ANIMAL HEALTH

0099 In silico identification of natural product inhibitors of *Brucella abortus* threonyl-tRNA synthetase. M. Li[1,2], N. Zheng[1,2,3], F. Wen[1,2], Y. Zhang[1,2], S. Li[1,2], S. Zhao[1,2], and J. Wang[*1,2,3], *[1]Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture–Milk and Dairy Product Inspection Center (Beijing), Beijing, China, [3]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Bovine brucellosis is mainly caused by the bacterium *Brucella abortus*, and represents a major problem to livestock industry development worldwide and is also a threat to human health in many developing and underdeveloped countries. Aminoacyl-tRNA synthetases (aaRSs), the central enzymes in protein translation, catalyze the covalent attachment of correct amino acids to their cognate tRNAs, yielding the aminoacyl tRNAs (aa-tRNA or charged tRNA) used for protein synthesis. Due to the pivotal role in protein synthesis, aaRSs have been considered as some of the most promising targets for antibiotics development in pathogenic species. In this study, a three-dimensional structural model of *Brucella abortus* threonyl-tRNA synthetase (BaThrRS) was constructed using computer-aided molecular modeling technique taking *Escherichia coli* threonyl-tRNA synthetase (EThrRS, PDB ID: 1QF6) as template. The ZINC natural product database including 11247 compounds was subjected for virtual screening based on molecular docking against the ATP binding site of the target using Autodock Vina program. Considering the mode of binding and affinities, seven leads, ZINC67910544 (–12.4 kcal/mol), ZINC72320615 (–12.3 kcal/mol), ZINC72320626 (–12.2 kcal/mol), ZINC27215482 (–12.6 kcal/mol), ZINC35270978 (–12.1 kcal/mol), ZINC35458951 (–12.1 kcal/mol), and ZINC42805205 (–12.4 kcal/mol) were selected on basis of binding energies in comparison to the selective inhibitor borrelidin (free energy of binding: –9.3 kcal/mol). Among them, ZINC27215482 (–12.6 kcal/mol) was best lead because of its highest inhibitory activity. The binding site of ZINC27215482 on BaThrRS was a pocket consisting of 22 residues: TYR316, ASN319, MET341, ASN342, CYS343, GLN374, MET383, ARG384, VAL385, PHE388, GLN390, ASP392, HIS394, TYR476, LYS479, GLN493, GLN498, THR496, GLN498, HIS525, SER531, and ARG534. Therefore, through a high throughput virtual screen we identified seven novel BaThrRS inhibitors that are used against bovine brucellosis with great potential for further development.

Key Words: *Brucella abortus*, threonyl-tRNA synthetase, virtual screening

0100 Evaluation of immune function markers in OmniGen-AF® supplemented steers. S. A. Armstrong[*1,2], D. J. McLean[2], T. H. Schell[1,2], G. Bobe[1], and M. Bionaz[1], *[1]Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, [2]Phibro Animal Health Corporation, Quincy, IL.*

The effect of OmniGen-AF® (OG) supplementation on the expression of immune function markers in circulating whole blood cells was investigated in the first 28 d of feeding healthy Angus steers. Steers were randomly assigned to control (n = 4/group) or OG (n = 5/group; supplemented daily with 56 g/head OG), and fed a diet including grass hay, alfalfa, and ground corn. Steers were housed in a freestall barn and fed via Calan Broadbent system. Blood was collected via jugular puncture before the study (–4 d) and on d 14, 21, and 28 of supplementation. Genes evaluated included *CXCR2*, *CD80*, *CD62L*, *IL10RA*, *IL10RB*, *MAPK8*, *NOD2*, and *TLR1*. Data were analyzed using LinReg software to account for efficiency of amplification and normalized by three internal control genes (*RPL19*, *RPS9*, and *TBP*) and corrected to d –4. The qRT-PCR data were log-transformed and the data points with Studentized residuals $t > 2$ removed (i.e., outliers). The final data set was subjected to ANOVA analysis with treatment, time, and treatment × time as main effect and animal as random effect using Proc GLIMMIX of SAS. Time, treatment, and time × treatment did not have an impact on *CD62L* gene expression. The expression of *CD80*, *CXCR2*, *IL10RA*, *IL10RB*, *MAPK8*, and *NOD2* was different through time ($P < 0.05$), and time had a tendency to influence *TLR1* gene expression ($0.05 < P < 0.10$). Compared with controls, OG supplementation down-regulated *CD80* and *IL10RB* gene expression ($P < 0.05$); OG supplementation had a tendency to down-regulate *CXCR2* and *MAPK8* expression compared with controls ($0.05 < P < 0.10$). A treatment × time effect was detected for *CXCR2* gene expression ($P < 0.05$) with control steers displaying higher *CXCR2* expression by the conclusion of the supplementation period compared with OG supplemented cattle. These results, when considered with previous data on immune function markers and OG supplementation, suggest OG may be regulating antigen presentation and signal transduction. Future studies may also consider using *CD80*, *CXCR2*, *IL10RB*, and *MAPK8* as markers of OG efficacy within the first 28 d of supplementation.

