ANIMAL HEALTH I: **MODELS OF DISEASE AND STRESS**

0064 Heat stress as a model to study the effect of a gut health concept (Presan-Fx) on the intestinal barrier function of weanling piglets. P. J. Roubos* and Y. M. Han, *Nutreco Research and Development, Boxmeer, Netherlands.*

An acute heat stress model is used to study effects on the intestinal barrier function. During heat stress, the animal redistributes the blood supply to the periphery, leading to increased gut permeability. To mitigate the impairment on barrier function, a mixture of Presan-Fx (synergistic blend of organic acids, medium chain fatty acids, butyrates, and a phenolic compound) was used in this model with weanling piglets. The objective of this study was to evaluate the effect of a heat stress challenge on intestinal barrier function and the role of gut health concept in it. Twenty-four piglets were distributed over 4 treatments in a 2 × 2 factorial design, with a control diet with or without Presan-Fx (2 kg/ton), under the condition with or without heat challenge. After a 6-d adaptation period, animals in the heat stress groups were given an acute heat stress of 40°C for 10 h followed by 28°C for 24 h. Animals were monitored for growth performance and the barrier function was evaluated by morphological assessment, tight junction proteins, and cytokine production. Heat stress decreased ADG, but the impact was reversed significantly in animals fed Presan-Fx (see Table 0064). For the animals fed Presan-Fx, villus height was higher and the crypt depth was lower after heat stress compared with the control, suggesting that enterocytes had fewer damaged villi and higher cell production. Heat stress decreased the overall expression of the tight junction proteins Claudin-4, Claudin-7, and Occludin had no effect on E-cadherin. The dietary treatments did not influence the expression of tight junction proteins. However, the expression of 5 inflammatory cytokines (Il-1α, IL-8, IL-10, IL-17, and IL-23) was decreased by the Presan-Fx as measured by Q-PCR. In conclusion, an acute heat stress impaired intestinal barrier function. Adding Presan-Fx supported the animals with a better resistance to a heat stress challenge.

Key Words: gut health, heat stress, intestinal barrier function

Table 0064. Average daily growth and histology data

	No stress		Heat stress		
	Control	Presan-Fx	Control	Presan-Fx	P-value
ADG 0 to 6, g	411.0	455.5	456.6	416.7	0.98
ADG 6 to 8, g			90.0[a]	341.7[b]	0.05
ADG 6 to 11, g	550.0	503.3			
Villus height, µm	ND	ND	327.3[a]	421.2[b]	0.07
Crypt depth, µm	ND	ND	74.5[a]	119.3[b]	0.08

ND = not determined.

0065 A dual challenge of corticotropin-releasing hormone and vasopressin alters immune cell profiles in beef heifers. J. A. Carroll*[1], N. C. Burdick Sanchez[1], J. O. Buntyn[2], S. E. Sieren[3], S. J. Jones[3], and T. B. Schmidt[3], *[1]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [2] Department of Animal Science, University of Nebraska, Lincoln, [3]University of Nebraska, Lincoln.*

The duration and magnitude of cortisol release can have different effects on the immune response. Over the last decade, studies have suggested that acute stress, when cortisol is elevated for a short duration of time, can be immuno-stimulatory rather than immuno-suppressive. This study was designed to determine the effect of an induced cortisol release, via a dual corticotropin-releasing hormone (CRH) and vasopressin (VP) challenge, on changes in immune cell profiles of beef heifers. Four days before the challenge, 10 heifers (605 ± 13 kg) were fitted with indwelling jugular cannulas and indwelling vaginal temperature (VT) recording devices that measured VT continuously at 5-min intervals. On d 0, heifers were challenged IV with 0.3 µg/kg BW bovine CRH and 1.0 µg/kg BW bovine VP concurrently. Two whole blood samples were collected at 30-min intervals from -2 to 8 h relative to the challenge at 0 h. One vacutainer containing EDTA was collected for complete blood cell count (CBC) analysis and the second was collected in a 9-mL monovette serum tube. After collection, serum was isolated and stored at −80°C until analyzed for cortisol concentrations by ELISA. There was a time effect ($P < 0.001$) for VT, cortisol, and CBC variables. A multiphasic response was observed for VT, with VT initially increasing ($P = 0.05$; relative to 0 h) within 15 min post challenge. Serum cortisol concentrations increased ($P < 0.001$) immediately after the challenge, reaching maximum concentrations between 0.5 and 2 h post challenge, and then continuously decreasing until reaching baseline concentrations at 6 h post challenge ($P = 0.17$; 0 vs. 6 h). Total white blood cell and lymphocyte concentrations increased ($P < 0.001$) 2 h after CRH/VP challenge and remained elevated for the duration of the blood collection period. Monocyte concentrations initially decreased 1 h post challenge ($P < 0.001$), and returned to baseline concentrations by 2 h post challenge ($P = 0.08$; 0 vs. 2 h). In contrast, neutrophil concentrations decreased ($P = 0.02$) 3 h post challenge and remained decreased throughout the duration of the blood collection period. These data demonstrate that immune cell populations are influenced by an acute activation of the hypothalamic-pituitary-adrenal axis. Additionally, the increase in circulating concentrations of lymphocytes and decrease in circulating concentrations of neutrophils observed in this study are indicative of an immunological priming event that could be beneficial to the animal.

