

POSTER PRESENTATIONS

Animal Health III

W1 Effects of low doses lipopolysaccharide infusion on plasma proteome in lactating cows using comparative proteomics. T. J. Yuan, J. Q. Wang*, Y. X. Yang, D. P. Bu, S. S. Li, and P. Sun, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Subacute ruminal acidosis (SARA) increases the concentration of lipopolysaccharide (LPS) in the rumen. Previous studies have indicated that LPS could translocate into the peripheral blood and increase acute phase proteins, such as serum amyloid A, haptoglobin. The objective of this study was to investigate the effects of infusing low dose LPS into external pudic artery on plasma proteome in lactating dairy cows using comparative proteomics, and to explore the mechanism of host response to LPS challenge. All cows ($n = 6$) were infused LPS ($0.01 \mu\text{g}/\text{kg}$ body weight) via external pudic artery. Milk and blood samples were collected at different times after infusing LPS. Two-dimensional electrophoresis gel coupled with MALDI-TOF-TOF spectrometry was used. Plasma proteins at 0, 6, 12 and 24 h after LPS infusion were separated by 2-dimensional electrophoresis. After visualization proteins with Coomassie Brilliant Blue G250 solution, protein spots were detected by PDQuest 8.0.1 software and differential proteins were identified by MALDI-TOF-TOF spectrometry. Milk SCS started to increase after infusing LPS and peaked at 6 h, and then gradually decreased to the level before infusing LPS at 24 h. Eight protein spots were upregulated at 6, 12 and 24 h in cows after infusing LPS, but there was not significantly different among 6 to 24 h, and were identified to be 4 proteins including vitamin D-binding protein precursor, serpin A3-6, α -1 antitrypsin and serpin A3-1 precursor. These results suggests that vitamin D-binding protein precursor, serpin A3-6, α -1 antitrypsin and serpin A3-1 precursor may play an important role in response to LPS challenge, and further study was needed.

Key words: lipopolysaccharide, dairy cow, plasma proteome

W2 Evaluation of endotoxin (LPS) activity in bovine blood using neutrophil dependent chemiluminescence. S. Kahl*¹, T. H. Elsasser¹, and C. V. Obiezu-Forster², ¹USDA, Agricultural Research Service, Beltsville, MD, ²Spectral Diagnostic Inc., Toronto, ON, Canada.

The purpose of this study was to evaluate the applicability of a neutrophil chemiluminescence-based assay for the measurement of LPS stimulatory activity in bovine whole blood. The assay is based on the capacity for LPS to trigger the respiratory oxidative burst activity (RBA) of autologous neutrophils. This RBA is then detected as photons released from oxidized added luminol in a chemiluminometer. In the protocol, chemiluminescence (CL) of blood samples without (CL_{BL}) and with an added reference quantity of LPS (100 ng/ml , $\text{CL}_{\text{BL+LPS}}$) was measured in luminol/zymosan solution and the relative LPS blood activity (EA_{R}) was expressed as a $\text{CL}_{\text{BL}}/\text{CL}_{\text{BL+LPS}}$ ratio. EDTA-stabilized whole blood was collected from 16 healthy steers, 9 cows diagnosed with advanced mastitis of gram(-) bacterial etiology, and 4 steers given an i.v. bolus of low ($0.25 \mu\text{g}/\text{kg}$ BW) or high

($2.0 \mu\text{g}/\text{kg}$ BW) LPS. In the in vitro recovery studies a linear relationship was observed between added LPS (up to 100 ng/ml) and EA_{R} ($R^2 = 0.99$, $P < 0.01$). Estimated mean (\pm SE) blood EA_{R} in healthy cows was 0.058 ± 0.010 and increased to 0.353 ± 0.062 ($P < 0.01$) in cows with mastitis. Individual EA_{R} values exceeded a calculated clinical cut-off value of 0.137 (upper limit of 95% confidence interval of observed EA_{R} in healthy group) in 78% of infected cows. After the in vivo injection of high LPS dose, estimated blood EA_{R} values exceeded clinical cut-off value at 2 min (0.317 ± 0.057 ; $n = 2$) and 5 min (0.163 ± 0.018), but returned to baseline at 10 min (0.084 ± 0.027). Within 10 min after low LPS dose injection, estimated EA_{R} values remained below cut-off value, although animals showed clinical symptoms of initial proinflammatory response. Results indicate that bovine neutrophil chemiluminescence assay has the potential to detect LPS stimulation of neutrophil respiratory burst activity in bovine blood and to discriminate between healthy cows and cows infected with gram(-) bacteria displaying clinical mastitis. Additional studies are needed to evaluate the specificity of the assay while further modifications to this protocol may result in increased sensitivity.

Key words: bovine, chemiluminescence, endotoxin assay

W3 Evaluation of yeast nucleotides on intestinal barrier function in vitro. A. Ganner*, M. Werner, S. Henikl, and G. Schatzmayr, *BIOMIN Research Center, Tulln, Lower Austria, Austria.*

Yeast contains between 3 and 18% of ribonucleic acid. During manufacturing process and autolysis, the RNA breaks down in its monomers. Nucleotides have been considered nutritionally as semi-essential, they are synthesized de novo using amino acids as precursors, or by salvage of dietary amino acids and nucleotide breakdown. Under field conditions, challenge and stress, the endogenous synthesis may not be capable of supplying the animal's actual nucleotide needs. Nucleotides are involved in various essential biochemical processes; in animal studies they have been shown to have positive effects on performance, growth, gut health and the immune system. Target of the present study was to evaluate the effect of purified nucleotides (adenosine monophosphate (AMP), adenine, adenosine, guanosine monophosphate (GMP), guanine, guanosine, inosine monophosphate (IMP), inosine, uridine, uracil, cytidine and cytosine) and commercially available yeast nucleotide products on intestinal barrier function using a porcine intestinal epithelial cell line (IPEC-J2). IPEC-J2 cells were incubated in 24-well plates with Transwell inserts in the presence of the respective test substance at 41°C and 5% CO_2 for a maximum of 15 days. Transepithelial electrical resistance (TER) was measured daily by a volt-ohm meter. Results were expressed as increase in TER compared to untreated cells (0%). TER was increased up to 200% by AMP. Adenine and adenosine increased TER up to 80% and 140%, respectively. GMP, guanine and guanosine showed an increase in TER over the first 2 days. IMP and inosine showed an increase in TER up to 100% and 60%, respectively. Uridine and uracil, cytidine and cytosine increased TER between 30% and 70%. Five commercially available yeast nucleotide or yeast extract samples with analyzed nucleotide profile were tested in different dos-

ages. Increase in resistance was noticed between 80 and 170%. Our results indicate that nucleotides improve the intestinal barrier function by increasing the TER in a dose-dependent manner. Thus, yeast nucleotides may have protective effects to the intestine and as a result to exert beneficial effects on animal's health.