Key Words: OmniGen-AF®, immunity, beef steers

0101 Influence of dietary supplementation with a *Saccharomyces cerevisiae* fermentation product prototype on the pathophysiological response to a combined intranasal bovine herpesvirus-1 and intratracheal *Mannheimia haemolytica* challenge in Holstein steers. K. P. Sharon[*1], Y. Liang[1], R. E. Hudson[1], I. Yoon[2], M. F. Scott[2], N. C. Burdick Sanchez[3], P. R. Broadway[3], J. A. Carroll[3], and M. A. Ballou[1], *[1]Texas Tech University, Lubbock, TX, [2]Diamond V, Cedar Rapids, IA, [3]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX.*

The objective of this study was to determine the effects of supplementing a *Saccharomyces cerevisiae* fermentation product prototype (Prototype) on the pathophysiological response during a combined viral-bacterial respiratory challenge. Holstein steer calves (126.5 ± 6.11kg; $N = 16$) were completely randomized to treatments including 0 (CON) or 20 g/head/d of Prototype ($n = 8$). Calves were housed in open, dry lot corrals with four calves per pen (2 pens/treatment). Calves were offered ad libitum access to a 50:50 total mixed ration of a commercially available 16% CP pelleted calf grower and 18% CP chopped alfalfa hay. Treatments were top dressed for 30 d. Orts were measured daily and the quantity of feed was adjusted for approximately 10% orts. Calves were moved to individual stanchions (2.13 × 0.76 cm) in an enclosed barn, fitted with rectal temperature monitoring devices, and allowed 24 h adaptation before initiating the respiratory challenge. All calves were challenged with 1.5×10^8 PFU·mL^{-1}·nostril^{-1} of bovine herpesvirus-1 cooper strain at –72 h using a mucosal atomizer and with 10^6 CFU of *M. haemolytica* (MH) intratracheal at 0 h. Blood samples were collected via jugular venipuncture at –96, –72, –48, –24, 0, 6, 24, 48, 72, 120, 168, and 240 h relative to the MH challenge. Total leukocytes counts tended ($P = 0.063$) to be greater at 24 h among CON steers. Neutrophil:lymphocyte also tended to be greater ($P \leq 0.095$) at 24 and 72 h among CON steers. Monocyte phagocytosis of an environmental *Escherichia coli* tended ($P = 0.056$) to be greater in steers fed the Prototype at 24 h. Neutrophil oxidative burst to an environmental *Escherichia coli* tended ($P = 0.071$) to be greater at 6 h and was greater ($P = 0.011$) at 168 h among steers fed the Prototype. However, monocyte oxidative burst tended ($P = 0.052$) to be greater among CON at 72h. Neutrophil L-selectin did not differ between treatments ($P = 0.515$). Neither serum haptoglobin concentrations ($P = 0.773$) nor rectal temperature ($P = 0.985$) differed between treatments. These data demonstrate that the *Saccharomyces cerevisiae* fermentation product prototype may influence some acute leukocyte responses during a viral-bacterial respiratory challenge, but did not have strong influences on measures of inflammation or disease.