Key Words: acute stress, cortisol, immune response

0066 Investigating innate immune response differences between Angus and Holstein cattle with the dermal fibroblast model. A. L. Benjamin[*1], W. J. Weber[2], S. D. McKay[1], B. A. Crooker[2], and D. E. Kerr[1], *[1]University of Vermont, Burlington, [2]University of Minnesota, St. Paul.*

Individual immune responses to pathogens can be variable, depending on environmental, genetic, and possibly epigenetic influences. Holstein and Angus cattle are selectively bred and managed for different traits, which could impact disease susceptibility between these breeds. A dermal fibroblast model was used to investigate potential genetic and epigenetic influences on the innate immune response in each breed. Skin biopsies were collected from the shoulder area of 5 Holstein and 12 Angus 19-mo-old heifers. Fibroblasts were isolated by collagenase digestion and cryopreserved. Revived cells were challenged with LPS (100ng/ml; 24h) and levels of secreted IL-8 were determined. The Holstein cultures produced ~3 times more ($P < 0.01$; unpaired t test) IL-8 then the Angus cultures (2190 ± 600 vs. 650 ± 370 pg/ml, respectively). Total RNA was collected from 4 Angus and 4 Holstein cultures that were cultured in parallel and challenged with LPS for 0, 2, and 8 h. Whole transcriptome analysis was performed by RNA-seq with an average of 46 million reads per sample aligned to the UMD 3.1 reference genome by NextGENe software. Breed differences in gene expression were determined with the edgeR statistical package. Between breeds, there were 849, 1014, and 751 genes differentially expressed genes (FDR < 0.05, fold change > 2) at h 0, 2, and 8 post-LPS, respectively. Immune response genes, such as TNF-a at 2,h and IL-8 and CCL20 at 8 h, were induced 6.9-, 4.5-, and 8.6-fold more in Holsteins compared with Angus, whereas expression at 2 h of CXCL12 and TRAIP, an inhibitor of TRAF2-mediated NF-kB activation, were 7.4- and 2.7-fold higher in Angus. Additionally, a semi-quantitative assessment of global DNA methylation was performed by methylated CpG island recovery assay (MIRA-seq) on genomic DNA extracted from these cultures. Read alignment (44 million reads per sample) and differential methylation region (DMR) analysis were performed similarly to RNA-seq. The genome was analyzed in consecutive 3 kb regions and revealed 51 DMR (FDR < 0.1, fold change > 2). Of these, 35 were more methylated in Angus and 16 were more methylated in Holsteins. Relationships among these DMR and the differential gene expression are not readily apparent, but are being further investigated. Our results reveal breed differences in the LPS response of dermal fibroblasts isolated from Angus and Holstein heifers. Given that the cells were cultured side by side in controlled environmental conditions, the observed differences are likely due to a combination of genetic and epigenetic factors.