Key words: yeast nucleotides, gut epithelial cell line, TER

W4 Oral treatment of pregnant cows with lipopolysaccharide and lipoteichoic acid modulated selected plasma metabolites and innate immunity in newborn calves. S. Iqbal*, Q. Zebeli, D. A. Mansmann, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

Immunization of pregnant dams is commonly used to enhance the newborn immune status. The aim of this study was to orally challenge prepartum dairy cows with repeated doses of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) and evaluate clinical disease, and metabolic and immune status of their newborn calves. Thirty pregnant Holstein dairy cows were blocked by parity and the anticipated day of calving, and were randomly allocated to 2 groups, 28 d before the expected day of parturition. Cows were orally administered 2 mL of saline solution (CTR), or 2 mL of saline solution containing 3 increasing doses of LPS from *Escherichia coli* 0111:B4 as follows: 1) 0.01 µg/kg BW on d -28 and -24, 2) 0.05 µg/kg BW on d -21 and -18, and 0.1 µg/kg BW on d -14 along with a flat dose of LTA from *Bacillus subtilis* (i.e., 120 µg/animal). Ten calves per group were randomly selected to determine the disease incidence and evaluate their health and metabolic and immune status collecting blood samples at wk 1 to 4 after birth and analyzing for glucose, lactate, β-hydroxybutyric acid (BHBA), nonesterified fatty acids (NEFA), cholesterol, and haptoglobin. Results demonstrated that the group of calves from treated cows had greater plasma cholesterol (140 vs. 120 mg/dL; $P = 0.02$), and a numerical increase of BHBA in the plasma (152 vs. 133 mmol/L; $P < 0.10$). Furthermore, this group of calves showed better energy status with greater plasma lactate (107 vs. 81 mmol/L; $P < 0.01$), although treatment did not affect concentration of plasma glucose and NEFA ($P > 0.05$). Interestingly, the calves from the treated cows had greater plasma haptoglobin (234 vs. 119 mg/dL; $P = 0.04$). No effect of treatment was observed on calf diarrhea, number of medications given to calves, and their mortality rate. In conclusion, oral treatment of dams with LPS and LTA modulated selected plasma metabolites and markers of innate immunity in their newborns suggesting that treatment of pregnant cows with bacterial immunogens might improve the immunity and wellbeing of newborn dairy calves.

Key words: lipopolysaccharide, lipoteichoic acid, newborn calves

W5 Repeated oral administration of lipopolysaccharide and lipoteichoic acid modulated post-treatment plasma metabolites and innate immunity of prepartal dairy cows. S. Iqbal*, Q. Zebeli, D. A. Mansmann, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

The transition period is critical for the health and productivity of dairy cows due to high incidence of metabolic diseases. The objective of this study was to investigate metabolic, immune, and clinical responses to repeated oral administration of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) in prepartal dairy cows. Thirty pregnant Holstein dairy cows were blocked by parity and the anticipated day of calving, and were randomly allocated to 2 groups 28 d before the expected day of parturition. Cows were orally administered 2 mL of

0.85% saline solution (CTR), or 2 mL of saline solution containing 3 increasing doses of LPS from *Escherichia coli* 0111:B4 as follows: 1) 0.01 µg/kg BW on d -28 and -24, 2) 0.05 µg/kg BW on d -21 and -18, and 0.1 µg/kg BW on d -14 along with a flat dose of LTA from *Bacillus subtilis* (120 µg/animal). Clinical responses including rectal temperature, respiration and rumen contraction rates were evaluated during the 5 h post-treatment and blood samples were collected from the tail vein before and after administration of each dose at 1, 3, and 5 h post-treatment. Blood samples were analyzed for plasma glucose, lactate, NEFA, BHBA, cholesterol, and haptoglobin (Hp). Results demonstrated that treatment did not affect rectal temperature and respiration rate; however, it numerically lowered rumen contraction rate ($P < 0.10$). Moreover, treatment increased plasma glucose (76 vs. 62 mg/dL; $P < 0.01$) and lactate (78 vs. 63 mmol/L; $P < 0.01$) especially at 1 h post-injection for lactate ($P = 0.02$) and 3 and 5 h for glucose ($P < 0.01$). In addition, treated cows tended to have greater concentration of circulating NEFA (102 vs. 92 mmol/L; $P = 0.06$) and lower plasma cholesterol (130 vs. 167 mg/dL; $P = 0.01$), and plasma Hp (554 vs. 753 mg/dL; $P = 0.04$). There was no effect of treatment on plasma BHBA ($P > 0.05$). Overall, repeated oral administration of LPS and LTA modulated post-treatment patterns of selected plasma metabolites, clinical responses, and markers of innate immunity in transition dairy cows.

