Animal Health: Immune Response Patterns

TH1 Fatty acid catabolism modifies hypothalamic metabolome to suppress inflammation and appetite. J. W. McFadden*[1,2], E. Kim[3], Q. Li[2], S. Aja[2], V. V. Bandaru[2], N. J. Haughey[2], F. P. Kuhajda[2], and G. V. Ronnett[2], *[1]West Virginia University, Morgantown, [2]Johns Hopkins University, Baltimore, MD, [3]Daegu Gyeongbuk Institute of Science and Technology, Daegu, South Korea.*

Overnutrition causes hypothalamic lipotoxicity, reduced fatty acid (FA) oxidation (FAO), and inflammation to support hyperphagia. Increasing neuronal FAO with C75, a carnitine palmitoyltransferase-1 stimulator, suppresses appetite during overnutrition. The objective was to define this mechanism in primary hypothalamic neurons (PHN) collected from embryonic Sprague-Dawley rats. Following differentiation, 9 d in vitro, PHN (cultured in 3 mM glucose, 5% O_2, 5% CO_2) were treated with vehicle, 200 μM palmitate (C16:0), 70 μM C75, or C75 with C16:0 for 3, 4, or 18 h. For targeted metabolomics, lipid extracts were analyzed by a liquid chromatograph electrospray ionization tandem mass spectrometer (LC/ESI/MS/MS). For untargeted metabolomics, methanol extracted metabolites were independently analyzed on a gas chromatograph/MS and an ultrahigh performance LC/MS/MS. Data were analyzed by ANOVA and Tukey's test. For metabolomics data, statistics were performed on the log of the normalized, median-scaled data. Treatment with C75 for 4 h increased ($P < 0.01$) [^{14}C]-palmitate oxidation and ATP levels, and decreased ($P < 0.05$) AMP-activated protein kinase activation in PHN. From targeted and untargeted profiling, 96 and 185 metabolites were detected, respectively. Of interest, levels of palmitate, as well as palmitoyl-linked ceramide, dihydroceramide, monohexosylceramide, cholesterol, and fatty acylglycerol increased ($P < 0.05$) in PHN treated with C16:0 for 3 h; outcomes reversed ($P < 0.05$) by augmented FAO with C75. Untargeted metabolomics revealed decreased ($P < 0.10$) acetyl-CoA and propionylcarnitine levels, and increased ($P < 0.05$) oxidized nicotinamide adenine dinucleotide and citrate levels in PHN treated with C75 for 18 h, indicative of increased tricarboxylic acid cycle flux and oxidative phosphorylation caused by upregulated FAO. Treatment with C16:0 for 18 h increased ($P < 0.01$) tumor necrosis factor-α and interleukin-1β mRNA in PHN, pro-inflammatory effects inhibited by C75. Increasing hypothalamic FAO prevents lipotoxicity and inflammation to improve energy sensing, a potential means to prevent hyperphagia during overnutrition.

Key Words: fat metabolism, hypothalamus, metabolomics

TH2 Effect of subcutaneous fat stores on fatty acid content of serum phospholipids fraction in periparturient dairy cows. C. M. Scholte*, K. C. Ramsey, S. L. Shields, and P. Rezamand, *University of Idaho, Moscow.*

During early lactation, the diet does not meet the elevated energy requirements of high producing dairy cows. This leads to elevated lipid mobilization and thus release of fat into the blood in the form of nonesterified fatty acids (NEFA). Large quantities of NEFA have shown to alter the profile of the phospholipids (PL) fraction of the serum fatty acids (FA). The PL fraction is involved in cellular plasma membrane integrity, lipoprotein synthesis and intercellular signaling. The objective of this study was to determine how the changes in lipomobilization, as assessed by body condition score (BCS) around time of calving, affect the FA profile of serum PL fraction. Twenty-two primiparous and multiparous cows were monitored from 4 wk prepartum through 4 wk postpartum. Based on BCS around calving, cows were dichotomized into 2 groups of over-conditioned (BCS ≥3.25) or control (BCS ≤3.0). Blood samples were obtained at −28, −7, +8, +18, and +28d relative to parturition. Serum PL fraction was separated and analyzed for the FA profile. Data were analyzed as repeated measures by using PROC MIXED (SAS 9.2) and significance was declared at $P < 0.05$. Several FA in the PL fraction of serum lipids varied significantly by BCS around the time of parturition, including palmitic (C16:0), sapienic (C16:1), margaric (C17:0), linoleic (C18:2), and eicosadienoic (C20:2) acids. Further investigation is warranted to fully understand the relationship between over-conditioning and FA profiles of serum and circulating immune cells and their response to pathogens.

Key Words: dairy cow, lipid mobilization, phospholipid

TH3 Productive performance and risk of fat cow syndrome of cows at peak lactation with or without clinical mastitis. C. F. Qin[1,2], P. H. Zhang*[1], J. Q. Wang[2], P. Sun[2], D. P. Bu[2], D. Zhu[1], Y. G. Chai[1,2], and T. Zhang[1], *[1]Hunan Provincial Key Laboratory for Genetic Improvement of Domestic Animal, College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Mastitis affects milk quality and quantity and induces metabolic disorders, causing great economic loss. This study evaluated the effects of clinical mastitis (CM) on productive performance and risk of fat cow syndrome in cows at peak lactation. Four Holstein cows without mastitis and another 4 diagnosed with CM were used in this study. All cows were from the same farm and with the same days in milk (57 DIM) and similar parities (1 to 3). Dry matter intake (DMI) and milk yield were recorded daily and body condition scoring (BCS) was conducted twice weekly. On 77 DIM, serum was sampled via jugular vein before morning feeding and milk samples were obtained. Data were analyzed using the MIXED procedure (SAS 9.1). In cows with CM, BCS increased ($R^2 = 0.87$) and milk yield decreased linearly ($R^2 = 0.94$) by DIM. Milk fat percentage (3.87 and 6.70%; $P > 0.05$) and yield (1.08 and 0.86 kg/d; $P > 0.05$), total solids proportion (12.48 and 15.13%; $P > 0.05$) and yield (3.50 and 2.19 kg/d; $P > 0.05$) and fat to protein ratio (1.34 and 1.90; $P > 0.05$) were not affected by CM. Composition of milk protein (2.94 and 3.50%; $P < 0.05$) and free fatty acid (1.46 and 2.75%; $P < 0.05$) increased when cows suffered from CM. DMI (20.78 and 12.55 kg/d; $P < 0.01$), milk yield (27.89 and 11.09 kg/d; $P < 0.01$), 4.0% fat corrected milk yield (27.38 and 19.09 kg/d; $P < 0.05$), energy corrected milk yield (29.11 and 20.11 kg/d; $P < 0.05$) and feed conversion rate (1.36 and 0.90; $P < 0.05$) decreased in cows with CM. In serum, level of total calcium (2.35 and 2.40 mmol/L; $P > 0.05$) and 25-hydroxyvitamin D_3 (31.28 and 25.67 ng/mL; $P > 0.05$) were not affected by suffering from CM. Albumin composition (35.43 and 29.43 g/L; $P < 0.05$) was lower and glucose level (2.86 and 4.48 mmol/L; $P < 0.05$) increased in serum of cows with CM, and concentration of serum β-hydroxybutyric acid (0.41 and 0.62 mmol/L; $P = 0.08$) tended to be higher in cows suffered from CM. Results indicated that productive performance was depressed and risk of fat cow syndrome increased when cows at peak lactation suffered from clinical mastitis.

Key Words: clinical mastitis, productive performance, fat cow syndrome

TH4 Association between hematological parameters and gender upon arrival with clinical bovine respiratory disease (BRD) risk in newly received beef calves. J. T. Richeson*[1], P. J. Pinedo[2], E. B. Kegley[3], J. G. Powell[3], M. S. Gadberry[3], P. A. Beck[3], and S. M. Falkenberg[4], *[1]Department of Agricultural Sciences, West Texas A&M University, Canyon, [2]Texas A&M AgriLife Research & Extension Center-College of Veterinary Medicine & Biomedical Sciences, Texas A&M University System, Amarillo, [3]Department of Animal Science, Division of Agriculture, University of Arkansas, Fayetteville, [4]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA.*

The primary objective of this study was to analyze the association between complete blood count (CBC) parameters and gender upon arrival at a stocker receiving facility with the risk of subsequent clinical BRD. A secondary objective was to evaluate the accuracy of CBC parameter thresholds on predicting the risk of BRD. A retrospective cohort model was designed using 1,179 crossbred bull and steer calves (initial BW = 197 ± 2.4 kg) participating in 4 studies at 2 University of Arkansas cattle facilities. Gender (bull or steer) was determined and whole blood samples were collected and analyzed for concentration and/or percentage of total and differential leukocytes, red blood cells (RBC), hemoglobin, and platelets using an automated hemocytometer. Calves were monitored daily for signs of respiratory illness and were considered morbid based upon the BRD case definition specific to each trial. For statistical analyses, area under the curve (AUC) values were determined to measure the accuracy of each CBC parameter in predicting BRD and for assessing sensitivity and specificity thresholds. Then, a chi-squared test and multivariable logistic regression models were used for BRD prediction and results were categorized into low, medium, or high quartiles. Individual animal was experimental unit for all analyses and after removal of outliers, 943 animals were included in the final model. The resulting AUC values for the CBC parameters showing significant contrasts were low to moderate, and ranged from 0.51 (neutrophils; $P = 0.69$) to 0.67 (eosinophils; EOS; $P < 0.001$). The only CBC parameters showing a consistent predictive value for BRD were EOS ($P \leq 0.002$) and RBC ($P \leq 0.007$). Bull calves upon arrival were at greater ($P < 0.001$) risk of developing subsequent clinical BRD than steers; the odds of being initially diagnosed with BRD for bulls were 3.32 times the odds for steer cohorts. Arrival gender, along with the combination of reduced EOS and increased RBC concentrations in peripheral blood could be useful in identifying animals at greater risk for BRD.