Key Words: epigenetics, variation

0067 Predictive models of lameness in dairy cows achieve high sensitivity and specificity with force measurements in 3 dimensions. J. T. Dunthorn[*1], R. M. Dyer[2], U. Tasch[3], N. Neerchal[3], P. Rajkondawar[4], and G. Steingraber[4], *[1]Step Analysis, Baltimore, MD, [2]University of Delaware, Newark, [3]University of Maryland, Baltimore County, Baltimore, [4]BouMatic, Madison, WI.*

Lameness remains a significant cause of production losses and a growing welfare concern across the dairy industry. Metabolic, nutritional, and housing factors interact to sustain a steady increase in the prevalence of lameness, driving a growing need for automated and continuous methods of lameness detection. A force-plate system restricted to the vertical (z) dimension yielded a high specificity but low sensitivity of detection. The objective of this study was to determine the effect of supplementing the vertical dimension with the transverse (x) and longitudinal (y) dimensions on detection accuracy. We used a parallel, force-plate system to measure the ground reaction forces (GRF) across 3 orthogonal directions (3D). The GRF for randomly selected cows ($n = 83$) were recorded and a clinical diagnosis of lameness was generated using locomotion score, lesion diagnosis, lesion score, and claw and interdigital integument pain score. Logistic regression was used to characterize the relationship between the clinical characteristics and GRF across all 3 orthogonal dimensions to generate a statistical algorithm for the probability of lameness. Misclassification error was estimated using a modification of the Leave-One-Out (LOO) method of cross validation. The LOO

Table 0067. Model performance using various combinations of measurement directions (1-degree, 4-knot spline transformation). Results have been ordered by increasing AUC

Measurement direction (including stance time)	TN	FP	FN	TP	Sensitivity	Specificity	AUC
x	213	44	94	45	0.32	0.83	0.59
z	212	45	87	52	0.37	0.82	0.62
x, z	210	47	61	78	0.56	0.82	0.73
y	218	39	77	62	0.45	0.85	0.75
y, z	222	35	53	86	0.62	0.86	0.79
x, y	221	36	50	89	0.64	0.86	0.83
x, y, z	239	18	14	125	0.90	0.93	0.98

x = transverse (medial-lateral) direction; y = longitudinal (cranial-caudal) direction; z = vertical (weight) direction.

cross validation trains the model using all but a single run. We modified LOO to leave out all runs except for those from a single cow, Leave-One-Cow-Out (LOCO), to use as the training data and tested the resulting model using the runs of the cow not used in model development. This preliminary study determined that 76 variables across all 3 dimensions resulted in a model with 90% sensitivity, 93% specificity, and 98% area under the receiver operating curve (AUC). Furthermore, all 3 dimensions were both necessary and sufficient to accurately establish the probability of lameness (Table 0067).

Key Words: animal welfare, lameness, 3-dimensional ground reaction forces

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0068 Performance trends in commercial livestock populations in the United States before and subsequent to the inclusion of genetically modified feed in livestock diets. A. L. Van Eenennaam*, *University of California-Davis, Davis.*

The first genetically modified (GM) crops were planted in the United S in 1996; by 2000, GM soy and cotton comprised > 50% of U.S. land devoted to these crops. Adoption increased steadily thereafter and in 2013 a total of 93% of soy, 90% of cotton, and 90% of all corn grown in the United States were GM varieties. It is estimated that 70 to 90% of harvested GM biomass is fed to food-producing animals, making the world's livestock populations the largest consumers of the current generation of GM crops. It has been purported by some that GM feed has deleterious effects on underlying animal health. The United States feeds billions of livestock each year, providing a very large uncontrolled GM feeding field data set. Considering that animal health is critical to optimizing production performance and animal production systems are managed to minimize disease, it would be reasonable to hypothesize that if animal feed derived from GM crops had deleterious effects on the billions of animals that have been fed on diets containing predominantly GM feed, then animal performance and health attributes in these large populations would have been negatively impacted. To test this hypothesis, data on livestock productivity and health (somatic cell count, percent mortality, percent postmortem carcass condemnation) were collated from publicly available sources for 2 time periods: 1983–1994, before the introduction of GM crops in 1996, and 2000–2011, a period with high levels of GM feed based on high rates of U.S. adoption and international trade of GM crops. These data on broilers, dairy and beef cattle, and swine revealed improving productivity and animal health trends. Productivity improvements in all livestock species continued in the positive direction they were trending before the introduction of GE feed, often at an accelerated rate ($P < 0.05$), and health parameters also improved over time. Available mortality and carcass condemnation data suggest that these rates actually decreased during the 2000–2011 time period when high levels of GM ingredients would be expected to be present in livestock feed. Field data sets representing billions of observations do not reveal disturbing trends in U.S. livestock health and productivity data. These data are in agreement with the many peer-reviewed, controlled animal feeding studies that have reported no biologically relevant difference between the nutritional attributes and safety of feed from GM plants, as compared with feed derived from conventional crop varieties.