Key words: lipopolysaccharide, lipoteichoic acid, metabolic and clinical response

W6 Diets enriched in barley grain treated with lactic acid and heat lowered rumen endotoxin and improved innate immunity in dairy cows. S. Iqbal*, Q. Zebeli, A. Mazzolari, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

Feeding early lactation dairy cows high grain based diets is associated with an inflammatory state and high incidence of various metabolic diseases. The aim of the present study was to investigate the effects of feeding barley grain steeped in lactic acid (LA) and heat on ruminal endotoxin and plasma biomarkers of innate immunity. Eight mid-lactation (170 DIM) rumen-fistulated Holstein cows were used in a 2 × 2 crossover design with 2 21-d periods, with the first 11 d used for diet adaptation and the last 10 d for measurements. Cows were fed once daily a total mixed ration containing barley silage and rolled barley grain (31.5% DM basis) steeped for 48 h in equal quantity of tap water (CTR), or in 1.0% LA and heat at 55°C (LAH). The rumen fluid and blood samples were collected on d 11, 15, and 21 shortly before the morning feeding of each experimental period. Postprandial patterns of rumen endotoxin and plasma haptoglobin (Hp) were evaluated collecting rumen fluid and blood samples every 2 h starting at 0800 to 2000 on the last day of each experimental period. The principal component analysis revealed that each of the 2 diets fed could be distinguished on the basis of the measured rumen and plasma variables. Data revealed that cows fed LAH diet had lower concentration of preprandial rumen endotoxin (472 vs. 793 ng/mL; $P < 0.01$), however, treatment had no effect on plasma Hp ($P > 0.05$). Results of postprandial responses showed that LAH diet had numerically lower concentration of plasma Hp (586 vs. 679; $P < 0.10$) and a treatment by time interaction for rumen endotoxin ($P < 0.01$), suggesting a role for both the treatment and the time of sampling on this variable. Interestingly, cluster analysis showed a cluster between rumen endotoxin and plasma Hp indicating strong interrelationship between these 2 variables. Overall, results of this study indicated that feeding barley grain steeped in LAH lowered concentration of rumen endotoxin and modulated postprandial innate responses in mid-lactation dairy cows.

Key words: barley grain, lactic acid and heat, innate immunity

W7 Oral administration of bacterial lipopolysaccharide and lipoteichoic acid modulated milk composition and efficiency in transition dairy cows. S. Iqbal*, Q. Zebeli, D. A. Mansmann, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

The transition period is characterized by high incidence of metabolic disorders, which influence subsequent milk production and composition of dairy cows. The objective of this study was to evaluate the production response of postpartal dairy cows to repeated oral administration of lipopolysaccharide (LPS) and lipoteichoic acid (LTA). Thirty pregnant Holstein dairy cows were blocked by parity and the anticipated day of calving, and were randomly allocated to 2 groups, 28 d before the expected day of parturition. Cows were orally administered 2 mL of 0.85% saline solution (CTR), or 2 mL of saline solution containing 3 increasing doses of LPS from *Escherichia coli* 0111:B4 as follows: 1) 0.01 µg/kg BW on d -28 and -24, 2) 0.05 µg/kg BW on d -21 and -18, and 0.1 µg/kg BW on d -14 along with a flat dose of LTA from *Bacillus subtilis* (i.e., 120 µg/animal) prepartum. Feed intake was obtained during the 4 wk before and 4 wk after parturition, whereas milk data were collected during 4 wk after calving to determine milk production and composition. The data showed that treatment tended to increase milk energy efficiency (1.30 vs. 1.06; $P = 0.06$) and was associated with a trend for lower feed intake (31 vs. 34 kg/d; $P = 0.10$). Furthermore, the overall variance analysis demonstrated that treatment group had greater fat to protein ratio (1.37 vs. 1.25; $P = 0.04$), and fat-corrected milk to feed intake ratio (milk fat efficiency; 0.82 vs. 0.68; $P = 0.01$). Milk lactose yield was higher only in primiparous cows in the treatment group (1.41 vs. 1.19 kg/d; $P = 0.01$). In addition, primiparous cows in this group, showed a tendency for greater fat yield and 4% fat corrected milk ($P < 0.10$). Treatment increased milk urea nitrogen in treated multiparous cows ($P < 0.01$) and lowered protein yield ($P = 0.04$). No effect of treatment was observed on other milk components and on the overall milk production ($P > 0.05$). In conclusion, the results indicated that repeated oral administration of LPS and LTA modulated milk production and composition in dairy cows postpartum.

Key words: oral lipopolysaccharide, lipoteichoic acid, milk production and composition

W8 Oronasal exposure to lipopolysaccharide differentially affected blood metabolites in multiparous dairy cows. A. Hosseini*, D. A. Mansmann, Q. Zebeli, S. Iqbal, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, Alberta, Canada.*

Translocation of endotoxin into blood circulation causes alterations in blood metabolites and immunity. In this study, we evaluated the hypothesis that repeated oronasal application of lipopolysaccharide (LPS) prepartum might improve the metabolic and immune status of periparturient dairy cows. One hundred primiparous (PP) and multiparous (MP) Holstein dairy cows (PP and MP with ~BW 620 and 720 kg, respectively) were randomly assigned into control (CTR; PP = 18; MP = 32) and treatment (TRT; PP = 19; MP = 31) groups. Either carrier alone (3 mL of 0.85% saline) or 3 increasing doses (0.01, 0.05, and 0.1 µg/kg BW) of LPS from *E. coli* 0111:B4 were applied oronasally (1 mL nasally and 2 mL orally) twice a week on wk -4, -3, and -2. Several blood variables including β-hydroxybutyric acid (BHBA), cholesterol, glucose, lactate, nonesterified fatty acids (NEFA) and hap-

toglobin (Hp) were measured during d -28, -25, -21, -14, -7, 2, 14, 21, and 28; however, HP was measured only in MP cows. All data were processed statistically by the MIXED procedure of SAS. Overall results indicated that TRT increased concentrations of cholesterol ($P = 0.06$) and lactate in the serum ($P < 0.01$) of all cows. Data also showed that parity affected ($P < 0.01$) concentrations of cholesterol, NEFA and lactate. Interestingly, concentration of NEFA in serum was greater in PP cows ($P < 0.01$). Furthermore, an effect of day ($P < 0.01$), and interactions of day × parity ($P = 0.03$) and TRT × parity ($P = 0.04$) were obtained for serum glucose with a decreased concentration postpartum in all cows. The results showed that serum BHBA was increased ($P < 0.01$) on d 14 in PP cows. Additionally, concentrations of glucose, cholesterol, and lactate were influenced by TRT × parity interactions ($P < 0.10$), while TRT × day interaction influenced ($P < 0.10$) serum Hp. In conclusion results of this investigation indicated potential involvement of LPS in alteration of blood metabolites in dairy cows. Moreover, the oronasal treatment with LPS might be used to improve the metabolic status and immunity of transition dairy cows.