Key Words: biomarker, bovine respiratory disease, complete blood count

TH5 Relationship between lying behavior and metritis in Holstein dairy cows. J. M. Huzzey*, A. Itle, D. M. Weary, and M. A. G. von Keyserlingk, *University of British Columbia, Animal Welfare Program, Vancouver, BC, Canada.*

Sick animals are often lethargic, as energy conservation helps facilitate recovery. The aim of this study was to test the prediction that Holstein cows with metritis would spend more time lying down. Data loggers were used to measure postpartum lying behavior (lying time, number of lying bouts, average lying bout duration) of cows on 2 commercial farms from d+1 to d+20 relative to calving: 150 cows from Farm 1 and 69 cows from Farm 2. Vaginal exams occurred twice per week from calving until 20 d in milk (DIM) and cases of metritis were diagnosed based on the presence of purulent and/or fetid vaginal discharge. Among cows diagnosed with metritis, 13 animals had at least 2-d of lying data recorded before the first observed clinical signs of infection; this data was used to create baseline data from which within-cow changes in lying activity could be described during the days following diagnosis. Lying behavior following diagnosis was averaged into 2 3-d periods for analyses: d 0 to 2 and d 3 to 5 post-diagnosis. Differences from baseline were analyzed using one sample *t*-tests. Clinical signs of metritis first appeared at 5.9 ± 2.4 DIM and the severity of the infection peaked at 7.5 ± 3.1 DIM (mean ± SD). The lying time (±SD) of metritic cows during the baseline period averaged 11.63 ± 2.87 h/d. Lying time tended to decrease by 1.08 ± 0.54 h/d from baseline during the first 3-d post-diagnosis and was 2.15 ± 0.75 h/d lower than baseline between d 3 to 5 post-diagnosis; the increased difference in lying time during the later days may have been associated with an increasing severity in the infection. Prior to metritis diagnosis the average (±SD) number of lying bouts and lying bout duration was 11.04 ± 2.15 bouts/d and 1.10 ± 0.34 h/d, respectively. These measures of lying activity did not change after metritis diagnosis. The reduction in lying times following metritis diagnosis is opposite to the lethargy-induced change originally predicted and may be due to animal discomfort and pain associated with the uterine infection.

Key Words: health, activity, welfare

TH6 Depleted serum vitamin E concentrations precede retained placenta in multiparous dairy cows. Y. Qu*, A. N. Fadden, M. G. Traber, and G. Bobe, *Oregon State University, Corvallis.*

Retained placenta (RP), defined as fetal membranes not being expelled within 24 h after calving, is a costly disease in multiparous dairy cows that is associated with perturbations in arachidonic acid metabolism. Vitamin E is an antioxidant that limits lipid peroxidation and has been shown to be lower one week before calving. We hypothesized that serum vitamin E (α-tocopherol) concentrations are depleted weeks before calving in cows that will develop RP. The objective of this study was to evaluate vitamin E status of multiparous dairy cows from a commercial dairy herd using a nested case-control design to compare cows that (a) were healthy after calving, (b) developed RP, or (c) developed in the first 28 d after calving other diseases, such as mastitis, metritis, laminitis, or ketosis. Blood samples were taken at postpartum d −21, −14, −7, −3, −1, and 0 from 96 multiparous Holstein cows (32 cows per group) and serum α-tocopherol and cholesterol concentrations were analyzed. Cows that later developed RP had 24% lower prepartal serum α-tocopherol concentrations (8.3 ± 0.6 vs. 10.9 ± 0.4 μ*M*; $P < 0.001$) and 17% lower α-tocopherol to cholesterol molar ratios (2.91 vs. 3.50 μ*M*/m*M*; $P = 0.003$) compared with cows that did not develop RP. Differences between cows that developed RP and those that did not were apparent as early as 3 wk before calving [α-tocopherol concentrations (8.0 ± 0.7 vs. 10.9 ± 0.5 μ*M*; $P = 0.002$) and α-tocopherol to cholesterol molar ratios (2.84 vs. 3.49 μ*M*/m*M*; $P = 0.003$)]. Healthy cows (concentration:12.3 ± 0.5 μ*M*, $P_{\text{Healthy vs. RP}} < 0.001$; ratio: 3.97 μ*M*/m*M*, $P_{\text{Healthy vs. RP}} < 0.001$) had the highest prepartal vitamin E status, whereas cows with other diseases had an intermediate vitamin E status (concentration: 9.5 ± 0.5 μ*M*, $P_{\text{Other Disease vs. RP}} = 0.10$; ratio: 3.45 μ*M*/m*M*, $P_{\text{Other Disease vs. RP}} = 0.66$). These findings suggest decreased vitamin E status is an early indicator for the development of RP in multiparous cows.

Key Words: dairy cow, retained placenta, vitamin E

TH7 Elevated serum visfatin concentrations precede retained placenta in multiparous dairy cows. A. N. Fadden, M. G. Traber, and G. Bobe*, *Oregon State University, Corvallis.*

Visfatin (also known as "pre-B colony enhancing factor" or "nicotinamide phosphoribosyl transferase") is a multifunctional protein associated with immune function and glucose homeostasis, which has not been previously examined in dairy cows. Visfatin is expressed in humans in fetal membranes and placenta and is elevated in serum during placental infections. Retained placenta (RP), defined as fetal membranes not being expelled within 24 h after calving, is a costly disease in multiparous dairy cows that is associated with placental inflammation. We hypothesized that serum visfatin concentrations are elevated in dairy cows that will develop RP. The objective of this study was to compare prepartal serum visfatin concentrations of multiparous dairy cows that either were healthy after calving (control) or developed RP (cases) on a commercial dairy herd. Using a nested case-control design, blood samples, taken at d −21 (−25 to −18), −14 (−17 to −11), −7 (−10 to −5), −3 (−4 to −3), −1 (−2 to −1), and 0 postpartum from multiparous Holstein cows that (a) were not treated for diseases within the first 4 weeks after calving (healthy cows, n = 22) or (b) developed RP (RP cows, n = 28) were analyzed for serum concentrations of visfatin. The RP cows had on average 26% lower prepartal serum visfatin concentrations (8.4 ± 0.6 vs. 6.2 ± 0.7 μg/L; $P = 0.009$) compared with healthy cows. Three weeks before calving, serum visfatin concentrations were significantly higher in RP compared with healthy cows (9.5 ± 0.7 vs. 7.5 ± 0.8 μ*M*; $P = 0.04$). Thus, elevated serum visfatin concentrations precede calving and suggest that serum visfatin concentrations could be used as a biomarker of the development of RP in multiparous cows.

Key Words: dairy cow, retained placenta, visfatin

TH8 Depleted serum vitamin E concentrations precede milk fever in multiparous dairy cows. Y. Qu*, A. N. Fadden, M. G. Traber, and G. Bobe, *Oregon State University, Corvallis.*

Milk fever (MF), defined as the clinical manifestation of hypocalcemia within the first 48 h after calving, is a costly disease in multiparous dairy cows that is associated with perturbations in calcium transport. We previously documented that depleted serum vitamin E (α-tocopherol) concentrations precede left displaced abomasum. We hypothesized that serum α-tocopherol concentrations are depleted before calving in cows that will develop MF, a gateway disorder for left displaced abomasum. Our objective was to compare prepartal serum α-tocopherol concentrations of multiparous dairy cows that (a) were healthy after calving, (b) developed MF, or (c) developed after calving other diseases on a commercial dairy herd. Using a nested case-control design, blood samples, taken on d −21, −14, −7, −3, −1, and 0 postpartum from 105 multiparous Holstein cows (35 cows per group) that (a) were not treated for diseases within the first 4 weeks after calving, (b) developed clinical signs of MF, or (c) developed in the first 28 d after calving other diseases, such as mastitis, metritis, laminitis, or ketosis, were analyzed for serum concentrations of α-tocopherol and cholesterol. Cows that later developed MF had on average 17% lower prepartal serum α-tocopherol concentrations (9.0 ± 0.5 vs. 10.8 ± 0.4 μ*M*; $P = 0.005$) and 15% lower α-tocopherol to cholesterol molar ratios (2.91 vs. 3.44 μ*M*/m*M*; $P = 0.002$) compared with cows that did not develop MF. These group differences were already significant 3 weeks before calving for α-tocopherol (8.6 ± 0.7 vs. 10.7 ± 0.5 μ*M*; $P = 0.01$) and α-tocopherol to cholesterol molar ratios (2.97 vs. 3.48 μ*M*/m*M*; $P = 0.03$). Healthy cows (concentration: 12.1 ± 0.5 μ*M*, $P_{\text{Healthy vs. MF}} < 0.001$; ratio: 3.91 μ*M*/m*M*, $P_{\text{Healthy vs. MF}} < 0.001$) had the highest prepartal vitamin E status, whereas cows with other diseases had an intermediate vitamin E status (concentration: 9.6 ± 0.5 μ*M*, $P_{\text{Other Disease vs. MF}} = 0.39$; ratio: 3.02 μ*M*/m*M*, $P_{\text{Other Disease vs. MF}} = 0.55$). These findings suggest lower serum α-tocopherol concentrations as potential early indicator for developing of MF in multiparous cows.

Key Words: dairy cow, milk fever, vitamin E

TH9 Assessment of shedding of *Mycobacterium avium* ssp. *paratuberculosis* into milk and colostrum of naturally infected dairy cows over complete lactation cycles. J. R. Stabel*[1,2], L. Bradner[1], S. Robbe-Austerman[3], and D. C. Beitz[2], *[1]USDA-ARS-NADC, Ames, IA, [2]Iowa State University, Ames, [3]USDA-ARS-VS, Ames, IA.*

Mycobacterium avium ssp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD). One mode of transmission of MAP is through ingestion of contaminated milk and colostrum by susceptible calves. The objective of this study was to determine if the amount of MAP shed into the milk and colostrum of infected cows was affected by severity of infection, as well as the number of days in milk (DIM). Milk was collected over the 305-d lactation period from 24 asymptomatic cows in the subclinical stage of disease, 20 symptomatic cows demonstrating clinical signs, and 6 noninfected control cows. Milk was assayed for MAP by culture with BACTEC 12B and Herrold's egg yolk (HEY) media and by direct PCR for IS900 target gene. MAP was detected in 1.1, 2.7, and 7.6% of milk samples collected from cows with subclinical JD and 9.5, 11.2, and 38.8% of milk samples collected from cows with clinical JD by culture in HEY medium, Bactec 12B medium, and direct PCR, respectively. None of the milk samples collected from control cows were positive for MAP by any detection method. Viable MAP was primarily isolated from milk and colostrum of subclinically and clinically infected cows collected in early lactation (DIM 0–60); with decreased incidence in mid (DIM 60–240) and late (DIM 240–305) lactation. This study demonstrates that shedding of MAP into milk is affected by infection status of the cow as well as DIM, providing useful information to producers to help break the cycle of infection within a herd.