Key Words: health, respiratory, *Saccharomyces cerevisiae* fermentation product

0102 Dose response effect of *Saccharomyces cerevisiae* fermentation product prototype on leukocyte functionality and ex vivo cytokine production during a dexamethasone challenge in Holsteins steer calves. K. P. Sharon[*1], Y. Liang[1], R. E. Hudson[1], I. Yoon[2], M. F. Scott[2], N. C. Burdick Sanchez[3], P. R. Broadway[3], J. A. Carroll[3], and M. A. Ballou[1], *[1]Texas Tech University, Lubbock, [2]Diamond V, Cedar Rapids, IA, [3]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX.*

The objective of this study was to determine the dose response effects of supplementing *Saccharomyces cerevisiae* fermentation product prototype on leukocyte functionality and ex vivo cytokine production during a dexamethasone (DEX) challenge. Holstein steers (125.1 ± 8.16 kg; $N = 32$) were assigned to treatments including 0, 20, 40, or 60 g/head/d of prototype ($n = 8$). Calves were housed for 21 d in dry lot corrals with four calves per pen (2 pens/treatment). Calves were offered ad libitum access to a 50/50 TMR of a commercially available 16% crude protein pelleted grower and 18% CP chopped alfalfa hay. Treatments were top dressed. The quantity of feed offered and orts were measured daily. After the 21 d adjustment to diets, calves were jugularly catheterized and moved into individual stations (2.13 × 0.76 cm) in an environmentally controlled barn and allowed 48 h to adapt before the first DEX injection. Blood samples were collected at –24, –6, 0, 6, 12, 18, 24, 48, and 72 h relative to the first DEX injection. DEX was administered via jugular catheter at 0.1 mg/kg BW at 0, 6, and 12 h. Peripheral blood neutrophil (PMN) concentrations increased ($P < 0.001$) at 6 h and remained elevated through 72 h in all steers. Neutrophil L-selectin and PMN and monocyte (MONO) oxidative burst (OB) and phagocytosis (PHAG) of an environmental *Escherichia coli* decreased ($P < 0.059$) at 6 h in all steers. L-selectin returned to baseline at 72 h while OB and PHAG failed to return to baseline by 72 h. Total leukocyte counts ($P < 0.001$) and PMN concentrations ($P = 0.001$) increased linearly with prototype dose. PMN L-selectin concentrations did not differ ($P = 0.684$) among treatments. Oxidative burst intensity in PMN ($P = 0.025$) and MONO ($P = 0.003$) increased linearly with prototype dose at 72 h, as well as in MONO PHAG intensity ($P = 0.004$) at 6 h. The percentage of PMN ($P = 0.012$) and MONO ($P = 0.013$) that were both PHAG and OB positive increased linearly with prototype at 72 h. Ex vivo whole blood lipopolysaccharide stimulated TNF-α concentrations was greater ($P = 0.026$) in prototype steers than control steers at –24 h. Overall, these data demonstrate that the dexamethasone challenge induced severe leukocyte dysfunction, and prototype supplementation influenced plasma neutrophil concentrations and may have increased recovery of neutrophil and monocyte function.

Key Words: dairy, health, yeast fermentation product

0103 Effects of climatic conditions before and after birth on growth rate of Holstein calves in a hot environment. E. L. Lopez-Rodriguez[*1], A. Martinez[2], and M. Mellado[3], *[1]Universidad Autonoma Agraria Antonio Narro, Torreon, Mexico, [2]UAAAN, Saltillo, Mexico, [3]Autonomous Agrarian University Antonio Narro, Saltillo, Coahuila, Mexico.*

Birth weight and growth records, representing 5938 Holstein calves from three large commercial dairy herds in northern Mexico (26° N; 24.2°C mean annual temperature) were analyzed to document the effects of environmental factors on growth traits of dairy calves from 2013 to 2015. Climate variables indicative of heat stress [e.g., maximum ambient temperature (MaxT) and temperature-humidity index (THI)] were considered, 1 or 2 mo before calving and at calving. Growth traits were birth body weight (bBW), weaning weight (WW), preweaning daily weight gain (DWG). The effect of season, MaxT, and THI the day of calving and MaxT one and 2 mo before calving were analyzed by the GLM and REG procedures of SAS. The relationship between bBW and MaxT on the day of calving was negative and curvilinear. When MaxT and THI reached 34°C and 80 units, bBW had a noticeable drop ($P < 0.01$) compared with lower ambient temperatures (38.6 ± 3.6 vs. 39.2 ± 3.9 kg for calvings at MaxT < or > 34°C, respectively). Birth body weight was lower ($P < 0.01$) in calves born in spring than the rest of the year (38.3 ± 3.9 vs. 39.1 ± 3.8 kg; mean ± SD). Maximum ambient temperature one or 2 mo before calving did not affect bBW. Body weight at weaning and DWG of calves decreased gradually ($P < 0.01$) when MaxT and THI reached 28°C and 73 units at calving, respectively. A season effect was detected ($P < 0.01$) for DWG and WW. These traits were 403 ± 117 and 450 ± 110 g and 66.0 ± 8.5 and 69.2 ± 8.1 kg for summer and winter, respectively. It was concluded that, in this particular environment (high heat load for most of the year), heat stress markedly affects bBW and growth rate of Holstein calves. Thus, environmental management of the late gestation cow during hot summer months is warranted to optimize calf growth rates.