Key words: oronasal lipopolysaccharide, blood metabolites, innate immunity

W9 Oral administration of lipopolysaccharide and lipoteichoic acid modulated plasma metabolites and decreased the risk of metabolic diseases in periparturient dairy cows. S. Iqbal*, Q. Zebeli, D. A. Mansmann, S. M. Dunn, and B. N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

The transition period imposes enormous stress on the dairy cow and may impair long-term herd health. The aim of this study was to investigate metabolic and health status of periparturient dairy cows repeatedly administered orally with lipopolysaccharide (LPS) and lipoteichoic acid (LTA). Thirty pregnant Holstein dairy cows were randomly assigned to one of the 2 treatment groups starting at 28 d before the expected day of parturition. Cows received orally either 2 mL of 0.85% saline solution (CTR), or 2 mL of saline solution containing 3 increasing doses of LPS from *Escherichia coli* 0111:B4 as follows: 1) 0.01 µg/kg BW on d -28 and -24, 2) 0.05 µg/kg BW on d -21 and -18, and 0.1 µg/kg BW on d -14 along with flat dose of LTA from *Bacillus subtilis* (i.e., 120 µg/animal). Blood samples were collected on d 1 and 3 of wk -4, and then once on wk -3, -2, -1, +1, +2, +3, and +4 around parturition and analyzed for glucose, BHBA, NEFA, lactate, and cholesterol. Cows were monitored for metabolic and infectious disease incidence, body condition score (BCS), manure score, and urine pH throughout the experimental period. Results showed that oral administration of LPS and LTA lowered plasma lactate in the treated cows (2.58 vs. 3.67 mmol/L; $P < 0.01$) and had a tendency to increase plasma cholesterol (152 vs. 137 mg/d; $P < 0.10$). Treatment did not affect concentrations of BHBA, NEFA, and glucose in the plasma ($P > 0.05$). Interestingly, repeated oral LPS and LTA showed a tendency for lower incidence of metritis, laminitis, retained placenta, and uterine discharges ($P < 0.10$). Furthermore, the incidence of uterine horn fluctuations was lower in the treated group ($P = 0.01$). No effect of oral treatment was obtained for BCS, manure score, and urine pH ($P > 0.05$). In conclusion, oral administration of LPS and LTA modulated selected plasma metabolites related to carbohydrate and lipid metabolism and lowered the incidence of multiple metabolic diseases in periparturient dairy cows.

Key words: lipopolysaccharide, lipoteichoic acid, blood metabolites and metabolic diseases

W10 Bovine acute-phase response following different doses of corticotrophin-releasing hormone (CRH) challenge. R. F. Cooke^{*1}, J. A. Carroll², F. N. T. Cooke¹, B. I. Cappellozza¹, C. Trevisanuto¹, V. D. Tabacow¹, J. Dailey², and D. W. Bohnert¹, ¹*Oregon State University—Eastern Oregon Agricultural Research Center, Burns,* ²*USDA—ARS Livestock Issues Research Unit, Lubbock, TX.*

Fourteen weaned, halter-trained Angus steers (BW = 191 ± 2.1 kg) were fitted with indwelling jugular catheter and rectal temperature monitoring device on d -1 of the study. On d 0, steers were ranked by BW and randomly assigned to receive 1 of 3 infusion treatments (i.v.): 1) 0.1 µg of bovine CRH/kg of BW (CRH1), 2) 0.5 µg of bovine CRH/kg of BW (CRH5), and 3) 10 mL of saline. Blood samples were collected via catheters, relative to treatment infusion (0 h), hourly from -2 to 0 h and 4 to 8 h, and every 30 min from 0 to 4 h. Rectal temperatures were recorded every 30 min from -2 to 8 h relative to infusion. Blood samples were collected via jugular venipuncture and rectal temperatures were assessed using a digital thermometer every 6 h from 12 to 72 h, and every 24 h from 96 to 168 h. Samples collected from -2 to 8 h relative to CRH infusion were analyzed for plasma concentrations of cortisol, ceruloplasmin, and haptoglobin, whereas samples collected from 12 to 168 h were analyzed for plasma concentrations of ceruloplasmin and haptoglobin only. Plasma cortisol peaked at 0.5 h for CRH1 steers (58.9 ng/mL) but returned to baseline levels at 1 h relative to infusion (time effect; $P < 0.01$). Within CRH5 steers, plasma cortisol peaked at 0.5 h (51.3 ng/mL) and returned to baseline levels 3 h relative to infusion (time effect; $P < 0.01$). Plasma cortisol concentrations did not change after infusion for saline steers (time effect; $P = 0.42$). Rectal temperatures were greater ($P < 0.05$) for CRH1 steers compared with CRH5 and saline steers at 36 and 42 h relative to challenge. Plasma haptoglobin concentrations in CRH1 steers increased significantly and were greater ($P < 0.02$) compared with CRH5 and saline steers from 48 to 96 h relative to challenge (time effect; $P < 0.01$). Conversely, plasma haptoglobin concentrations were similar ($P > 0.23$) and did not change across time for CRH5 and saline steers (time effect; $P > 0.48$). No treatment effects were detected on plasma ceruloplasmin concentrations. In conclusion, both CRH5 and CRH1 increased plasma cortisol concentrations, but only CRH1 elicited an acute-phase protein response in beef steers.