Key Words: *Mycobacterium avium* ssp. *paratuberculosis*, Johne's disease, milk

TH10 Monitoring response to vaccination with an inactivated BVDV vaccine by RNAseq transcriptome analysis in cattle. W. Demasius[1], R. Weikard[1], F. Hadlich[1], K. Müller[2], and Ch. Kühn*[1], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Freie Universität Berlin, Department of Veterinary Medicine, Berlin, Germany.*

Bovine neonatal pancytopenia (BNP) is a fatal disease of newborn calves that is characterized by severe hemorrhages and a depletion of thrombocytes, leucocytes and myeloid stem cells. Epidemiological studies confirmed an association between BNP and a specific inactivated Bovine virus diarrhea virus (BVDV) vaccine. Genetically preconditioned cows vaccinated with this vaccine elicit BNP in newborn calves via colostrum. Although alloantigens against MHC class I molecules have been detected in the serum and colostrum of the respective cows, the pathogenesis of BNP is still not fully clear. Deep RNA sequencing (RNaseq) offers the opportunity of holistic monitoring of quantitative and structural differences between whole transcriptomes of a cell or tissue. In our analyses, we included 12 lactating and nonlactating cows, all except one from a German Holstein × Charolais crossbred population. Blood samples for RNA isolation were taken prior and 2 weeks after vaccination with the BVDV vaccine associated with BNP. RNaseq of transcript libraries was performed in a paired-end 61-bp multiplexed run. The reads obtained were demultiplexed, evaluated for quality and aligned to the bovine reference genome with the Bowtie/Tophat software package. Cufflinks options were used to assemble transcripts and to obtain read counts per transcribed locus. Read counts of loci were tested for differential expression in response to vaccination applying

a threshold of q < 0.05. Our experiment yielded 57.8 to 79.7 million mapped reads/sample. Transcript assembly showed that 4,596 of the 18,181 loci expressed in all samples had not been identified before. Bioinformatic enrichment analyses of transcripts differentially expressed due to vaccination identified the pathways of allograft rejection, graft-versus-host disease, cytokine-cytokine receptor interaction, natural killer cell mediated cytotoxicity, and RIG-I-like receptor signaling as significantly affected by vaccination, which indicates a major immune response to an alloantigen and to RNA virus infection.

Key Words: RNAseq, vaccination, cattle

TH11 *Cryptosporidium parvum* in Holstein calves at the State of Jalisco, México. I. Vitela-Mendoza[1], L. Medina-Esparza[1], C. Cruz-Vazquez[1], M. Ramos-Parra[1], I. Mejía-Haro[1], and S. S. González-Muñoz*[2], *[1]Instituto Tecnológico El Llano, Aguascalientes, Aguascalientes, México, [2]Colegio de Postgraduados, Montecillo, Estado de México, México.*

Cryptosporidium parvum, a parasite that causes diarrheas in young calves, is typically spread by ingestion of oocysts excreted in feces. Therefore, the objective of this study was to detect *Cryptosporidium* species in Holstein calves in Los Altos Norte, State of Jalisco. Between July and December 2012, 400 samples of feces were taken: 200 from 7- to 14-d-old male calves and 200 from 4- to 6-mo-old heifers. These samples were individually analyzed using a microscope (Leica LCD Digital) to identify *Cryptosporidium* oocysts, using Kinyoun staining. We also carried out molecular diagnosis to further characterize the species in samples identified as positive by the microscope analysis. *Cryptosporidium parvum* was found in 80% of 7- to 14-d-old male calves, and in 20% of 4- to 6-mo-old heifers. Nested PCR was used in 50 samples, in which 830-bp fragment corresponding to the 18S rRNA gene *Cryptosporidium* was identified in 36 samples (76%) of 7- to 14-d-old male calves, and in 14 samples (28%) of 4- to –mo-old heifers. Genetic analysis allowed to determining that isolates have 99 to 100% similarity with *C. parvum* bovine genotype. The high prevalence of cryptosporidiosis detected in this study is the first report of genetically identified *C. parvum* in Holstein calves in the State of Jalisco.

Key Words: *Cryptosporidium parvum*, nested PCR, Holstein calf

TH12 Evidence of seasonality and birth clusters of *Mycobacterium avium* subspecies *paratuberculosis* infection in US dairy herds. Y. Zare*[1], G. E. Shook[2], M. T. Collins[3], and B. W. Kirkpatrick[1,2], *[1]College of Agricultural and Life Sciences, Department of Animal Science, University of Wisconsin-Madison, Madison, [2]College of Agricultural and Life Sciences, Department of Dairy Science, University of Wisconsin-Madison, Madison, [3]School of Veterinary Medicine, Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison.*

Paratuberculosis (Johne's disease) is a contagious intestinal infection of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Young calves are at the highest risk for acquiring the infection mainly through ingestion of MAP from contaminated milk, colostrum, and feces or environmental contacts. The objectives were to assess (1) seasonality of MAP infection incidence and (2) whether MAP-infected animals were randomly distributed or clustered by date of birth within herds. Data consisted of ELISA and fecal culture test results of 9,200 mature cows from 30 US Jersey herds and 4 Wisconsin Holstein herds. ELISA results were available for all animals; however, fecal cultures were performed selectively. Within-herd apparent prevalence of MAP infection ranged from 0.01 to 0.30. Two definitions for infected animals were utilized. In the first infection (case) definition (CD1) animals testing positive and negative to ELISA were considered as infected and non-infected, respectively. In the second case definition (CD2) animals testing positive to either test were considered infected and cows testing negative to ELISA or to both ELISA and fecal culture were regarded as non-infected. For objective 1, 28 herds for CD1 and 24 herds for CD2 met specific criteria and were pooled for analysis. The effects of age, breed, herd, and season of birth were examined using logistic regression analysis. Season of birth was significant for both CDs; highest incidence was in summer ($P < 0.05$). For objective 2, 14 herds for CD1 and 2 herds for CD2 met specific criteria. A temporal clustering approach implemented in SaTScan software v 9.1 was used to detect clusters of birth cohorts using animals' birthdates and windows of 4, 10, 60, 90 and 120 d. Significant clustering of MAP-infected animals occurred in multiple herds. Existence of clusters necessitates matching cases and controls on birthdates to control for non-uniform exposure to MAP in genome wide association, candidate gene studies, or on-farm disease intervention trials.

Key Words: Johne's disease, dairy cattle, temporal clustering

TH13 Association between bovine viral diarrhea virus (BVDV) vaccine response and birth and weaning weights in crossbred beef calves. X. Sheng*, J. Walker, and M. Gonda, *South Dakota State University, Brookings.*

Antibody response following bovine viral diarrhea virus (BVDV) vaccination varies among individuals in beef herds. Some of this variation could be caused by genetic differences among individuals. If genetic selection were to be used to improve individual antibody response to vaccination, we must first understand whether BVDV vaccine response associates with other economically important traits in beef production. Our objective was to estimate the phenotypic correlation between BVDV-specific antibodies post-vaccination and birth and weaning weights in crossbred beef cattle. Crossbred calves (n = 208) raised at Antelope Range and Livestock Research Station in Buffalo, SD were vaccinated with Bovi-Shield GOLD 5 (Pfizer Inc., NY). Blood samples were collected at 3 time points: time of initial vaccination (d 0; $\mu = 193$ d of age), booster vaccination (d 34), and 50 d after the booster vaccination (d 84). Weights were recorded within 48 h of birth and when weaned (time of booster vaccination). A BVDV-specific antibody ELISA was used to measure BVDV-specific antibodies. The BVDV vaccine response was defined as (1) final BVDV-specific antibody concentration (d 84) subtracted from initial BVDV-specific antibody concentration (d 0) and (2) booster BVDV-specific antibody concentration (d 34) subtracted from initial BVDV-specific antibody concentration. A regression model including effects of gender and age was applied to estimate the association between BVDV-specific antibody response to vaccination and birth and weaning weights. Gender and age was significantly correlated with weaning weight and birth weight ($P < 0.0001$). Higher final BVDV vaccine responses (d 84 – d 0) were significantly correlated with heavier birth weights ($P = 0.0176$). Neither final nor booster BVDV vaccine response was significantly correlated with weaning weight. Our results suggested that no unfavorable phenotypic correlation between BVDV-specific antibodies post-vaccination and weaning weight existed in cattle. However, a significant, unfavorable phenotypic correlation between BVDV vaccine response and birth weight was found.

Key Words: BVDV, vaccine response, beef cattle

TH14 The effect of feeding endophyte-infected fescue on the acute phase response to lipopolysaccharide in beef heifers. A. W.

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Angus heifers (n = 22; 292 ± 9.0 kg BW) were paired by BW and randomly placed on either an endophyte-infected (E+) or endophyte-free (E−) diet for 10 d to determine the influence of feeding endophyte-infected fescue on the physiological and acute phase responses of beef heifers to a lipopolysaccharide (LPS) challenge. Heifers were individually penned (thermoneutral; 3.0 × 3.7 m stalls) and fed at 1.8 × NEm. Diets contained 20% fescue seed, 30% cottonseed hulls, 36% cracked corn, 10% supplement, and 4% molasses, and were balanced to meet protein and mineral requirements. On d −16, heifers were fitted with vaginal temperature probes and on d −1 heifers were fitted with indwelling jugular cannulas. On the day of challenge, sickness behavior scores were recorded and blood samples were collected from heifers at 0.5 h intervals from −2 to 8 h, and again at 24 h relative to LPS administration (0.5 μg/kg BW at time 0 h). Serum was isolated and analyzed for cortisol, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) concentrations. Data were analyzed separately within pre- and post-challenge periods with the Mixed Procedure of SAS, using repeated measures in a completely randomized design. Within period, no treatment by time interactions were detected ($P > 0.10$). Cortisol, IFN-γ, TNF-α, and IL-6 concentrations increased for both groups from pre-LPS to post-LPS. Basal cortisol concentrations were unaffected ($P = 0.31$) by endophyte treatment pre-LPS, but were greater ($P = 0.01$) in E+ heifers during the post-LPS period (50.1 vs 55.0 ng/mL). TNF-α was greater in E+ heifers during both the pre- ($P < 0.01$; 6.2 vs. 6.0 pg/mL) and post-LPS periods ($P < 0.01$; 102.0 vs. 62.7 pg/mL). Neither IFN-γ nor IL-6 was affected by endophyte treatment during either period ($P \geq 0.13$). Sickness behavior scores were greater for E+ post-LPS ($P < 0.01$). Vaginal temperatures were greater for E- heifers pre-LPS ($P < 0.01$; 38.70 vs. 38.61°C) and greater for E+ heifers post-LPS ($P < 0.01$; 38.98 vs. 39.05°C). These data indicate that endophyte status affects the acute phase response when heifers are challenged with LPS.