Key Words: animal health, feed, genetically modified, GMO

0069 Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. S. M. Deelen[1], T. L. Ollivett[1], D. M. Haines[2], and K. E. Leslie*[1], *[1]University of Guelph, ON, Canada, [2]University of Saskatchewan, Saskatoon, Canada.*

The objective of this study was to evaluate the utility of a digital Brix refractometer for the assessment of success of passive transfer of maternal immunoglobulin, as compared with the measurement of serum total protein (STP) by refractometry. Blood samples (n = 400) were collected from calves 3 to 6 d of age. Serum IgG concentration was determined by radial immune-diffusion (RID) and STP and percent Brix (%Brix) using a digital refractometer. The mean (± SD) IgG concentration was 24.1 ± 10.0 g/L, with a range from 2.1 to 59.1 g/L. The mean STP concentration was 6.0 ± 0.8 g/dL, with a range from 4.4 to 8.8 g/dL. The mean %Brix concentration was 9.2 ± 0.9%, with a range of 7.3 to 12.4%. Brix percentage was highly correlated with IgG [correlation coefficient (r) = 0.93]. Test characteristics were calculated for the assessment of failure of passive transfer (FPT; serum IgG < 10 g/L). The sensitivity and specificity of STP at 5.5 g/dL were 76.3% and 94.4%, respectively. However, it is noteworthy that relatively few samples had IgG levels < 10 g/L. As such, further evaluations of different populations are warranted. A receiver operating characteristic curve was created to plot the true positive rate against the false positive rate for consecutive %Brix values. The optimal combination of sensitivity (88.9%) and specificity (88.9%) was at 8.4% Brix. Serum total protein was also positively correlated with %Brix (r = 1.00) and IgG (r = 0.93). The results of the current project suggest that dairy producers can successfully monitor their colostrum management and the overall success of passive transfer using a digital Brix refractometer to estimate IgG concentration of colostrum and calf serum.

Key Words: calf, passive transfer, refractometer

0070 Associations of serum haptoglobin in newborn dairy calves with future health, growth, and mortality up to 4 mo old. C. F. Murray[1], C. Windeyer[2], T. F. Duffield[1], K. M. Waalderbos[1], and K. E. Leslie[*1], *[1]University of Guelph, ON, Canada, [2]University of Calgary, AB, Canada.*

The objective of this research was to investigate factors associated with serum haptoglobin (Hp) levels in newborn calves. In addition, the associations between serum Hp levels in newborn calves with future growth, morbidity, and mortality in calves up to 4 mo of age were investigated. A total of 1365 Holstein heifer calves from 15 dairy farms were enrolled in this study during 2008. Following calving, a birth record was completed, including information on the calving event, colostrum administration, and other details. During weekly farm visits, each calf was assessed at 1 to 8, 15 to 21, 36 to 42, and 90 to 120 d of age. At these times, each calf was assessed using a standardized clinical score for general health and height and weight were measured. At the first sampling event, a blood sample was collected for the determination of serum total protein and Hp. Treatment events and death loss were recorded by the farm staff throughout the study. Data analysis was conducted using a multivariable linear regression model to evaluate associations of explanatory variables with serum Hp. Separate multivariable logistic models were used to determine associations of various factors with treatment for disease and mortality. Serum Hp concentration in the first week of life was not significantly associated with the degree of calving difficulty. However, serum Hp was higher in calves with a higher rectal temperature and depressed attitude at the first sampling event. Calves with higher Hp in their first week of life had a significantly higher total health score throughout the entire sampling period. Haptoglobin was not significantly associated with ADG or treatment for bovine respiratory disease. Yet, for every 1 g/L increase in serum Hp in the first week of life, the odds of being treated for any other disease during the study period increased by 7.6 times. In addition, Hp concentration in the first week of life was associated with mortality in calves up to 4 mo of age. The optimal cut point for Hp was determined to be 0.13 g/L for the prediction of disease and death, although the sensitivity of Hp concentration as a diagnostic test for individual calves was low. Monitoring serum Hp in the first week of life shows considerable promise at the group level for overall assessment of calving management and the impact of calving events on future health and mortality.