Key words: acute-phase, bovine, CRH

W11 Feasibility of high immune response technology as a health management tool to characterize immune response profiles of dairy cattle. L. C. Wager^{*}, S. Cartwright, and B. A. Mallard, *Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.*

High immune response (HIR) is a patented evaluation technology that has the potential to improve the health and food quality of dairy cattle through the reduction of antibiotics and enhanced resistance to economically important diseases such as mastitis. The test includes a blood sample to evaluate antibody-mediated immune response (AMIR) and a skin thickness measurement to evaluate cell-mediated immune response (CMIR) together. Dairy cattle with high immune response following immunization with specified test antigens are at a lower risk for developing disease compared with average and low responding animals. To determine if HIR technology would be utilized by the dairy industry, 2 focus groups were conducted within 2 important Ontario dairy regions. Qualitative market information gathered from those focus groups indicated a significant interest in using HIR to test calves or cows for HIR (75% of producers), and that they would use this information for culling decisions, grouping, breeding and/or treat-

ing animals. Pre-commercialization research is currently underway to test the transferability of HIR to the dairy marketplace and includes: 1) a quantitative market assessment of interest in HIR throughout Ontario (n = 400 producers; 10% of Ontario herds) to build on previous qualitative focus group data; 2) a validation study of previous research to rank cattle based on antibody from milk in lieu of blood (n = 26 cows); 3) β-testing the application of HIR on one to 2 Ontario dairy herds to demonstrate the economic value of HIR (n = 2; 350–450 animals per herd); 4) HIR testing of Gencor AI cull-sires to demonstrate the ability of the sire immune system to respond to test antigens without adverse reactions and to confirm no cross-reactivity to governmental health testing. In 10 cull-sires tested to-date no cross-reactivity was noted for the tuberculosis DTH test and other serological tests thus indicating that HIR should benefit all sires including young sires before entering a bull-test facility. All research including analysis should be completed by June 2011.

Key words: high immune response, health management, breeding for health

W12 Influence of blood sample storage temperature and latency until analyzed on various ex vivo innate immune response assays in Holstein heifers. M. A. Ballou^{*1} and L. E. Hulbert^{1,2}, ¹*Department of Animal and Food Sciences, Texas Tech University, Lubbock,* ²*Department of Animal Science, University of California at Davis, Davis.*

Objectives were to determine the influence of peripheral blood sample storage temperature and how quickly the blood samples needed to be processed for ex vivo innate immune responses. Eight Holstein heifers, approximately 1 yr old, were briefly restrained in self-locking stanchions and 2, 10 mL heparinized vacutainers were collected via jugular venipuncture. One sample from each heifer was placed immediately on ice (ICE; 0.18 ± 0.39°C) while the other was placed in an ice chest with no-ice (NI; 21.88 ± 0.51°C). Samples were serially analyzed for ex vivo innate immune parameters at 2 (baseline), 4, 6, 8, 10, and 24 h after collection. Data were analyzed by ANOVA with the fixed effects of heifer, storage temperature (ST), time, and the interactions of heifer x ST and ST x time. The ICE samples had more leukocytes than NI samples (ST, $P < 0.001$). All samples had decreased total leukocyte counts (time, $P < 0.05$) from baseline measurements at 10 and 24 h for ICE and NI samples, respectively (ST x Time $P < 0.01$). Neutrophil proportions in ICE samples did not change with time, but NI samples were lower at 2 h and increased over time (ST x time, $P < 0.03$). The NI samples had greater neutrophil proportions at 8 h compared with 2 h (time $P < 0.01$). Hematocrits were lower (ST, $P < 0.01$) in ICE samples and were above baseline (ST x time $P < 0.01$) at 24 h after collection. More (ST, $P < 0.01$) TNF-α was secreted after NI whole blood samples were stimulated with LPS than ICE samples. NI samples had lower neutrophil L-selectin expression compared with ICE samples, except at 24 h (ST x time, $P < 0.03$). At 2 h, samples stored on ice had a greater neutrophil oxidative burst response than NI samples (ST x time, $P < 0.01$). There were no differences ($P < 0.10$) in neutrophil oxidative burst response between ICE or NI samples from 4 to 10 h, but at 24 h, samples stored on ice had less intense oxidative burst (ST x time, $P < 0.01$). These data indicate that ST and latency until analyzed influences ex vivo innate immune response measurements and should be controlled for when designing experiments.

Key words: immune, time, temperature

W13 Caprylic acid fractionation of serum followed by refractometry to predict serum IgG in preweaned calves. C. Rodríguez¹, N. Saborido¹, L. Castillejos², M. Rodríguez², A. Lago^{*3}, J. Campbell³, J. Quigley³, and J. Polo¹, ¹APC Europe, S.A., Granollers, Spain, ²Animal Nutrition and Welfare Service, Autonomous University of Barcelona, Barcelona, Spain, ³APC Inc., Ankeny, IA.

We evaluated the use of caprylic acid (CA) fractionation of IgG from serum followed by refractometry of supernatant as a rapid method to predict IgG concentration in young calves. Jugular blood samples were collected from calves (n = 100) between 2 and 60 d of age from 4 farms in Girona, Spain. Calves were managed according to standard management practices on each farm. Serum was separated by centrifugation and total protein (TP; automatic analyzer Mira Plus, ABX Diagnostics), IgG (radial immunodiffusion) and refractive index (nD; Refractometer 30PX Refractometer, Mettler Toledo) were measured. Serum (1 mL) was placed in a 1.5 mL tube and 0.06 mL of CA was added. The tube was left at room temperature for 15 min and stirred vigorously every 5 min; thereafter, it was centrifuged and nD of the supernatant was measured. Mean nD of CA supernatant was 1.338 ± 0.0002 and ranged from 1.333 to 1.343. Mean serum IgG was 17.74 ± 0.60 mg/mL and ranged from 4.01 to 33.21. Mean serum TP was 4.73 ± 0.12 g/dL and ranged from 2.81 to 7.60. Mean serum nD was 1.345 ± 0.0001 and ranged from 1.343 to 1.349. The nD of the CA supernatant was highly correlated with serum IgG ($r = 0.86$; $\text{IgG} = 3591.3 \times \text{nD} - 4787.3$) and serum TP ($r = 0.87$; $\text{TP} = 446.1 \times \text{nD} - 591.53$). Similarly, nD of whole serum was highly correlated with serum IgG ($r = 0.90$; $\text{IgG} = 4405 \times \text{nD} - 5908.3$). Finally, serum TP was less well correlated with serum IgG ($r = 0.74$; $3.877 \times \text{TP} - 0.699$). The CA fractionation method was useful for rapidly measuring IgG level in serum of young calves and was highly correlated with serum IgG and TP. Direct measurement of nD to predict serum IgG was also precise. Serum TP, currently the most common on-farm test to estimate serum IgG, was a less precise method to estimate serum IgG than both nD of whole serum and nD of the CA supernatant.