Key Words: endophyte, cytokine, LPS

TH15 Oronasal administration of lipopolysaccharide and oral administration of lipopolysaccharide along with lipoteichoic acid enhanced salivary immunoglobulin A in periparturient dairy cows. S. Iqbal*[1], Q. Zebeli[1,2], D. A. Mansmann[1], S. M. Dunn[1], and B. N. Ametaj[1], *[1]University of Alberta, Edmonton, AB, Canada, [2]University of Veterinary Medicine, Vienna, Austria.*

The objective of this study was to evaluate whether repeated oronasal or oral administration of dairy cows to increasing doses of lipopolysaccharide (LPS) or a combination of LPS with lipotechoic acid (LTA) prepartum, respectively, would improve their mucosal immune responses. Two experiments were conducted. In experiment 1, 100 dairy cows were treated oronasally with increasing doses (0.01, 0.05, and 0.1 μg/kg BW) of LPS from *Escherichia coli* 0111:B4 dissolved in saline solution (TRT), or with saline solution alone (CTR). In experiment 2, 30 dairy cows were treated orally either with saline solution alone (CTR), or saline solution containing 3 increasing doses of LPS from *E. coli* 0111:B4 (0.01, 0.05, and 0.1 μg/kg BW) along with a flat dose of LTA from *Bacillus subtilis* (i.e., 120 μg/animal; TRT). Both treatments were applied for 3 consecutive weeks started at d −28, and then applied on d −25, −21, −18, and −14 prepartum. Saliva samples were collected on d −28, −7, +7, and +28 around parturition and analyzed for total IgA antibodies. Data was analyzed by the mixed procedure of SAS. Overall results indicated that TRT group in experiment 1 had greater concentrations of salivary total IgA antibodies ($P < 0.01$), with higher values started at d −7, and peak levels attained at d +7 compared with CTR group. Cows in experiment 1 had no treatment by wk interaction ($P > 0.05$); however measurement wk alone showed an influence for salivary IgA antibodies ($P < 0.01$). Interestingly, salivary total IgA antibodies were 2-fold greater in TRT cows in experiment 2 ($P < 0.01$), with higher values starting from d −7 until d +28 compared with CTR group. Cows in experiment 2 also showed an effect of measurement wk, as well as a treatment by wk interaction for salivary IgA antibodies ($P < 0.05$). In conclusion, periparturient dairy cows treated with repeated oronasal LPS alone or oral LPS along with LTA before calving enhanced concentrations of saliva total IgA antibodies against the 2 main bacterial endotoxins.

Key Words: lipopolysaccharide, lipoteichoic acid, saliva immunoglobulin A

TH16 Repeated oral exposure to lipopolysaccharide and lipoteichoic acid prepartum decreased uterine horn fluctuation and the incidence of abnormal discharges in postparturient dairy cows. S. Iqbal*[1], Q. Zebeli[1,2], D. A. Mansmann[1], S. M. Dunn[1], and B. N. Ametaj[1], *[1]University of Alberta, Edmonton, AB, Canada, [2]University of Veterinary Medicine, Vienna, Austria.*

The postpartum uterus of the transition dairy cow is highly susceptible to infection with numerous bacterial strains. The aim of this study was to evaluate whether repeated oral administration of lipopolysaccharide (LPS) along with a flat dose of lipoteichoic acid (LTA) to dairy cows would affect uterine horn fluctuation and the incidence of abnormal discharges. Thirty pregnant Holstein cows received orally either 2 mL of saline solution alone (CTR), or saline solution containing 3 increasing doses of LPS from *E. coli* 0111:B4 (0.01, 0.05, and 0.1 μg/kg BW) along with a flat dose of LTA from *Bacillus subtilis* (i.e., 120 μg/animal; TRT) on d −28 −25, −21, −18, and −14 before the expected day of parturition. Cows were monitored for rectal temperature starting 2 wk before until 2 wk after the expected day of parturition. Examination of the reproductive tract of each cow was conducted via rectal palpation on a weekly basis until +28 d postpartum. Data was analyzed by categorical modeling procedure of SAS. Results indicated that uterine horn fluctuations were present in CTR cows in a greater number of cases (8 vs. 1) compared with the TRT cows ($P < 0.01$). The symmetry of the uterine horns was not uniform in 4 cows in the CTR group vs. 1 cow in the TRT group; although data was not significant ($P > 0.05$). The number of cows with uterine discharges also tended to be greater in the CTR cows (6 vs. 1) compared with the TRT group ($P = 0.08$); although both groups had only 1 cow with bad odor of uterine discharge ($P > 0.05$). No difference in the cervix size and rectal temperature was evidenced between the 2 treated groups ($P > 0.05$). In a companion abstract we are reporting greater total salivary IgA antibody in cows treated with LPS and LTA. In conclusion, cows administered orally with LPS and LTA showed lower cases with uterine horn fluctuations and uterine discharges suggesting that the treatment might confer some protection against bacterial infections. More research is warranted to prove these preliminary findings.

Key Words: lipopolysaccharide, lipoteichoic acid, uterine horn fluctuation

TH17 Environmental heat stress modulates thyroid status and its response to repeated endotoxin (LPS) challenge in steers. S.

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Thyroid hormones are important in the adaptation to heat stress, allowing the adjustment of metabolic rates in favor of decreased energy utilization and heat production. Thyroid status is compromised in a variety of acute and chronic infections and toxin-mediated disease states. Our objective was to evaluate in cattle, the activity of pituitary-thyroid axis during adaptation to heat stress and the response of this thyroid status to immune stress modeled by LPS challenge. Ten steers (318 ± 49 kg BW) housed in climate chambers were subjected to either a thermoneutral (TN: constant 19°C) environment or heat stress (HS) conditions (cyclical daily temperatures: 32.2 to 40.0°C) for 9 d. To minimize confounding effects of altered plane of nutrition, TN were pair-fed to HS. On d 4 and 7, steers received a LPS challenge (LPS1 and LPS2; E. Coli 055:B5, 0.2 µg/kg BW, i.v.) with blood samples collected at 0, 1, 2, 4, 7, and 24 h relative to the start of each challenge. Plasma concentrations of thyrotropin (TSH), thyroxine (T_4), triiodothyronine (T_3), and reverse-triiodothyronine (rT_3) were measured by RIA. Before the start of LPS1, HS decreased ($P < 0.01$ vs. TN) plasma TSH (40%), T_4 (45.4%), and T_3 (25.9%), but did not affect rT_3 concentrations. In TN group, LPS1 challenge decreased ($P < 0.01$ vs. 0 h) plasma concentrations of TSH between 1 and 7 h and T_4 and T_3 at 7 and 24 h. In HS steers, LPS1 injection reduced plasma TSH at 2 h only ($P < 0.05$), decreased plasma T_3 at 7 and 24 h ($P < 0.01$) but did not affect already depressed plasma T_4. In all steers, LPS1 increased ($P < 0.01$) plasma rT_3 concentrations at 4, 7, and 24 h. The patterns of T_4, T_3, and rT_3 changes during LPS2 were similar to those in LPS1 with less evident response in plasma TSH after LPS2. The data are consistent with the concept that HS adaptation in cattle results in the depression of pituitary-thyroid axis with preserved normal extrathyroidal T_3 production. The data also suggest that LPS challenge suppresses both pituitary-thyroid axis and peripheral T_3 generation, responses that are more apparent in steers subjected to previous HS exposure.

Key Words: endotoxin, heat stress, thyroid hormone

TH18 Proinflammatory responses to repeated endotoxin (LPS) challenges are augmented in Brahman cattle compared to Angus cattle following the second LPS challenge. T. H. Elsasser*[1], C. Chase[2], and S. Kahl[1], *[1]USDA-ARS, Beltsville, MD, [2]USDA-ARS, Clay Center, NE.*

The ability to express a robust proinflammatory response (PIR) to a pathogen is essential in protecting against pathogen proliferation. However, failure to actively terminate PIR in due course can lead to excessive tissue damage from the overproduction of reactive compounds being generated in responding cells. *Bos taurus* and *Bos indicus* cattle are known to present different sensitivities to pathogen susceptibility. However, few data if any specifically address the characteristics of their respective host PIR after exposure. To determine whether *Bos taurus* and *Bos indicus* cattle differ in their PIR characteristics, a validated repeated LPS challenge procedure was performed with the respective biomarkers assessed specifically in regard to the degree to which the respective breeds mounted a tolerance response to the second LPS challenge. Healthy Brahman (BR) and Angus (AN) steers (n = 4/breed; 312 kg Av BW) were fed ad libitum and group housed by breed. Each animal was challenged twice with LPS (LPS-1, LPS-2; *E. coli* 055:B5, 0.2 µg/kg BW) with challenges separated by 3 d. Blood samples were obtained by venipuncture before and at 1, 2, 3, 4, 6 and 24 h following LPS. Concentrations of tumor necrosis factor-α (TNF), nitric oxide response ([nitrate + nitrite], NOx) and xanthine oxidase (XO) were measured in plasma. Rectal temperatures (RT) were recorded at the times of blood sampling. After LPS-1, TNF responses (AUC, area under the concentration × time curve) were greater in AN than BR but decreased only in AN after LPS-2 ($P < 0.05$). The total NOx response after both LPS challenges were significantly greater in BR than AN ($P < 0.05$) and augmented in BR after LPS-2. Mean XO concentrations were more than twice as high in BR then XO at any time before or after LPS ($P < 0.001$, breed), marginally increased after LPS-1 in both breeds, and only attenuated in response afterLPS-2 in AN. Increases in RT did not abate in BR following the LPS-2 ($P < 0.03$ vs. AN). The data indicate that fundamental differences in tolerance development in PIR were apparent between BR and AN.

