongabba, Australia,

<sup>4</sup>University of Queensland Diamantina Institute, Brisbane, Australia.

Methane production is a typical occurrence within the digestive tracts of warm blooded animals, including humans. Methanogens and methane emissions however, means something different to each host. Methane emissions from ruminant livestock has been a major focus of research over the last decade because of the attribution of ~10–20% of the annual anthropogenic methane output to these animals. This has led in part to methane emission measurements for a broad range of animal species and breeds, including Australia's native herbivores, to identify "low emitters"; and resulted in comparative studies to define how the gut microbiota in these animals might account for the differences. The presence of methanogens within the human gut has long been recognized via a combination of breath methane measurements and microbiota surveys, and linked to a range of functional gastrointestinal disorders and non-communicable diseases. Historically much of the focus has been directed to the numerically most predominant species, principally assigned to the genus *Methanobrevibacter*, which are canonically involved with the conversion of gaseous substrates (CO<sub>2</sub> and H<sub>2</sub>) arising from bacterial fermentation. However, recent studies by our group and others have revealed that, perhaps, the heterotrophic methanogens (i.e., those capable of using small organic molecules such as alcohols and methylated amines) warrant closer attention if "methane matters" in animals and man are to better managed. Here, we provide a brief overview of recent research on these heterotrophic archaea, with a specific focus on the genus *Methanosphaera*, which so far has been found only in the gastrointestinal tracts of animals, by using specific examples of our own and others recently published research with livestock, Australian macropodids (kangaroos and wallabies) and human clinical and nutritional studies. We propose this specific guild of methanogenic archaea not only warrants further attention, but provides new opportunities for the better management of the "animal-methane" axis.

**Key Words:** methane and methanogens, gut function, human health and animal productivity

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**0225 Sub-acute ruminal acidosis (SARA): A tale of two microbiomes.**

C. A. McCartney<sup>1</sup>, R. C. Cernat<sup>2</sup>, H. H. C. Koh-Tan<sup>3</sup>, H. J. Ferguson<sup>3</sup>, E. M. Strachan<sup>4</sup>, W. Thomson<sup>4</sup>, T. J. Snelling<sup>1</sup>, C. M. Harvey<sup>4</sup>, I. Andonovic<sup>5</sup>, C. Michie<sup>5</sup>, N. N. Jonsson<sup>3</sup>, G. W. Horgan<sup>6</sup>, and R. J. Wallace<sup>7</sup>, <sup>1</sup>University of Aberdeen, UK, <sup>2</sup>Chr. Hansen A/S, Hoersholm, Denmark, <sup>3</sup>University of Glasgow, UK, <sup>4</sup>Harbro Ltd., Turriff, UK, <sup>5</sup>Strathclyde University, Glasgow, UK, <sup>6</sup>BIOSS, Aberdeen, UK, <sup>7</sup>Rowett Institute of Nutrition and Health, Aberdeen, UK.

Most mechanistic studies of sub-acute ruminal acidosis (SARA) in cattle involve the experimental induction of SARA by dietary starch or legume. Our aim was to determine how these observations relate to on-farm conditions in northeastern Scotland. In six beef farms, management practices, feed composition/particle size and animal activity using motion sensor collars were monitored using 20 animals per farm. At slaughter, rumen wall condition was assessed under four categories: color, papillae integrity, papillae shape, and post-washing blackness. Ruminal fluid and cecum contents were collected for VFA and soluble lipopolysaccharide (LPS) analysis, and also for microbial community analysis. Eighty-six ss rRNA amplicon libraries were generated by PCR, which were subsequently sequenced in equimolar concentrations using Illumina MiSeq.

Close examination of the feed indicated that the process of mixing grain with forage was critical. Rumen wall damage did not appear to be correlated with the particle size of the total mixed ration, but rather with the dustiness of the barley component of the feed. Motion sensor data showed that the rate of change of movement appeared to be correlated with the condition of the rumen wall across farms. Ruminal LPS concentrations were significantly different ( $P < 0.001$ ) between farms, and caecal LPS concentrations were significantly higher (up to 27-fold) than corresponding ruminal LPS concentrations, and exhibited a stronger relationship with the rumen damage scores. We therefore postulate that the hindgut has a greater effect on ruminal health than previously thought.

Microbial community and subsequent PCA analysis revealed significant ( $P < 0.001$ ) clustering of ruminal fluid and caecal content communities. Within each sample type, there was also significant clustering by farm; however, the clustering was weaker when grouped by damage score. Bacteroidetes, Firmicutes and Proteobacteria were the predominant phyla in the ruminal digesta. In the caecal content, Proteobacteria were barely detected and comprised mainly the Moraxellaceae family (known pathogens, "pink eye" in cattle). This was surprising as LPS from Enterobacteriaceae has been implicated with SARA. It is possible that growth of Proteobacteria is not supported in the cecum and the bacteria therefore lyse, causing the high LPS concentrations. In conclusion, the particle size of the barley component of the feed appears to have a relationship with SARA related pathology. Further-

more, this study has highlighted the role of the hindgut in the pathology of SARA, which warrants further investigation.