Key words: calves, immunoglobulin, caprylic acid

W14 Development of a rapid method to estimate IgG in bovine colostrum. K. M. Morrill^{*1}, J. D. Quigley², A. Lago², and H. D. Tyler¹, ¹Iowa State University, Ames, ²APC Inc., Ankeny, IA.

Caprylic acid (CA) has been utilized to fractionate colostrum IgG for further laboratory purification and analysis. The objective of this study was to develop a rapid, cow-side test for determining colostrum IgG concentration using CA fractionation followed by refractometry of the IgG-rich supernatant. Frozen colostrum samples (n = 85) obtained from Holstein cattle, were warmed to room temperature in a water bath and treated with varying concentrations of CA and acetic acid (AA). Samples were then centrifuged or allowed to sit for an allotted time to precipitate non-IgG proteins. Supernatant liquid was then analyzed with a digital refractometer (SPER Scientific, model 300034) to determine refractive index (nD). The nD of Ig-rich fraction was compared with total colostrum IgG concentration determined by radial immunodiffusion (Triple J Farms; Bellingham, WA). The nD of supernatant was positively correlated ($r = 0.96$) to RID when 1 mL of colostrum was added to a tube containing 75 μL CA and 1 mL 0.06 MAA, shaken for 10 s and not centrifuged. Refractive index was measured within 1 min of addition of CA. Decreasing AA to 1 mL or increasing AA to 2 mL decreased the correlation ($r = 0.73$ and $r = 0.63$, respectively) between nD and IgG. For centrifuged samples, altering the sitting time before centrifugation from 30 min to 0, 10 or 20 min numerically

increased correlation ($r = 0.82$ to 0.87 , 0.85 and 0.87 , respectively), but these were not statistically different. When the centrifuge step was removed, nD after samples sat for 1 min was highly correlated ($r = 0.96$) with IgG; however visible separation of supernatant and precipitate did not occur in samples with IgG concentrations >20 mg/ml until after 10 min. Total protein (TP) was measured on a subset of 45 samples and weakly correlated ($r = 0.41$) with IgG; this suggests that TP is a poor method to determine colostrum IgG concentration. These results indicate that a simple procedure requiring only CA, AA and a refractometer may rapidly and effectively estimate colostrum IgG concentration in bovine colostrum.

Key words: colostrum, refractometer, IgG

W15 The effect of treatment with long-acting antibiotic upon arrival at a custom heifer rearing facility on non-specific fever, otitis media, neonatal calf diarrhea complex and growth. A. L. Stanton^{*1}, S. J. LeBlanc¹, L. K. Fox², J. Wormuth³, D. F. Kelton¹, and K. E. Leslie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Washington State University, Pullman, ³CY Heifer Farm, Elba, NY.

The primary objective of the study was to evaluate a single subcutaneous injection of tulathromycin (TUL) in the early postnatal period, administered upon arrival at a commercial heifer rearing facility, on the incidence of disease in young dairy calves. A second objective was to describe the risk factors for morbidity and the impact of disease on growth of these calves. The third objective was to investigate the role of *Mycoplasma bovis* in the incidence of otitis media in this population of calves. Calves (n = 788) were randomly assigned to study treatment with TUL or a placebo (CONTROL) upon arrival at the heifer raising facility and were observed for disease daily for 8 weeks by farm staff. Microbiological culture and speciation of *M. bovis* was performed on nasal swabs collected from a subset of (n = 66) calves at 0, 2 and 4 weeks of age. All analyses were conducted using SAS v9.1 and controlled for source farm and group as random effects. Linear mixed models were used to analyze ADG. Generalized linear mixed models with a logit-transformation were used to analyze morbidity. CONTROL calves were 1.7 (CI: 1.2–2.6; $P < 0.01$), 3.7 (CI: 1.6–9.1; $P < 0.005$), and 1.7 (CI: 1.2–2.5; $P < 0.005$) times more likely than TUL calves to be treated for neonatal calf diarrhea complex, unilateral ear droop and bilateral ear droop, respectively. The ADG of TUL calves was 0.03 ± 0.01 kg greater than CONTROL calves ($P < 0.01$). Failure of passive transfer (FPT), non-specific fever, bovine respiratory disease complex and neonatal calf diarrhea complex decreased ADG. The largest decrease in ADG (0.14 ± 0.04 kg) associated with disease was seen in calves with non-specific fever ($P < 0.05$). Of animals sampled for *M. bovis*, 26% tested positive, and 4 different strains were identified. In summary, TUL was associated with decreased the incidence of several common calfhood diseases in the early post-natal period. *M. bovis* was present in this population but not clearly associated with otitis media.

Key words: tulathromycin, otitis media, calfhood disease

W16 Immune status of calves that naturally suckle their dams in dairy farms of Costa Rica. J. A. Elizondo-Salazar^{*1}, J. Sánchez-Salas¹, J. Rodríguez-Zamora¹, and A. J. Heinrichs², ¹Estación Experimental Alfredo Volio Mata, Facultad de Ciencias Agroalimentarias, Universidad de Costa Rica, ²The Pennsylvania State University, University Park.

The measurement of serum total protein (STP) in dairy calves by refractometer as an estimate of serum immunoglobulin concentration is the simplest test to give an indication of adequate passive transfer of immunity. A value of 5.0 g/dL has been established as the cutoff point for assessment of passive transfer status. Since there is no data on the immune status of dairy calves in Costa Rica, the objective of this study was to determine STP concentration in neonatal dairy calves that naturally suckle their dams. Blood samples were collected between d 1 and 7 of age from 417 heifer and 105 bull calves from more than 40 farms in different regions of Costa Rica. All blood samples were collected into serum (red top) Vacutainer tubes, refrigerated overnight, centrifuged, and the serum separated from clot within 24 h of collection. A hand-held refractometer (Atago Master-Sur/Na, Bellevue, WA) was used to measure STP. GLM procedure was used to establish differences between parity and breed of the dams, and sex of the calf. Descriptive statistics were generated to define percentage of failure of passive transfer by sex of the calf and parity of the dam. Calves coming from dams of first and second parity had statistically ($P < 0.05$) higher STP concentration (6.40 ± 0.14 and 6.21 ± 0.14 g/dL) than those coming from cows with 3 or ≥ 4 calvings (6.01 ± 0.15 and 5.91 ± 0.12 g/dL). There was no significant difference between sex of the calves (heifers 6.13 g/dL, bulls 6.14 g/dL). Jersey calves had higher STP concentrations than Holstein, Holstein-Jersey cross, or other breeds (6.43 ± 0.11 vs. 6.18 ± 0.11 , 6.01 ± 0.21 and 6.01 ± 0.16 , respectively). Overall, 21.5% of calves had failure of passive transfer (heifers 22.5%, bulls 17.1%).