Key Words: proinflammatory response, breed, acute phase response

TH19 Effects of intrauterine infusion of endometritic cows with *E. coli* lipopolysaccharide on endometrial gene expression and reproductive performance. J. Moraes*[1], P. Silva[2], A. Scanavez[1], L. Mendonca[1], J. Silva[1], K. Galvao[3], and R. Chebel[1], *[1]Department of Veterinary Population Medicine, University of Minnesota, St Paul, [2]Department of Animal Science, University of Minnesota, St Paul, [3]Department of Large Animal Clinical Sciences, University of Florida, Gainesville.*

Objectives were to evaluate the effects of intrauterine infusion of endometritic cows with *E. coli* lipopolysaccharide (LPS) on endometrial gene expression, cortisol and haptoglobin concentration, and reproductive performance. Cows (n = 2931) were examined for clinical endometritis at 31 ± 3 d postpartum using the Metricheck. Endometritic cows (n = 267) were assigned to receive intrauterine infusion of 20 mL of phosphate-buffered saline with 0µg (NC, n = 87), 150µg (150LPS, n = 91), or 300µg (300LPS, n = 89) of *E. coli* LPS. Healthy cows (CON = 2664) were used as positive control. A sub-sample of cows (NC, n = 16; 150LPS, n = 17; 300LPS, n = 17) had uterus biopsied 6h after infusion and another sub-sample of cows (NC, n = 18; 150LPS, n = 17; 300LPS, n = 14) had uterus biopsied 24h after infusion. Blood from all endometritic cows was collected before treatment (0h) and 24, 48 and 168h after treatment for determination of haptoglobin concentration. A sub-sample of endometritic cows (NC, n = 43; 150LPS, n = 45; 300LPS, n = 42) had blood sampled at 0, 2, 4, 6 and 24 h for determination of cortisol concentration. Binary data were analyzed by SAS PROC LOGISTIC, rate at which cows became pregnant was analyzed by Cox proportional hazard ratio using SAS PROC PHREG, and fold-change in expression of endometrial genes was analyzed by a non-parametric method (Kruskal-Wallis). Treatment affected ($P < 0.01$) the rate at which cows became pregnant. Pregnancy rate of CON cows was greater than that of NC cows [AHR (95% CI) = 0.59 (0.41, 0.84); $P < 0.01$] and pregnancy rate of CON tended to be greater than that of 300LPS cows [AHR (95% CI) = 0.75 (0.54, 1.05); $P = 0.09$]. The rate at which CON and 150LPS cows became pregnant was not different ($P = 0.15$). Interestingly, the expressions of mRNA for IL1-beta, IL-6, IL-8, IL-10, TLR-4, TNF-alpha at 6 h and 24 h after treatment were not ($P > 0.15$) affected by treatment. Treatment with 150 µg of LPS improved reproductive performance, but had no effect on endometrial gene expression.

Key Words: lipopolysaccharide, uterus, endometritis

TH20 Phagocytic activities of leukocytes, monocytes and neutrophils of dairy cows fed with n-3 and n-6 fatty acids sources in the transition period and early lactation. L. C. Verdurico*, J. R.

Gandra, R. D. Mingoti, R. V. Barletta, T. S. Canaes, L. Oliveira, G. D. Calomeni, R. Gardinal, C. S. Takyia, T. H. Vendramini, and F. P. Renno, *Universidade de Sao Paulo, Universidade de Sao Paulo, Pirassununga, Sao Paulo, Brazil.*

The aim of this study was to evaluate effect of omega 3 and omega 6 supplementation, on expression of adhesion molecules of Holstein cows during transition period and early lactation. Forty-eight Holstein cows were divided in 4 experimental groups in randomized design. Animals were assigned to receive one of 4 treatments: (1) control (C; n = 12), without fat sources in pre and postpartum; (2) flaxseed (FS; n = 12), fed 60 and 80 g/kg of DM of flaxseed in pre and postpartum; (3) whole raw soybeans (WS; n = 12), fed 120 and 160 g/kg of DM of whole raw soybeans in pre and postpartum; (4) calcium salts of unsaturated fatty acids (CSFA; n = 12; Megalac-E), fed 24 and 32 g/kg of DM of calcium salts of unsaturated fatty acids in pre and postpartum. Experimental diets were fed from 35 d before the estimate calving until 84 d of lactation, formulated to meet nutritional requirements of each period. Blood samples were taken −21, −14, −7 d in relation to prediction of birth, at birth and +7, +14, +21, +42, +84 d postpartum. Data were analyzed using the PROC MIXED of SAS 9.1 with fixed dietary effect, time effect, interaction between diet and time. Data were analyzed by orthogonal contrasts C vs. WS+CSFA+FS (C1); FS vs. WS+CSFA (C2); and WS vs. CSFA (C3). We measured phagocytic activities of leukocytes, monocytes and neutrophils of dairy cows. There was a higher percentage of leukocytes and monocytes positive for phagocytosis for diets FS, WS, and CSFA compared with the C diet, (36.0; 33.1; 30.2 vs. 21.3), (37.2; 31.4; 29.5 vs. 32.6) respectively for leukocytes in pre and postpartum; and (64.6; 61.4; 48.2 vs. 32.7), (51.2; 49.1; 47.6 vs. 41.2) respectively for monocytes in pre and postpartum. For neutrophils in pre and postpartum and postpartum monocytes was observed higher percentage of positive cells for FS diet compared with diets CSFA and WS. The inclusion of sources of fatty acids n-3 and n-6 in the diet of dairy cows improved the phagocytic activity in the transition period.

Key Words: dairy cow, fat source, immune function

TH21 Effect of IgG binding on expression of Fc receptors and SYK activation in bovine neutrophils. J. Williams*, M. Worku, A. Alston, R. Noble, and T. Hanner, *North Carolina Agricultural & Technical State University, Greensboro.*

In cattle the immunoglobulin G (IgG) binding FcR (FcγRI, -II, and -III) plays an important role in clearance of mastitis causing bacteria. Activating (CD32a) and inhibitory (CD32b) isoforms of Fcγ receptors, FcγRII (CD32) have been identified. Variation in FcRs may impact gene expression and downstream intracellular signaling. The objective of this study was to evaluate the effect of IgG binding on transcription of FcRI, FcRII and its sub isoforms and to evaluate changes in endogenous levels of phosphorylated Syk kinase. Blood was collected from 6 lactating cows (3 Holstein Friesian and 3 Holstein × Jersey). Isolated blood neutrophils were incubated with IgG or maintained in PBS. Total RNA was then isolated and cell lysates prepared. Real time PCR was used to evaluate changes in the expression profile of FcRI, FcRII and FcRII sub-isoforms using GAPDH as a reference control. Amplified products were separated on a 1% agarose gel and observed following staining with ethidium bromide. All genes were transcribed. Variable gene expression was observed. A significant (80-fold) increase in FcRII transcript was observed in Holstein × Jersey as compared with Holstein Friesian breeds. Binding of IgG to neutrophils for Holstein × Jersey cows significantly increased expression of SYK ($P < 0.007$). Following the binding of IgG to neutrophil variation in the transcription of immunoreceptors and activation of intracellular signal mediator SYK may play a role in the resolution of infection. Further characterization using a larger sample size is needed.

Key Words: Fc receptor, neutrophil, transcription

TH22 Modulation of the intestinal immune response of calves by *Bacillus cereus* var. *toyoi* (Toyocerin). A. Aris*[1], F. Fabregas[1], S. Pares[1], M. Terre[1], M. Castillo[3], and A. Bach[1,2], *[1]IRTA, Caldes de Montbui, Spain, [2]ICREA, Barcelona, Spain, [3]Rubinum SA, Rubi, Spain.*

It has been previously shown that *Bacillus cereus* var. *toyoi* (Toyocerin) improves the humoral response to vaccination in calves. The objective of this study was to further evaluate the effects of Toyocerin on the immune system response of calves. Twenty-four Holstein calves (75.2 ± 1.87 kg of BW and 77 ± 0.7 d of age), were randomly distributed according to BW in 2 groups: 12 calves were assigned to a conventional concentrate feed (CTR), and the other 12 calves were fed the same concentrate feed supplemented with 2×10^8 cfu *Bacillus cereus* var. *toyoi*/kg of concentrate (TOY). Animals were euthanized at 60–67 d of the study. Immediately after sacrifice, calves were abdomen-opened, and the whole gastrointestinal tract was removed. Jejune fragments were sampled to quantify cytokine and enzyme expression by qPCR and intestinal contents were processed to quantify production of IgA by ELISA. Data were analyzed by ANOVA using the treatment as the main effect. The TOY calves presented a greater ($P < 0.05$) amount of secretory IgA in jejune content than CTR animals (6434 ± 478 mg/mL vs. 4673 ± 499 mg/mL, respectively); whereas no differences in IgA were observed at cecum level. Calves in the TOY group showed a clear increase ($P < 0.05$) in the expression level of Th1 cytokines such as IFN-γ ($3.49 \times 10^{-3} \pm 0.519 \times 10^{-3}$) compared with calves in the CTR group ($1.34 \times 10^{-3} \pm 0.519 \times 10^{-3}$), which would explain an improvement in cellular activity. Accordingly, gene expression of nitric oxide synthase increased ($P < 0.05$) and that of myeloperoxidase tended ($P < 0.1$) to increase in TOY ($3.21 \times 10^{-3} \pm 0.398 \times 10^{-3}$ and $6.53 \times 10^{-4} \pm 0{,}68 \times 10^{-4}$, respectively) compared with CTR calves ($2.12 \times 10^{-3} \pm 0.382 \times 10^{-3}$ and $3.06 \times 10^{-4} \pm 0.65 \times 10^{-4}$,respectively). In conclusion, these results confirm the positive effects of Toyocerin in enhancing the humoral response of calves, expanding its modulatory effects at both the cellular and humoral level of intestinal immune response.