Key Words: birth body weight, growth traits, heat stress

0104 The hidden cost of a hidden disease: growth performance of calves as affected by bovine respiratory disease diagnosed using ultrasonography. C. Tejero[*1] and A. Bach[2,3], *[1]Rancho Las Nieves, Mallen, Spain, [2]ICREA, Barcelona, Spain, [3]IRTA, Caldes de Montbui, Spain.*

The aim of this study was to assess the consequences of bovine respiratory disease (BRD) during the first 60 d of life on subsequent growth performance. One thousand sixty-six calves (42.5 ± 6.9 kg of BW and 12.4 ± 5.6 d old) were raised, fed, and managed under exactly the same protocols in a contract heifer operation. Five hundred and thirty-three calves were diagnosed with BRD using an ultrasound and a 8–5 MHz linear probe with 12 cm scan depth, and the remaining coetaneous 533 calves were healthy and never diagnosed with BRD (NBRD). Ultrasonographic scans evaluated the right lung from the 10th intercostal space (ICS) cranial to the first ICS, and the left lung from the 10th ICS cranial to the second ICS. Calves were considered BRD positive when ≥1 cm of consolidation was present. Calves diagnosed with BRD were immediately treated with antibiotics. Respiratory disease was classified as lobular pneumonia type I (1 cm consolidation), type II (2 cm consolidation), and type III (3 cm consolidation), or lobar pneumonia (consolidated lobe). The potential impact of BRD and its severity on growth performance was assessed using a mixed effects model. The cranial area of the right cranial lung lobe was most commonly affected, followed by the right middle lung lobe, and the caudal area of the right lung lobe. Most BRD cases were diagnosed between 5 and 56 d of age (average 26.2 ± 12.6 d). Daily growth between 12.4 ± 5.6 and 50.8 ± 5.8 d was greater ($P < 0.001$) in NBRD (742 ± 4.9 g/d) than in BRD Type I (649 ± 8.4 g/d), and the latter had a greater ($P < 0.001$) ADG than BRD Type II calves (604 ± 13.9 g/d). Between 49.7 ± 2.5 and 111.6 ± 3.5 d, NBRD calves also grew (1176 ± 7.6 g/d) more ($P < 0.01$) than BRD calves (1084 ± 12 g/d), independently of the severity of the lung lesion. However, from 113.3 ± 7.3 to 162.8 ± 5.4 d, there were no differences in ADG (1116 ± 18 g/d) between NBRD and BRD calves. Therefore, BW at 50, 113, and 163 d was lower ($P < 0.01$) in BRD (66 ± 0.29, 135 ± 0.56, and 192 ± 0.76 kg, respectively) than in NBRD (70.9 ± 0.3, 143 ± 0.67, and 201 ± 0.98 kg, respectively) calves. It is concluded that having a lobular pneumonia with a lesion of ≥1 cm within the first 2 mo of life induces close to 10 kg of BW lag at 23 wk of age despite the application of antibiotic treatment on BRD diagnosis.

Key Words: health, lung, pneumonia

0105 Serum and colostrum antibody titers in Holstein cows, and the relationship between these titers and serum antibody titers in their calves. D. J. McLean[*1], J. D. Chapman[1], A. Woolums[2], D. J. Hurley[3], and L. O. Ely[3], *[1]Phibro Animal Health Corp., Quincy, IL, [2]Mississippi State University, Starkeville, [3]University of Georgia, Athens.*