Table 1. Failure of passive transfer (%) by parity of dam and sex of neonates

Parity	Heifers	Bulls	Both
1	7.6	10.3	8.3
2	21.0	22.2	21.1
3	28.6	18.2	26.3
≥ 4	28.2	19.4	26.6

Key words: calves, passive transfer of immunity, serum total protein

W17 Determining the heritable component of dairy cattle foot lesions. A. M. Oberbauer*, S. L. Berry, J. M. Belanger, and T. R. Famula, *Department of Animal Science, University of California, Davis.*

Lameness and hoof health impacts dairy producers both as an animal welfare issue as well as being implicated in lowered milk production. Further, it is one of the top 3 reasons dairy cattle are culled prematurely, following infertility and mastitis, thus contributing to the overall carbon footprint of the dairy industry due to the environmental costs associated with raising replacement dairy heifers. Selection schemes for dairy cattle focus on sire contribution to milk production with little consideration to the cow's physical structure. On a commercial California Holstein dairy, 2 binary hoof phenotypic traits, hoof lesions (sole lesion/upper limb lameness) and lameness due to abscesses or ulcers, were recorded. Monthly lactation records were collected from December 2006-April 2009 with weekly hoof health evaluations. Data on cows ($n = 2247$), in addition to hoof information, included birth date, freshening date, lactation number, and sire ($n = 235$) and dam information; total animals including those to build pedigrees were 5809. Lesions had a prevalence of 7.0% and abscess/ulcer lameness had a prevalence of 16.5%. The probability of any lameness (both conditions considered together) increased with increasing lactation number (0.018, 0.055, 0.082, 0.168 for first, second, and third plus

lactations, respectively). Using a threshold model with a genetic term, σ_G^2 , a permanent environment term, σ_{PE}^2 , and residual term, σ_E^2 , $h^2 = (\sigma_G^2)/(\sigma_G^2 + \sigma_{PE}^2 + \sigma_E^2)$ were calculated for each binary trait. The narrow sense heritability for lameness risk was estimated to be 0.24 for hoof lesion and 0.26 for abscess/ulcer lameness. The data suggest a significant genetic contribution to hoof health that is coupled with a greater risk of lameness with increasing lactation number (or age which cannot be ascertained by this study). The genetic component lends support for undertaking a SNP genome wide association study to identify loci contributing to the phenotype.

Key words: lameness, dairy cow, heritability

W18 Effects of cold pasteurizing colostrum with formic acid on bacteria counts and calf IgG absorption. L. A. Vickers*¹ and D. M. Veira², ¹*Animal Welfare Program, University of British Columbia, Vancouver, British Columbia, Canada,* ²*Agriculture and Agri-Food Canada, Agassiz, British Columbia, Canada.*

Colostrum is vital to the health of the newborn calf; however, farm practices often lead to bacterial contamination. We set out to determine if 'cold' pasteurizing colostrum with formic acid (FA) in combination with refrigeration would lower bacteria counts; further, we tested the effects of feeding FA treated colostrum on IgG absorption by newborn calves. Fresh colostrum from 8 cows was subjected to 1 of 4 treatments: 1) addition of FA to achieve a pH of 4.3 and refrigerated; 2) addition of FA to a pH of 4.3 and left at 20°C; 3) no FA and refrigerated; or, 4) no FA and left at 20°C. Samples from each treatment were frozen at 0, 24, 48, 96 and 192 h. The addition of FA immediately lowered aerobic bacteria counts (2.7 ± 0.2 vs 4.2 ± 0.2 log₁₀ cfu/ml; $P < 0.001$) and continued to do so for up to 192 h (1.1 ± 0.4 vs. 7.6 ± 0.42 log₁₀ cfu/ml; $P < 0.001$) compared with untreated colostrum. Untreated colostrum had lower aerobic bacteria counts over 192 h when refrigerated compared with being left at 20°C (5.2 ± 0.22 vs. 7.7 ± 0.2 log₁₀ cfu/ml; $P < 0.0001$). Colostrum IgG was not affected by the addition of FA (62.9 ± 15.2 g IgG/L $P = 0.99$). In a separate trial, 24 Holstein bull calves were fed 3L of colostrum through an esophageal feeder 2 h after birth from to 1 of 3 treatments: A) harvested and frozen immediately B) left at 20°C for 4 h then frozen or C) left at 20°C for 4hr then treated with FA to a pH of 4.3. IgG level of colostrum fed to calves did not differ between treatments (69.7 ± 18.1 g IgG/L; $P = 0.99$). A blood sample was taken 24 h after feeding to determine serum IgG. The addition of FA lowered bacteria counts compared with untreated colostrum (2.0 ± 0.3 vs 4.9 ± 0.3 log₁₀ cfu/ml; $P < 0.0001$). There was no difference in 24 h calf serum IgG (20.1 ± 6.6 mg/L; $P = 0.9$) or apparent efficiency of absorption ($41.6 \pm 6.2\%$; $P = 0.8$) between treatments. In conclusion, the use of formic acid as a way to cold pasteurize colostrum is effective at lowering aerobic bacteria counts in colostrum and does not interfere with IgG levels or absorption in calves.