Key Words: immunity, inflammation, gut

TH23 Decomposing between-cow and within-cow variation in hematology and leukocyte responses in dairy cows during the periparturient period. M. D. Sellers*, C. R. Nightingale, A. R. Pepper-Yowell, T. L. Harris, and M. A. Ballou, *Department of Animal and Food Sciences, Texas Tech University, Lubbock.*

Previous research has shown that most variation in immune variables following calving is residual, within-cohort variation. The objective of the current study was to decompose total variation in hematology and leukocyte responses into time, between-cow, and residual (within-cow) variation. Twenty-five Holstein cows were sampled at −60 (end of previous lactation), −30, 0, 15, and 30 d relative to parturition for hematology and various leukocyte responses. A linear model was fitted with the effects of cow and time, with within-cow variation being a major source of residual variation. Percentage of variation attributable to a given effect was estimated by dividing the sums of squares attributable to any effect by the total sums of squares and is reported as η^2. Bootstrapped resampling was used to estimate confidence intervals around η^2 estimates. Time effects were observed for all variables except TNF ($P = 0.148$) and lymphocyte count ($P = 0.704$). Between-cow effects

were observed for percentage of neutrophils positive for and intensity of oxidative burst response ($P < 0.007$ and $P < 0.0142$, respectively.), and total leukocyte count ($P < 0.003$). Across all variables, within-cow variation was higher than variation between cows or due to time (Table 1). Variation due to time was highest in plasma haptoglobin concentration ($\eta^2 = 0.345$), which elevated following parturition (Table). High within-cow variation may indicate that cows with high or low values at certain time points likely do not maintain high or low values for the duration of the peripartum period.

Table 1.

	η^2 - Time		η^2 - Cow		η^2 - Residual	
	Estimate	Interval	Estimate	Interval	Estimate	Interval
Oxidative burst intensity	0.181	[0.128–0.285]	0.271	[0.191–0.370]	0.548	[0.434–0.621]
L-Selectin expression	0.246	[0.102–0.505]	0.169	[0.089–0.229]	0.585	[0.363–0.713]
Plasma haptoglobin	0.345	[0.203–0.546]	0.083	[0.047–0.122]	0.572	[0.398–0.687]
Tumor necrosis factor-α	0.045	[0.018–0.159]	0.274	[0.161–0.373]	0.681	[0.558–0.758]
Total leukocytes	0.208	[0.146–0.322]	0.283	[0.191–0.366]	0.509	[0.395–0.600]
Neutrophil count	0.247	[0.160–0.387]	0.186	[0.079–0.283]	0.567	[0.421–0.705]

Key Words: peripartum, immune, variation

TH24 Leukocyte responses immediately following calving are not predictive of first test day milk yield or somatic cell count in multiparous Holstein cows. M. D. Sellers, C. R. Nightingale, R. Y. Liang*, T. L. Harris, A. R. Pepper-Yowell, B. S. Obeidat, and M. A. Ballou, *Department of Animal and Food Sciences, Texas Tech University, Lubbock.*

The objective of the current study was to determine if hematology or leukocyte responses immediately following calving were predictive of first test day milk yield or somatic cell count (SCC). Twenty-four multiparous Holstein cows within 72 h post-calving were sampled for measurement of hematology and many leukocyte responses, which included: neutrophil expression of the adhesion protein L-selectin, neutrophil oxidative burst capacity against an *Escherichia coli*, plasma concentrations of the acute phase protein haptoglobin, and whole blood secretion of tumor necrosis factor-α and interferon-γ when stimulated with lipopolysaccharide and phytohemagglutinin-P, respectively. At 29 ± 5 d in milk, milk was collected and analyzed for yield and somatic cell count. First test day milk (mean ± SD) was 47.4 ± 6.72 kg and SCC was 2.6 ± 1.69 Log_2(SCC/100,000). Regression analysis with stepwise backward elimination was utilized to estimate the best-fitting predictive model based on the lowest AIC. The best-fitting predictive model for first test day milk production included the dependent variables: percentage of neutrophils positive for producing an oxidative burst as well as tumor necrosis factor-α and interferon-γ secretion from whole blood cultures, but there were no nonzero coefficients ($P > 0.077$). The multiple R^2 for the model was 0.370 ± 0.181. The best-fitting model for first test day SCC included the dependent variables: percentage of neutrophils positive for producing an oxidative burst, mean fluorescence intensity of oxidative-burst positive neutrophils, and tumor necrosis factor-α and interferon- γ secretion from whole blood cultures. There was a tendency for secretion of interferon-γ secretion to have a nonzero slope ($P = 0.058$). The multiple R^2 for the model was 0.278 ± 0.190. These data suggest that measures of hematology or leukocyte responses obtained immediately following calving are not largely predictive of first test day milk yield or somatic cell count.

Key Words: immune, performance, peripartum

TH25 Differential effects of stimulation on ruminant neutrophils. K. Gyenai* and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro.*

Plants and their extracts have traditionally been used to improve animal health and welfare. Research is needed to determine the effectiveness and toxicity of traditionally used plants and other natural substances. In this study, we investigated the effects of *Moringa oleifera* leaf powder, bacterial LPS and peptidoglycan (PGN) on ovine, caprine and bovine neutrophils. Six animals each, treatment (n = 3) and control (n = 3) from the 3 different species were used. Isolated neutrophils were treated and incubated at 37°C, with 85% humidity and 5% CO_2 for 15 min with 100 μg PGN, 100 μg LPS or 100 μg of *Moringa* either individually or in combinations and PBS as control. Pro-inflammatory cytokine levels were evaluated in supernatants from treated neutrophils using commercial assays. Real time PCR using 84 different innate and adaptive immune markers was conducted using arrays. In ovine *Moringa*-treated samples, interferon gamma-γ and granulocyte-colony stimulating factor were (300 and 600% fold) increased compared with other cytokines and treatment respectively, ($P < 0.05$). Bovine neutrophil supernatants cytokine levels were significantly (1 to 3 fold) increased in tumor necrosis factor-α for LP, PGN and LPS in combination; PGN and *Moringa* in combination. However, in caprine neutrophils a 10 to 600% fold reduction was observed for all cytokines when compared with the other 2 species. Real-time analysis of innate and adaptive immune markers showed differential responses to each treatment. *Moringa*-treated ovine neutrophils had the highest expression profile with 16 genes up and 25 genes downregulated respectively. Treatment with Lps resulted to the least expression in all species, with ovine having smallest expression 3 genes down and 3 upregulated. Differential effects of stimulation were observed on ruminant neutrophils. Species-dependent effects may affect the use of plants such as *Moringa* and their extracts for animal health.

Key Words: *Moringa*, neutrophil, ovine

TH26 Evaluation of TLR2 surface expression on blood mononuclear cells (BMC) in high immune response (HIR) biased cows. L. Wagter-Lesperance*, M. Paibomesai, R. Opsteen, and B. Mallard, *University of Guelph, Guelph, ON, Canada.*

Dairy cattle with high immune response (HIR) following immunization with specified test antigens have been shown to be at a lower risk for developing disease compared with average and low responding animals. The host relies on both the first-line innate response and long-lasting adaptive immune response to effectively recognize and eliminate pathogens. Innate mechanisms of host defense are important in the initiation of adaptive immune responses and consist of a variety of cells and proteins. Cells express pattern recognition receptors (PRRs) such as toll-like receptor (TLR) that recognize pathogen-associated molecular patterns (PAMPs). Toll-like receptors recognize several PAMPs from both intracellular and extracellular pathogens. Blood mononuclear cells (BMC) from 8 cows were evaluated to identify differences in the expression of TLR2, in addition to CD3, CD5, CD14, and Anti-B cell receptor using flow cytometric analysis. Four of the 8 cows were previously classified as high for cell-mediated immune response (CMIR) and low for antibody-mediated immune response (AMIR) and the other 4 cows were classified as high for AMIR and low for CMIR. Descriptive

statistics and a Student's *t*-test of TLR2+ expression between HIR-biased groups was performed. Data will be analyzed using GLM SAS with significance at $P < 0.05$. Results (reported as the mean percent ± standard error) for all 8 cows were 19.85% ± 2.25 for TLR2+, 16.03% ± 1.37 for TLR2+CD14+, 18.69% ± 1.12 for TLR2+AntiBcell+, 9.015% ± 1.15 for TLR2+CD3+, and 3.065 ± 0.71 for TLR2+CD5+. Cows with a high AMIR bias (H-AMIR), had higher percentage of BMC expressing TLR2, in contrast to cows with a high CMIR bias (H-CMIR). H-AMIR bias cows tended to have more double stained cells in contrast to H-CMIR cows, but this was not significant. The sample size for this study was small, and so the evaluation of more cows may more clearly identify differences in the expression of TLR2 on BMC and specific subpopulations among cattle classified with high or low adaptive immune response.

Key Words: HIR, innate, toll-like receptor

TH27 Effects of an immunomodulatory dietary supplement on the global gene expression profile of neutrophils from periparturient dairy cows. X. S. Revelo[1], J. W. Davis[2], R. D. Schnabel[2], A. L. Kenny[2], N. M. Barkley[2], and M. R. Waldron*[2], *[1]University Health Network, Toronto, ON, Canada, [2]University of Missouri, Columbia.*

The importance of dietary strategies to improve the immune competency of periparturient dairy cows is well-recognized. We investigated the effects of a dietary supplement containing B-vitamins, dehydrated yeast, and *Trichoderma longibrachiatum* fermentation products on the global gene expression profile of neutrophils (PMNL). Cows received 56 g/day of OmniGen-AF (n = 5) or sham control (n = 5) mixed into total mixed rations from d 46 ± 1 prepartum until d 31 postpartum. Blood PMNL were harvested on d 7 postpartum and incubated with 0 or 50 μg/mL of *Escherichia coli* lipopolysaccharide (LPS) for 120 min. Poly-A enriched mRNA from the PMNL was converted into library templates, sequenced and mapped to the bovine reference genome. Digital count data was then analyzed to determine the effects of dietary supplementation and LPS incubation on PMNL gene expression. Feeding OmniGen-AF altered mRNA contents of 43 genes (7↑ and 36↓; $P < 0.05$, 20% FDR) in LPS-activated PMNL. Functional annotation analysis indicated that the genes with lower expression enriched the lysosome pathway. Independent from LPS activation, OmniGen-AF altered the expression of 53 transcripts (12↑ and 41↓; $P < 0.05$, 20% FDR) relative to non-supplemented controls. Genes with lower expression due to OmniGen-AF enriched the oxidative phosphorylation pathway. Gene ontology terms for this pathway included oxidation-reduction, electron transport chain, mitochondrial electron transport, ATP synthesis coupled electron transport, and cellular respiration. These results suggest potential mechanisms by which OmniGen-AF may influence the activity of PMNL in periparturient dairy cows. The effect of LPS incubation on PMNL gene expression was also tested independent of dietary supplementation. Regardless of dietary treatment, 50 μg/mL of LPS altered the mRNA contents of 333 genes (211↑ and 122↓; $P < 0.05$, 20% FDR), relative to 0 μg/mL. Genes with increased expression enriched the TOLL-like receptor signaling and hematopoietic cell lineage pathways. These results highlight molecular mechanisms involved in the PMNL response to LPS.