Key Words: haptoglobin, health, mortality

0071 Dynamics of culling for Jersey, Holstein, and crossbred cows in large multi-breed herds. P. J. Pinedo[*1], A. Daniels[2], J. Shumaker[3], and A. De Vries[4], *[1]Texas A&M AgriLife Research, Amarillo, [2]Circle H Headquarters LLC, Dalhart, TX, [3]Magnolia Veterinary Services, Amarillo, TX, [4]University of Florida, Gainesville.*

The objective of this observational study was to describe and compare the dynamics of reason-specific culling risk for the genetic groups Jerseys (JE), Holsteins (HO), and Jersey × Holstein crossbreds (JH), considering parity, stage of lactation, and milk yield, among other variables, in large multi-breed dairy herds in Texas. The secondary objective was to analyze the association between survival and management factors, such as breeding and replacement policies, type of facilities, and use of cooling systems. After edits, available data included 202,384 lactations in 16 herds, ranging from 407 to 8773 cows calving per year during the study period from 2007 to 2011. The distribution of lactation records by genetic group was 58%, 36%, and 6% for HO, JE, and JH, respectively. Overall culling rates across breeds were 30.1%, 32.1%, and 35.0% for JH, JE, and HO, respectively. The dynamics of reason-specific culling were dependent on genetic group, parity, stage of lactation, milk yield, and herd characteristics. Early lactation was a critical period for "died," and "injury-sick" culling. The risk increased with days after calving for "breeding" and, in the case of HO, "low production" culling. Open cows had a 3.5 to 4.6 times greater risk for overall culling compared with pregnant cows ($P < 0.01$). The odds of culling with reason "died" within the first 60 d in milk (DIM) were not significantly associated with genetic group. However, both JE and JH had lower odds of live culling within the first 60 DIM compared with HO cows [OR = 0.72 ($P < 0.001$) and 0.82 ($P = 0.002$), respectively]. Other cow variables significantly associated with the risk of dying within the first 60 DIM were cow relative 305 mature equivalent (MEQ) milk yield, parity, and season of calving. Significant herd-related variables for death included herd size and origin of replacements. In addition to genetic group, the risk of live culling within 60 DIM was associated with cow relative 305 MEQ milk yield, parity, and season of calving. Significant herd-related variables for live culling included herd relative 305 MEQ milk yield, herd size, type of facility, origin of replacement, and type of maternity. Overall, reason-specific culling followed similar patterns across DIM in the 3 genetic groups.

Key Words: culling, death, Holstein, Jersey

0072 Relationship of ocular and rectal temperatures to indicators of stress in mature horses.

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Rectal temperature has commonly been used as an indicator of health in many species of livestock, including horses. However, collection of a rectal temperature can be difficult and stressful on the animal. New technology, such as thermal imaging cameras, have recently become more prevalent and have been used to collect the body temperature of animals at other, less-invasive sites, including the ocular globe. The objective of this research was to determine the relationship between rectal temperature and ocular temperature, and to evaluate the efficacy of these measurements as indicators of the stress level of horses. To accomplish this, ocular temperature (OT), rectal temperature (RT), neutrophil count, lymphocyte count, heart rate (HR), and respiration rate (RR) were recorded before and during an immune challenge, using a novel vaccination of 30 mature horses (413 to 551 kg and 5 to 10 yr). Neutrophil to lymphocyte ratio (N:L) has been shown to be a good indicator of systemic inflammation and overall stress in an animal, and was used as the primary indicator of stress in this study. To determine the relationship between temperatures and among indicators of stress, the PROC CORR procedure of SAS was used. The relationship between OT and RT was found to have a weak correlation ($r = 0.37$; $P < 0.01$), illustrating that OT is not a good substitute for traditional RT measurements. Additionally, OT had very little relationship with N:L ($r = -0.01$; $P = 0.94$), HR ($r = 0.19$; $P = 0.31$), or RR ($r = 0.17$; $P = 0.38$). While still very weak, RT had stronger relationships than OT with N:L ($r = -0.11$; $P = 0.40$) and RR ($r = 0.26$; $P = 0.17$) and a similar correlation to HR ($r = 0.19$; $P = 0.31$). Of all the non-invasive measurements (OT, HR, and RR), RR had the strongest correlation to N:L as an indicator of systemic stress, ($r = -0.24$; $P = 0.21$). While OT is less invasive than RT, a direct measurement of OT is not a reliable predictor of RT in an animal. Still, it may hold some value to livestock and wildlife producers due to its ease of measurement, but without further investigation into the relationship of OT and indicators of stress, its utility remains limited.