**Key Words:** cecum, rumen, sub-acute ruminal acidosis (SARA)

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#### 0226 Dietary manipulation of canine and feline gut

**microbiome.** K. S. Swanson\*, *Department of Animal Sciences, University of Illinois, Urbana.*

Dogs and cats evolved as Carnivora and have traditionally relied on high-protein, high-fat diets containing relatively low fiber concentrations. Despite having a simple gastrointestinal tract designed to digest such diets, a rich microbial community exists. Today's pet dogs and cats live in close proximity to humans and have similar environmental exposures, serving as potential vectors for pathogen exposure. Dogs and cats are also afflicted by many of the complex diseases present in humans, including obesity, diabetes, inflammatory bowel diseases, and cancers, all of which may be influenced by diet and the gut microbiota. Given their proximity to humans, similar disease incidence and etiology, and unique metabolism, microbiome research in dogs and cats may not only lead to improved pet nutrition and veterinary care, but may increase our understanding of host-microbe interactions, with relevance to human metabolism and diseases and public health at large. Molecular techniques, including high-throughput sequencing, have dramatically changed the research landscape in regards to gastrointestinal microbiology. These techniques have been used to characterize the phylogeny and functional capacity of the canine and feline gastrointestinal microbiota and identify the effects of diet, age, and disease on these communities. Several hundred bacterial phylotypes, predominated by members of Firmicutes, Fusobacteria, Proteobacteria, Bacteroidetes, and Actinobacteria, are now known to inhabit the dog and cat gastrointestinal tract. Recent studies have revealed that the functional capacity of the gastrointestinal microbiota in dogs and cats is quite broad and similar to that of humans and rodent models. Although these populations are quite stable over time, our laboratory has demonstrated that macronutrient profile (e.g., dietary protein:carbohydrate ratio), dietary fiber amount and type, and the form of food consumed (e.g., raw vs. extruded diets) may have dramatic effects on the gastrointestinal microbiome of these host species. These dietary changes have not only been reported to impact microbial diversity and richness, gene content, and metabolic activity, but to alter host physiology and metabolism as well. Unfortunately, the majority of research has been performed in healthy animals housed in research colonies rather than free-living pets. Continued research on the composition and activity of the canine and feline gastrointestinal microbiomes, and how they are impacted by dietary intervention and other environmental exposures, is needed to increase our understanding of the host-microbe interactions that occur in the gastrointestinal health and their

relevance to health and disease.

**Key Words:** canine nutrition, feline nutrition, gut microbiota

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### BEEF SPECIES I

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#### 0227 Relationship between forage quality parameters and mineral intake in grazing beef cattle.

J. D. Rivera\*, M. L. Gipson, and R. G. Gipson,  
*Mississippi State Univ. South Branch Exp. Sta., Poplarville.*

One hundred ninety-two forage samples were collected over a 2-yr winter grazing season from cool-season pastures grazed by beef cattle (avg. BW = 294 kg) to determine the effects of periodicity and forage quality on mineral intake. Beef cattle minerals were offered to each pasture ( $n = 24$  pastures) every 28 d from December through June. Refusals were weighed at the end of each interval, and forage samples were collected at the same time. Mineral refusals were used in conjunction with offered mineral to calculate mineral DMI. Forage samples were weighed and dried for 72 h at 50°C to determine DM; dried samples were analyzed using NIR technology to determine ADF and CP content. Data were analyzed to determine correlation (PROC CORR) of periodicity, CP, ADF, DM and mineral intake. As expected, as the season progressed, sampling period was highly correlated to forage quality ( $P < 0.01$ ) with decreasing CP ( $r^2 = -0.92$ ), increasing ADF ( $r^2 = 0.96$ ) and DM ( $r^2 = 0.80$ ) associated with periods later in the grazing season. No effect ( $P = 0.25$ ) of sampling period was observed for DMI of mineral. Average mineral intake through the grazing season was  $81.6 \text{ g} \pm 1.7 \text{ g}$ . Crude protein averaged  $18.7\% \pm 8.4\%$ ; ADF averaged  $31.3\% \pm 11.7\%$ . Crude protein had no effect on mineral intake ( $P = 0.34$ ). Acid detergent fiber tended to negatively correlate ( $P < 0.06$ ;  $r^2 = -0.14$ ) with mineral DMI; greater mineral DMI was associated with lower ADF. Forage DM had a negative impact on mineral DMI ( $P = 0.001$ ;  $r^2 = -0.38$ ); with greater forage DM associated with lower mineral DMI. Results suggest that mineral intake of beef cattle grazing cool-season forages is not affected by forage quality (ADF, CP), nor sampling period, but rather more likely affected by forage DM content.

**Key Words:** beef cattle grazing mineral intake

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#### 0228 Feeding antibodies against interleukin-10 improved gain efficiency in beef steers.

M. R. Schaefer\*, M. E. Cook, D. M. Schaefer,  
*University of Wisconsin, Madison.*

Recent studies showed that oral anti-interleukin-10 antibody (aIL-10) is protective against gastrointestinal pathogens. The current objective was to evaluate oral aIL-10