Key Words: neutrophil, gene expression, OmniGen-AF

TH28 Effects of recombinant bovine somatotropin (rbST) treatment during the peripartum period on innate immune responses and hemogram parameters. P. Silva*[1], J. Moraes[2], A. Dresch[2], K. Machado[2], and R. Chebel[2], *[1]Department of Animal Science, University of Minnesota, St Paul, [2]Department of Veterinary Population Medicine, University of Minnesota, St Paul.*

Objectives were to evaluate the effect of peripartum treatment with rbST on innate immune responses and hemogram parameters of Holstein cows. Holstein cows (256 ± 3 d of gestation and body condition score >3.5) were enrolled in this study and randomly assigned to one of 3: CON-0 mg/d rbST (n = 21), 35%-12.5 mg/d rbST (n = 21), or 50%-17.9 mg/d rbST (n = 23). Cows assigned to the 35% and 50% were treated every 7 d. Cows received a body condition score and were weighed at −28, −14, and 0 d prepartum. Blood was sampled weekly from 14 d pre to 21 d postpartum. Polymorphonuclear leukocyte (PMNL) phagocytosis (PHAGO) and oxidative burst (OB) was determined by flow cytometry after an ex vivo challenge with *E. coli*. Furthermore, expression of CD18 and L-selectin were determined by flow cytometry and complete blood count was performed. Cows were examined daily for metritis daily until 14 d postpartum. Dichotomous data were analyzed by logistic regression using the PROC GLIMMIX and continuous data were analyzed by ANOVA using the PROC MIXED procedure. Body condition score ($P = 0.42$) and weight ($P = 0.85$) were not affected by treatment. Incidence of metritis tended ($P = 0.06$) to be different among treatments (CON = 14.3, 35% = 14.3, 50% = 4.4%). There was no effect of treatment on percentage of PMNL PHAGO+ ($P = 0.60$), OB+ ($P = 0.15$), and PHAGO+OB+ ($P = 0.60$). Treatment, however, tended to increase PHAGO intensity of PHAGO+ PMNL (CON = 3,159.3 ± 129.6 GMFI; 35% = 3,505.6 ± 136.6; 50% = 3,559.4 ± 134.5; $P = 0.07$) and affected OB intensity of OB+ PMNL (CON = 5,321.4 ± 489.1; 35% = 6,712.3 ± 515.6; 50% = 7,131.3 ± 507.8; $P = 0.03$). Similarly, among PHAGO+OB+ PMNL, treatment tended to affect PHAGO intensity (CON = 3119.4 ± 132.8; 35% = 3465.1 ± 140.0, 50% = 3536.8 ± 137.8, $P = 0.07$) and affected OB intensity (CON = 9991.7 ± 776.5, 35% = 12216 ± 819.3, 50% = 12581 ± 806.0; $P = 0.05$). Treatment did not affect percentage of PMNL CD18+ ($P = 0.34$) and L-selectin+ ($P = 0.26$) and intensity of CD18 ($P = 0.80$) and L-selectin ($P = 0.33$) expression. Treatment did not affect granulocyte:lymphocyte ratio ($P = 0.91$). Treatment of cows with rbST resulted in improved PMNL activity that led to reduced incidence of metritis.

Key Words: bovine somatotropin, prepartum cow

TH29 Immune status of dairy calves in the Northern Plains of Costa Rica: Year 2. J. A. Elizondo-Salazar*[1], D. Benavides-Varela[1], and A. J. Heinrichs[2], *[1]Estacion Experimental Alfredo Volio Mata, Facultad de Ciencias Agroalimentarias, Universidad de Costa Rica, San Rafael, Costa Rica. [2]The Pennsylvania State University, University Park.*

The objective of this study was to characterize for the second consecutive year the immune status of dairy calves in the Northern Plains of Costa Rica. The data correspond to total serum protein measurements (TSP) obtained during the period of August and November 2011 in 33 dairy farms. 671 female and 47 male calves were sampled. Dam breeds were classified into Holstein, Jersey, Holstein × Jersey crosses and other. For the purpose of this study, failure of passive immunity was considered when TSP concentration was less than 5.5 g/dL. TSP concentration ranged from 2.0 to 10.0, with an overall mean of 5.7 g/dL. Of all the animals evaluated, 43.7% had failure of passive transfer. When sex of the calves was considered, 44.1% of females and 38.3% of males failed to obtain adequate levels of immunity and concentration of TSP showed no significant differences (6.0 vs. 6.1 g/dL, respectively). Calves born to Holstein × Jersey crosses had significantly higher TSP concentrations than calves born to other breeds. When considering calving of the dam, offspring born to first-time heifers had TSP concentrations of 6.3 g/dL and showed the lowest percentage of animals with inadequate transfer of immunity. The findings of this study suggest that colostrum

management practices should be placed to minimize the risk of failure of passive transfer in dairy herds in the Northern Plains of Costa Rica.

Key Words: total serum protein, immunoglobulin, passive immunity

TH30 Effect of disease in one lactation on the incidence of disease in the subsequent lactation in dairy cows. A. Vieira-Neto*[2], C. A. Risco[1], J. E. Santos[1], and K. N. Galvão[1], *[1]University of Florida, Gainesville, [2]Universidade do Estado de Santa Catarina, Lages, SC, Brazil.*

Objective was to evaluate if having disease in one lactation (Lac1) would affect the risk of developing disease in the subsequent lactation (Lac2). Five hundred forty-four lactation pairs were created from 1088 lactations from 303 Holstein cows from a 500-cow herd from 2006 to 2012. Data on calving related problems (abortion, dystocia, stillbirth, twins, retained placenta), metabolic diseases [clinical hypocalcemia, ketosis, displaced abomasum (DA)], metritis, and mastitis were collected from the farm management software. Data was evaluated using chi-squared when no other variables could logically affect the outcome or using the LOGISTIC procedure of SAS when more than one variable could affect the outcome. Having calving related problems in Lac1 increased the likelihood of having it in Lac2 (29.3 vs. 20.7%; $P = 0.04$). Having clinical hypocalcemia in Lac1 increased the likelihood of having it in Lac2 (45.5 vs. 3.7%; $P < 0.001$). Having calving related problems in Lac2 also increased the likelihood of having clinical hypocalcemia in Lac2 (10.5 vs. 2.9%; $P < 0.001$). Having ketosis in Lac1 increased the likelihood of having ketosis in Lac2 (52.5 vs. 34.6; $P < 0.001$). Having calving related problems in Lac2 also increased the likelihood of having ketosis in Lac2 (46.8 vs. 36.2; $P = 0.04$). Having metritis in Lac1 increased the likelihood of having it in Lac2 (26.3 vs. 18.4%; $P = 0.02$). Other factors affecting the incidence of metritis in Lac2 were induced parturition (42.1 vs. 18.8%; $P = 0.005$), retained placenta (85.0 vs. 17.9%; $P < 0.001$), twins (76.9 vs. 19.0%; $P < 0.01$), stillbirth (46.2 vs. 19.1%; $P = 0.01$), clinical hypocalcemia (44.0 vs. 19.3%; $P = 0.01$), and ketosis (30.5 vs. 14.1%; $P < 0.001$). Having DA in Lac1 increased the likelihood of having it in Lac2 (23.5 vs. 3.8%; $P = 0.01$). Ketosis in Lac2 also increased the likelihood of having DA in Lac2 (11.0 vs. 0.3%; $P < 0.001$). Mastitis in Lac1 did not affect the likelihood of having it in Lac2. In conclusion, with the exception of mastitis, disease in Lac1 affected the incidence of disease in Lac2.

Key Words: disease incidence, disease recurrence, dairy cow

TH31 Cortisol, interleukin 8, and immunoglobulin G ratios predict treatment for bovine respiratory disease in feedlot cattle. S. E. Speidel[1], R. R. Cockrum*[1], J. L. Salak-Johnson[2], C. C. L. Chase[3], M. G. Thomas[1], K. G. Prayaga[6], R. K. Peel[1], R. L. Weaber[4], H. Van Campen[1], G. H. Loneragan[5], J. J. Wagner[1], and R. M. Enns[1], *[1]Colorado State University, Fort Collins, [2]University of Illinois, Urbana, [3]South Dakota State University, Brookings,, [4]Kansas State University, Manhattan, [5]Texas Tech University, Lubbock, [6]Zoetis, Kalamazoo, MI.*

Bovine respiratory disease (BRD) is one of the most harmful illnesses affecting the beef industry in North America. Factors such as antibodies, chemokines and glucocorticoids can be used to assess the immune status of individual animals. We hypothesized that these factors could be used as indicators of BRD in feedlot cattle. The objective of this study was to determine if these parameters were predictive of the incidence of BRD as determined by treatment of individual animals. Crossbred steers (n = 2869) group housed in a commercial feedlot in Southeastern Colorado were used in the evaluation. Jugular blood samples were collected upon receiving for immune response parameter analyses. Data included feedlot treatment records (TRT), cortisol, immunoglobulin G (IgG), immunoglobulin G1 (IgG1), immunoglobulin G2 (IgG2), IgG1 to IgG2 ratio (IgG1:IgG2), and interleukin 8 (IL8). Six fixed logistic regression models were used to determine relationships between immune response parameters and BRD treatment in R. Binomial observations were used for TRT (0 = no treatment and 1 = treatment for BRD). Predictor variables included the fixed effects of sire, receiving weight, a feedlot pen by ranch interaction, and each individual immune response parameter. Results indicated that unit increases in cortisol and IL8 increased the odds of being treated for BRD by 0.83% ($P = 0.079$) and 0.054% ($P = 0.039$), respectively. Alternately, a unit increase in IgG1:IgG2 decreased the odds of being treated for BRD 3.67% ($P = 0.038$). A weak trend was found with IgG2 showing an increased probability (0.79%, $P = 0.155$) of being treated for BRD. No relationships between treatment for BRD and IgG or IgG1 were detected. These results indicate a relationship exists between cortisol, IL8, IgG1:IgG2 and treatment for BRD.