Key Words: ocular temperature, rectal temperature, stress

0073 Enhancement of the acute phase response to lipopolysaccharide in feedlot steers supplemented with OmniGen-AF.

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This study was designed to determine the effect of supplementing feedlot steers with OmniGen-AF on the acute phase response to a lipopolysaccharide (LPS) challenge. Steers ($n = 18$; 270 ± 5 kg BW) were separated into 2 treatment groups ($n = 9$/treatment): 1 group was fed a standard receiving diet (Control, Cont) and the other group was fed the same receiving diet supplemented with OmniGen-AF at 4 g/45.4 kg BW for 29 d (OmniGen-AF). On d 27, steers were fitted with indwelling jugular cannulas and rectal temperature (RT) monitoring devices, and placed in individual stalls. On d 28, steers were challenged IV with LPS (0.5 μg/kg BW at 0 h). Sickness behavior scores (SBS) and 2 whole blood samples were collected at 30-min intervals from -2 to 8 h relative to the challenge at 0 h. One vacutainer containing EDTA was collected for complete blood cell count (CBC) analysis and the second was collected in a 9-mL monovette serum tube; after collection, serum was isolated and stored at −80°C until analyzed for cortisol and cytokine concentrations. Rectal temperature, SBS, and cortisol were affected by time ($P < 0.001$). Before the challenge, RT was greater ($P < 0.001$) in Cont steers (39.31 ± 0.03°C) than OmniGen-AF steers (39.14 ± 0.03°C). Therefore, post-challenge RT was analyzed as the change in response from baseline values. The change in RT relative to baseline values increased ($P < 0.001$) in both groups in response to LPS challenge but was not affected by treatment ($P = 0.49$). Sickness behavior scores increased ($P < 0.001$) after LPS challenge and tended ($P = 0.09$) to be greater in Control (1.57 ± 0.02) than OmniGen-AF steers (1.51 ± 0.02). Cortisol concentrations were affected by treatment ($P = 0.005$) and time ($P < 0.001$). For both groups, cortisol increased ($P < 0.001$) in response to LPS challenge. Cortisol was greater in Cont (29.2 ± 0.9 ng/mL) than OmniGen-AF steers (25.5 ± 0.9 ng/mL). White blood cell and lymphocyte concentrations were greater ($P \leq 0.004$) in Cont than OmniGen-AF steers throughout the study. Neutrophils were decreased ($P = 0.04$) in Cont steers (0.7 ± 0.2 K/uL) compared with OmniGen-AF steers (1.3 ± 0.2 K/uL) before the LPS challenge. There was a treatment ($P \leq 0.02$) and time ($P < 0.001$) effect for tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ). Specifically, TNFα and IFNγ concentrations increased ($P < 0.001$) in response to LPS challenge. Furthermore, concentrations of TNFα and IFNγ were decreased in ($P \leq 0.02$) in Cont steers compared with OmniGen-AF steers. These data suggest that OmniGen-AF supplementation served to prime the immune

system before the LPS challenge, allowing for an enhanced response to LPS challenge.