Vaccination of cows in late gestation is sometimes used to improve maternal antibody titers in their calves. However, scant published research has reported the relationship between serum antibody titers to specific infectious agents in vaccinated cows, the colostrum of these cows, and the serum of calves consuming their colostrum. As part of a larger study, the relationship between cow serum and colostrum antibody titers and calf titers was evaluated. Fifty-four multiparous Jersey and Jersey-cross cows were vaccinated between dry-off and calving with commercially available vaccines containing bovine herpesvirus-1 (BHV-1), bovine viral diarrhea

virus (BVDV), bovine respiratory syncytial virus (BRSV), rotavirus, coronavirus, *E. coli* J-5, and *Salmonella* siderophore receptor and porin (SRP); blood was collected at dry off, mid-dry, and at calving. Calves born to enrolled cows were fed colostrum from only their dams; calf serum was collected at 7 and 30 d of life. Antibody titers against agents in the vaccine were measured in serum and colostrum of cows by standard neutralizing techniques or ELISA, and correlations between cow serum antibodies at 30 d before calving, cow colostrum, and calf serum antibodies at 7 d of life were evaluated. Correlations between cow serum antibodies and colostrum antibodies for different agents were significant ($P < 0.05$) but only moderately strong (Pearson correlation coefficient [PCC] range: 0.32–0.7), and varied for different agents. Similarly, correlations between cow colostrum antibodies and calf serum antibodies were usually significant, but only moderate (PCC range: 0.36–0.77). The R^2 value for the correlation between colostrum antibodies and calf antibodies ranged from 0.11–0.59, indicating that for most agents, the colostrum antibody titer to a given agent did not explain a majority of variation in the calf serum antibody titer to that agent. Antibody titers to specific agents in cows are significantly, but not strongly, correlated with their colostrum antibody titers, and colostrum antibody titers are significantly but not strongly, related to antibody titers in calves. These data suggest that, in addition to maternal antibody concentration, other factors have an important impact on serum antibody titers to specific infectious agents in young dairy calves.

Key Words: colostrum, antibody transfer, vaccination

0106 Evaluating preweaned calf housing and its impact on calf respiratory parameters on New York dairy farms. K. M. Morrill*, *Cornell University, Ithaca, NY.*

The objectives of this project were to (1) evaluate environmental and air quality parameters across different types of calf housing facilities; (2) evaluate rates of respiratory illness in preweaned calves; and (3) determine the impact of environmental factors, air quality, and housing type on calf health. This was an observational study in which calf facilities were evaluated on a single visit during June 2015. Housing included hutches ($n = 9$), individual pens in a barn ($n = 11$), and group pens in a barn ($n = 9$). Facility and calf pen evaluations included wind speed, temperature, relative humidity, heat stress index, bedding type, bedding composite sample for bacteria counts, nesting score of calf pens, calf health scoring, and airborne bacteria. Data were analyzed using SAS 9.3 to determine the impact of housing type, environmental, and air quality variables on calf respiratory score. A total of 29 facilities and 437 preweaned calves were evaluated. Calf facility temperature averaged 24.2°C (range 15.5 to 30.6°C) with a relative humidity of 21.5% (range 10 to 78%) and a heat index of 21.5°C (range 6 to 30.9°C). Temperature and airborne bacterial counts were greater in hutches as compared with individual and group pens ($P < 0.01$). Humidity was similar for hutches and group pens, but greater than individual pens. Gram negative airborne bacterial counts were lowest in individual pens. No difference in heat index was observed across housing type. Mean calf respiratory scores was 2.5 (range of 0 to 9) on a 12 point scale; 13.33% of calves evaluated scored greater than 5, indicating a respiratory challenge. Prevalence of respiratory illness in preweaned calves ranged from 0 to 50% of calves on a per farm basis (mean = 11.05% of calves/farm), with 44.82% of farms having no respiratory illness and 10.32% of farms having 30 to 50% of evaluated calves exhibiting signs of respiratory illness. There was a negative correlation between respiratory score and pen temperature ($R^2 = 0.90$). There was no influence on respiratory score by housing system, bedding type, ventilation system, relative humidity, airflow, or airborne bacterial counts. Data collected from this study suggests that respiratory illness continues to be a challenge, even when weather is temperate. Additional research is needed to evaluate rates of respiratory illness during cold stress and transitional weather, as well as to evaluate management factors that increase the risk of infection.