Key words: colostrum, formic acid, dairy calf

W19 Allelic variations in the bovine vitamin D receptor gene: Correlations with periparturient hypocalcemia? M. Reiche, C. Deiner, A. Mösch, and H. Martens*, *Institute of Veterinary Physiology, Faculty of Veterinary Medicine, FU Berlin, Institute of Veterinary Physiology, Faculty of Veterinary Medicine, FU Berlin, Berlin, Germany.*

Periparturient hypocalcemia (milk fever) is a disorder of the Ca metabolism in dairy cattle primarily affecting multiparous cows. The major reasons of the rapid decrease of blood Ca concentration are the prompt

increase of Ca secretion into the colostrum and the delayed activation of Ca regulation mechanisms including calcitriol, a metabolite of vitamin D. Vitamin D receptor (VDR) gene polymorphisms are reported to be associated with variations of Ca metabolism in man. The present study investigated the potential existence of VDR gene polymorphisms in German Holstein Friesian cows and correlated resulting variations with the incidence of hypocalcemia. Blood DNA was isolated from 26 high-yielding cows in their 4th to 6th lactation, out of which 17 had experienced hypocalcemia with ionized serum calcium levels < 0.9 mmol/l at least once, whereas nine cows had never undergone periparturient hypocalcemia in their lifetime. The 10 VDR exons and parts of adjacent introns were sequenced and compared with the *Bos taurus* VDR sequence published on NCBI based on breed Hereford. In total, 8 sequence alterations were detected in the fragments, which were primarily heterozygous. However, only 4 of them were located on exons with a potential change of the encoded amino acid. Calculated *P*-values (Fisher's exact probability test) were all >0.05, hence, the sequence variations found in this study were not correlated with the incidence of periparturient hypocalcemia.

Key words: periparturient hypocalcemia, vitamin D receptor, gene polymorphism

W20 Strategies to control the cattle tick, *Rhipicephalus microplus*, in dairy herds in the Brazilian Southwestern Amazon region: Technical recommendations. L. G. Brito*¹, F. da Silva Barbieri¹, and M. C. de Sena Oliveira², ¹Embrapa Rondônia, Porto Velho, RO, Brazil, ²Southeast Embrapa, São Carlos, SP, Brazil.

One of the most serious sanitary problems faced by dairy farmers in Brazil is infestation of their herds by the cattle tick *Rhipicephalus microplus*. This infestation is closely related to the region's climate conditions, mainly the high average temperature and rainfall. During the period between October 2004 and November 2009, 14 dairy cows (cross-breeds) were monitored at the Porto Velho experimental field of the Embrapa Rondônia research center, and climate data were obtained from the local weather station. From October 2004 to March 2006 the animals were not treated with any pesticides, to enable obtaining the seasonal fluctuation of *R. microplus* under the prevailing climate conditions of the Brazilian Southwestern Amazon region. Multiple linear regression analysis was used to reveal the influence of climate factors on the tick infestation in the herds. During this period without use of pesticides, the cattle had mean infestation of 201 ticks/animal. There was a significant decline in this rate during the dry season (May to September), when it was 72 ticks/animal, as opposed to 237 ticks/animal in the rainy season. Rainfall was the climate factor with the greatest influence on the tick infestation rate ($R^2 = 0.64$). In April 2006 integrated control strategies were implemented for the entire herd at the experimental station, composed of 111 animals of variable genetic composition, including *B. taurus* × *B. indicus* and *B. indicus*. The integrated tick control strategies entailed application of high-efficacy

pesticides, as demonstrated by the adult immersion test, utilization of animals to attract larvae and pasture rotation of lactating cows. After implementing these integrated control measures, the mean infestation of *R. microplus* on the lactating cows fell from 201 ticks/animal to only 1.1 ticks/animal. By using the strategies listed was obtained an effective control of the cattle tick population in CEPV, where in the period between October 2007 and October 2009 did not require any treatment directed to control of ticks in the dairy herd in this period.

Key words: control, cattle tick, Brazilian Southwestern Amazon

W21 Ruminal binding characteristics of Mycopurge against various aflatoxins in vitro. M. R. Akkaya¹, M. A. Bal¹, and V. Akay*², ¹Kahramanmaraş Sutcu Imam University, Turkey, ²Global Nutritech Ltd., Kocaeli, Turkey.

The objective of this experiment was to determine the ruminal binding characteristics of modified yeast extract and HSCAS containing mycotoxin adsorbent (MP, Mycopurge) against various aflatoxins in an in vitro study. Ninety milliliters of certified aflatoxin mixture [aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2)] in a liquid form was mixed with 30 mL of ruminal in vitro medium providing the final concentrations of 6 ng AFB1, 1.5 ng AFB2, 6 ng AFG1 and 1.5 ng AFG2, respectively. Treatments were: 1) aflatoxin mixture + water (Control); 2) aflatoxin mixture + rumen fluid (AR); 3) aflatoxin mixture + MP (6 mg) + rumen fluid (ARMP). Wheat starch was used as a substrate for AR and ARMP treatments. After various incubation time points (0, 3, 6, 12, 24 h) at 39°C, aflatoxin concentrations in ruminal medium were detected with HPLC. Although AFB1 concentration at 0 h was 6 µg/L, it was reduced to 2.50 and 1.68 µg/L in Control, 0.86 and 0.50 µg/L in AR, and 0.34 and 0.20 µg/L in ARMP at 3 and 12 h, respectively ($P < 0.001$). In addition, AFB1 concentration in ARMP treatment was in a steady-state after 3 h of incubation compared with Control and AR treatment where AFB1 concentrations became stabilized after 12 h of incubation ($P < 0.001$). Similar type of binding pattern was observed for ARMP treatment in ruminal incubation of AFB2, where the concentration was reduced down to the lowest level at 6 h (0.21 µg/L) compared with Control (0.74 µg/L) and AR (0.36 µg/L) treatment. In addition, concentrations of both AFG1 and AFG2 were in a steady-state condition for AR (0.67 and 0.48 µg/L) and ARMP (0.46 and 0.38 µg/L) treatments after 12 h of ruminal incubation. However, binding capacity of MP for AFG1 and AFG2 was always in favor of ARMP treatment at all time points ($P < 0.001$). There was no treatment effect on ruminal in vitro gas production across all treatments, averaging 53.5 mL at 24 h. Results indicate that aflatoxins can be degraded by the heat of incubation medium along with microbial degradation. In addition, MP can help to bind those respective aflatoxins and reduce their concentrations in the rumen before they enter into the blood stream.

Key words: modified yeast extract, aflatoxin, ruminal binding