Key Words: cattle, immune response, OmniGen-AF

0074 Age-dependent changes in heifer fibroblast DNA methylation and LPS-induced gene expression.

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To determine how the innate immune response develops in dairy calves, dermal fibroblasts were isolated from 15 heifers at 3 stages of development (5, 11, and 16 mo of age) and challenged with 100 ng/ml of lipopolysaccharide (LPS) for 36 h. The secretion of interleukin- (IL) 8 protein into media in response to LPS increased significantly ($P < 0.01$) at each age as measured by a paired 1-way ANOVA with average IL-8 levels of 300 ± 220, 1340 ± 530, and 1750 ± 560 pg/ml at 5, 11, and 16 mo, respectively. To investigate a potential involvement of DNA methylation in this differential responsiveness, cultures from 3 of these animals obtained when they were young (5 mo) and older (16 mo), underwent DNA de-methylation through exposure to 10mM 5-aza-2'-deoxycytidine (AZA) for 4 d before 24-h LPS exposure ($n = 3$/group). The AZA treatment abolished the differential IL-8 response to LPS seen under control conditions ($P < 0.01$), primarily due to an increase in production by the young cultures (control young vs. old 240 ± 20 vs. 1350 ± 290 pg/ml, respectively; AZA Young vs. Old 1580 ± 50 vs. 1690 ± 70 pg/ml, respectively). The role of DNA-methylation in the gene expression response to LPS was further investigated by comparing the findings of methylated -CpG island recovery assay (MIRA-seq) on DNA from 3 pairs of young and older cultures to RNA-seq findings on the same cultures at 0, 2, and 8 h post-LPS exposure. The resulting libraries averaged 71 and 49 million reads per sample for RNA-seq and MIRA-seq, respectively. Sequence reads were aligned to the UMD3.1 reference genome using NextGENe software and expression and methylation analysis were performed with EdgeR. The overall response to LPS was robust with 617 and 414 genes displaying differential expression (> twofold difference; FDR < 0.05) at h 2 and 8, respectively, as compared with h 0. Older cultures had greater expression than younger cultures of many immune-associated genes, such as IL-8, IL-6, TNF-a, and CCL20 at 2 h post-LPS exposure (5.8-, 10.5-, 10.4-, and 18.1-fold, respectively). In addition, whole genome MIRA-seq analysis of consecutive 3 kb regions identified 20 differentially methylated regions between young and older cultures (> twofold difference; FDR < 0.1). Further analysis of these candidate regions is being conducted to determine how DNA methylation changes within animals over time and its potential role in development of the innate immune response.

Key Words: epigenetics, innate immunity, methylation

0075 Effect of trace mineral supplementation on clinical signs, immune response variables, and mineral balance of calves following exposure to bovine viral diarrhea virus and subsequent *Mannheima haemolytica* infection.

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The objective was to determine the influence of copper, manganese, and zinc supplementation on the clinical signs, immune response variables, and mineral balance of calves, following a bovine viral diarrhea virus (BVDV) and *Mannheima haemolytica* (MH) immune challenge. Steers ($n = 16$; BW = 225 ± 20 kg) from a single ranch were processed, weaned, and randomly pairwise assigned to either the mineral supplemented (MIN) or control (CON) experimental treatments. The MIN calves received 150 mg of Cu, 130 mg of Mn, and 360 mg of Zn daily, whereas CON calves received the basal diet with no additional Cu, Mn, or Zn supplementation. The basal diet contained sufficient Mn and Zn, but inadequate Cu based on published nutrient requirements. After 46 d on the experimental treatments, all calves were naturally exposed to BVDV type 1b for 4 d and subsequently infected with *MH*. Data were analyzed using the GLM procedure of SAS with sampling time serving as a repeated measure. Calf served as the experimental unit. The immune challenge was validated via increased ($P < 0.001$) BVDV antibody titers, MH whole cell (WC) and leukotoxin (LKT) antibody titers, rectal temperatures (TEMP), and subjective clinical scores (CS). A time by treatment interaction was observed for BVDV and MHWC antibody titers ($P \leq 0.04$). Calves receiving MIN had reduced ($P = 0.03$) BVDV antibody titers but increased ($P = 0.02$) MHWC antibody titers compared with CON calves. Mineral supplementation did not impact CS, TEMP, or MHLKT antibody titers ($P \geq 0.48$). There was a significant ($P < 0.001$) time by treatment interaction observed for liver Cu levels. Time significantly impacted the concentrations of Cu, Mn, Fe, and Zn within the liver and Cu, Zn, and Fe within the muscle and serum ($P \leq 0.03$). Calves receiving MIN had greater liver Cu ($P < 0.001$) and Mn ($P = 0.04$) concentrations compared with CON calves. In contrast, serum Cu concentrations were increased ($P = 0.02$) in CON calves compared with MIN calves. Mineral supplementation did not impact mineral levels within the muscle samples ($P \geq 0.20$). The supplementation of Cu, Mn, and Zn may potentially impact the antibody response to a BVDV and MH immune challenge in calves. When Cu is supplemented to calves receiving a marginally Cu deficient diet, Cu status within the body can be altered.

Key Words: bovine respiratory disease, mineral supplementation, immune challenge