Key Words: bovine respiratory disease, cattle, immunity

TH32 Citrus-derived oil (CDO) kills both *Staphylococcus aureus* and *Escherichia coli* in bovine MAC-T mammary epithelial cell lines in vitro. K. M. Moyes*, J. A. Almario, S. Salaheen, D. Hewes, and D. Biswas, *University of Maryland, College Park.*

Both *E. coli* and *S. aureus* are major mastitis-causing pathogens in dairy herds and are associated with reduced animal health and economic losses to farmers. Management strategies to prevent and control *E. coli* mastitis are lacking whereas cure rates using antibiotics for *S. aureus* mastitis vary considerably. However, antibiotic use is coming under increasing public scrutiny due to the possible development of resistant pathogens and risk of residues appearing in the milk. Therefore, new strategies to control or treat mastitis are warranted. Recent studies have shown that 0.1% and 0.2% CDO inhibited growth of both *S. aureus* and *E. coli* bacteria. Therefore, our objective was to determine the effect of CDO on the inhibition of *S. aureus* and *E. coli* in bovine MAC-T mammary epithelial cell lines in vitro as a potential new strategy to control bovine mastitis. MAC-T cells (n = 6 replicates/treatment) were seeded into cell culture plates and then inoculated with ~10^7 cfu/ml of either *E. coli* or *S. aureus*. After infection, cells were washed and treated with CDO (Control [0%], 0.025, 0.05, 0.1 or 0.2% CDO) for 1 h. MAC-T cells were subject to lysis after treatment and bacteria were enumerated. When compared with control, treatment with either 0.05% 0.1% and 0.2% CDO decreased association of *S. aureus* by 4 log whereas 0.025% CDO decreased association by 2 log in MAC-T cells. Results were similar for *E. coli* where treatment with 0.1% and 0.2% CDO decreased the number of cells associated *E. coli* by 4.5 log whereas 0.05%; and 0.025% decreased *E. coli* by 3 log and 1 log, respectively, compared with control. Our results clearly show that CDO kill mastitis-causing pathogens in bovine MAC-T mammary epithelial cell lines in vitro. The use of CDO may be a promising new therapeutic option for improving control and treatment of *E. coli* and *S. aureus* mastitis of dairy cows during lactation.

Key Words: citrus oil, MAC-T, mastitis

TH33 Evaluation of on-farm colostrum quality measurement tools. A. Bartier*, C. Windeyer, and L. Doepel, *University of Calgary, Calgary, AB, Canada.*

Newborn calves acquire immunity passively through consumption of colostral IgG. The colostrometer and Brix refractometer are both

available as on-farm tools to indirectly assess colostral IgG content. The objective of this study was to determine which of these tools is a more accurate predictor of colostral IgG content compared with that determined by RID. Fourteen commercial dairy farms in central Alberta, Canada, with herd sizes ranging from 60 to 300 lactating cows were used. 572 colostrum samples were collected by the producers between February and July, 2012. These samples were from the cows' first milking and represented the colostrum that was fed to the calves at their first feeding. Colostrum quality was measured with a colostrometer (specific gravity measurement) and a digital Brix refractometer (dissolved solids content). IgG content was determined using RID at a commercial laboratory. The minimum, maximum and mean ± SEM for the Brix refractometer, colostrometer, and RID were 6.8, 41.4, 25.5 ± 0.21° Brix; 0, 140, 82 ± 1.3 mg/mL; and 8.3, 128.6, 55.3 ± 1.1 mg/mL, respectively. Spearman correlation was used for the colostrometer data due to a non-normal distribution whereas Pearson correlation was used for the Brix refractometer. The colostrometer data were better correlated with RID results (r = 0.77, $P < 0.01$) than were the Brix refractometer data (r = 0.62, $P < 0.01$). The proportion of samples that each tool correctly identified as good quality (≥50 mg/mL IgG) or poor quality (<50 mg/mL) at different tool cut-off values were evaluated using chi-squared tables. Intervals of 10 mg/mL from 40 to 100 mg/mL for the colostrometer and 2° from 16 to 28° Brix were tested. The highest proportion of correctly identified good and poor quality samples occurred at 80 mg/mL for the colostrometer (84.1% sensitivity and 77.0% specificity, $P < 0.01$), and at 23° Brix (66.9% sensitivity and 82.4% specificity, $P < 0.01$). At these cut-offs the positive and negative predictive values were 60.6% and 92.0% for the colostrometer ($P < 0.01$), and 61.1% and 85.8% ($P < 0.01$) for the refractometer. This study indicates that both tools are adequate at indirectly determining colostrum IgG content.

Key Words: IgG, Brix, colostrometer

TH34 The Wnt/Frizzled pathway in bovine neutrophils. H. Ismael and M. Worku*, *North Carolina Agricultural and Technical State University, Greensboro.*

The Wnt proteins are secreted members of the wingless family of signaling molecules. These proteins bind to 2 receptors on the cell surface, single transmembrane low density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) and 7 transmembrane Frizzled receptors. There is accumulating evidence that the Wnt/Frizzled pathway may play a distinct role in inflammation. Expression of these genes in bovine neutrophils may play a role in mastitis related losses. The objectives of this study were to determine expression of members of Wnt signaling pathway in bovine neutrophils for long-term definition of their role in mastitis. Blood was collected from 3 Holstein Friesian cows. Neutrophils were isolated by differential centrifugation and hypotonic lysis of red blood cells. RNA was isolated using Tri-reagent. RNA samples were reverse-transcribed and the cDNA was obtained using the Ambion-Retroscript as per the manufacturer's protocol. Specific primers for Wnt1, Frizzled, secreted frizzeled and LRP were used for PCR amplification. Amplified products were run on a 1% agarose gel with PCR markers. Gels were stained with ethidium bromide and visualized using a Gel documentation system. GAPDH was used as loading control and primers in the absence of DNA were used as negative controls. Genes encoding Wnt(100–800bp), Frizzled (600bp) and LRP (400bp) were expressed in neutrophils from 3 cows. A larger sample size would allow statistical evaluation and significance in combating mastitis causing pathogens.

Key Words: gene, neutrophil, wingless

TH35 Genome-wide association of white blood cell types during vaccination. R. J. Leach*, C. G. Chitko-McKown, G. L. Bennet, S. A. Jones, J. W. Keele, W. M. Snelling, R. M. Thallman, and L. A. Kuehn, *U.S. Meat Animal Research Center, Clay Center, NE.*

Infectious disease of livestock continues to be a cause of substantial economic loss and adverse welfare. Breeding for disease resistant livestock could improve both the economic burden and animal welfare. We aim to identify key genes and pathways that control variation in immune response; knowledge that may aid both breeders and vaccinologists. The herd used during this investigation (n = 3500) were the product of multiple-sire matings of crossbred cows to F1 bulls of varying half-blood composition. The resulting animals consisted of variable fractions of 9 breeds: Angus, Hereford, Red Angus, Brahman, Charolais, Gelbvieh, Limousin, Simmental, and MARCIII composite (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgauer). Each animal was vaccinated for the 5 major viral causes of BRD: BRSV, PI3, BVDVI/II and IBR. Multiple immune related phenotypes (counts of: white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils) were measured pre and post booster vaccination (a delta measurement was also calculated [post-pre]). Every animal in the herd was also genotyped with 50K SNP, with founding sires genotyped with 770k SNP, allowing the imputation of every animal to density of 770k SNP. After a genome wide association study was conducted, regions associated with the immune responses were discovered. Twenty-two SNP in the current study were associated with levels pre, post and delta basophils and post eosinophils, at the genome wide significance level. These SNP were located on 11 separate chromosomes, at least in part, highlighting the complexity of eliciting an immune response. Further, each trait had at least one SNP associated with it at the chromosome wide significance level. In addition, the estimation of SNP effects in different breeds was possible due to the multi-breed composition of the herd. We conclude that key pivotal pathways may be shared in eliciting and maintaining an immune response to differing types of antigens. The USDA is an equal opportunity provider and employer.

Key Words: immune response, beef cattle, GWAS

TH36 Associations among vaginal-vulvar laceration, vaginal discharge early postpartum, and prevalence of uterine disease. A. Vieira-Neto*[2], F. S. Lima[1], J. E. Santos[1], R. D. Mingoti[3], G. S. Vasconcellos[3], C. A. Risco[1], and K. N. Galvão[1], *[1]University of Florida, Gainesville, [2]Universidade do Estado de Santa Catarina, Lages, SC, Brazil, [3]Universidade de São Paulo, São Paulo, SP, Brazil.*

Objective was to evaluate the associations among vaginal-vulvar laceration score (VLS), vaginal discharge score (VDS) early postpartum and prevalence of clinical endometritis (CE). Holstein cows (n = 1028) had VLS (0: no laceration; 1: laceration <2 cm at dorsal commissure or internal vaginal wall; 2: vaginal-vulvar laceration >2cm) evaluated at 4 DIM. Cows had VDS (1: clear; 2: flecks of pus; 3: <50% pus; 4: >50% pus; 5: watery, reddish/brownish foul smelling) evaluated at 4, 6 and 8 DIM. The highest score identified on d 4, 6 or 8 was the final classification for each cow. Metritis was diagnosed between 4 and 8 DIM when VDS = 5 and CE at 32 DIM when VDS ≥ 3. Data were analyzed using PROC LOGISTIC of SAS, and models were adjusted for the effects of parity, calving problems (dystocia, twin, stillbirth, retained placenta), and calf sex. Metritis incidence was increased ($P \leq 0.02$) in cows having VLS1 (43.3%) and VLS2 (63.0%) compared with VLS0 (35.2%). Metritis was also increased in cows having calving problems (78.9 vs. 45.7%; $P < 0.001$) and primiparous cows (59.0 vs. 47.8%; $P = 0.05$). Prevalence of CE was increased ($P \leq 0.04$) in cows with VDS3 (25.4%), VDS4 (34.7%), and VDS5 (61.1%), compared with VDS2 (9.3%). Only

one cow had VDS1, so it was combined with VDS2. Prevalence of CE was also increased in cows with calving problems (65.1 vs. 40.3%; $P < 0.001$). Based on the strong association between VLS and metritis and CE, and the association between VDS and CE, VLS and VDS can be considered risk factors for the development of uterine disease.

Key Words: vaginal-vulvar laceration, vaginal discharge, metritis