Key Words: calves, housing, respiratory

0107 Differential primary and secondary immune responses in calves fed heat-treated or unheated colostrum. S. L. Gelsinger* and A. J. Heinrichs, *The Pennsylvania State University, University Park.*

An experiment was conducted to compare immune responses between calves that received unheated or heat-treated colostrum. Half of a single, pooled batch of colostrum was frozen without heating; the other half was heated and maintained at 60°C for 60 min before freezing. Bull calves ($n = 26$) were randomly assigned to receive 8% of their birth weight as either unheated or heat-treated colostrum. Blood samples were collected at birth and 48 h of age to assess passive transfer. At 14 and 35 d of age, all calves received a subcutaneous injection of 0.2 mg ovalbumin per kg birth weight. Blood was sampled at 0, 4, 8, and 12 h, and daily on days 2 to 10 after each injection. Plasma was collected for analysis of total IgG, ovalbumin-specific IgG, interferon γ (IFNg), tumor necrosis factor α (TNFa), and interleukin 1 β (IL1b). Area under the curve was calculated for plasma cytokine and antibody concentrations, and all data were analyzed using Proc Mixed in SAS. Colostrum treatment, time point, and their interaction were included as fixed effects with calf as a repeated random effect. All calves achieved successful passive transfer of IgG. Calves fed unheated colostrum at birth had greater plasma IL1b and nonovalbumin IgG ($P < 0.05$) during the first challenge and tended to have greater nonovalbumin IgG during the second challenge ($P = 0.08$). Calves fed heat-treated colostrum at birth tended to have greater plasma IFNg during the second challenge ($P = 0.09$). These results imply that calves

fed heat-treated colostrum exhibit improved T-cell mediated but reduced innate and B-cell mediated immune response. Despite changes in cytokine and antibody production, neither body weight, body temperature, weekly feed (milk and starter) intake, nor total IgG concentration were different between groups through 45 d of age.

Key Words: calf, colostrum, immune response

0108 The effect of novel antiseptic compounds on umbilical cord healing and infection rates in the first week of life in dairy calves. A. L. Robinson*, L. L. Timms, K. J. Stalder, and H. D. Tyler, *Iowa State University, Ames.*

The objective of this study was to compare the effect of four umbilical dips on the healing rate and incidence of infection of umbilical cords using newborn Holstein and Jersey calves (n = 76). Calves were alternately assigned by birth order to four treatment groups: 7% iodine, a dry dip created using an antibacterial peptide (nisin) mixed with talc (formulation concentration = 3.105 g nisin per 100 g talc on a weight per weight basis), liquid nisin (64 ug/mL), and chlorhexidine mixed with alcohol in a 50:50 solution. Umbilical cords were dipped 30 min after birth. Before initial dipping, diameter of the umbilical cords (as an indicator of cord drying) were determined using digital calipers. As a potential indicator of umbilical infections, surface temperature of the umbilical stump (along with a reference point at the midpoint of the sternum) was measured using a dual laser infrared thermometer (Model 42570, Extech Instruments Nashua, NH). The IR and caliper measurements were all repeated at 24 ± 1 h, 48 1 h, and 72 1 h of age. Measurements of calf umbilical diameter were continued until the umbilical cord healed to the point of detachment. All data were analyzed using mixed model methods (PROC MIXED, SAS Version 9.2). Age at umbilical cord detachment tended to be different between treatments (P = 0.105); calves dipped with chlorhexidine mixed with alcohol detached at a mean age of 20 d compared with 15.5 d for the other three treatments. No treatment differences were noted (P > 0.05) between dips on drying rate of umbilical cords. Mean umbilical cord diameter was 17.81 ± 5.73 mm at birth and they healed to a mean diameter of 8.10 ± 5.07 mm at 24 h of age. Similarly, there were no treatment effects (P > 0.05) on incidence of umbilical infections (umbilical infection rate for all calves was 18.4%). Mean surface temperature of the umbilical stump was 27.4 ± 4.0°C at birth (0.1 ± 0.2°C lower than the sternal reference temperature). At 24 ± 1 h of age the mean temperature of the umbilical stump was 29.2 ± 4.3°C (0.4 ± 0.1°C lower than the sternal reference temperature). These data suggest that all four dips are effective in preventing umbilical infections and permitting healing of the umbilical cord when used within 30 min of birth.

Key Words: calves, umbilical cord, umbilical dip

0109 Effects of OmiGen-AF® and Provia 6086 on growth, leukocyte, and hematological variables of preweaned and immediately postweaned Holstein calves. Y. Liang*[1], R. E. Hudson[1], T. L. Harris[1], K. P. Sharon[1], J. P. Jarrett[2], D. McLean[2], J. D. Chapman[2], J. A. Carroll[3], and M. A. Ballou[1], *[1]Texas Tech University, Lubbock, TX, [2]Phibro Animal Health Corporation, Quincy, IL, [3]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX.*

The objective of this study was to determine the effects of OmniGen-AF (OG) and Provia 6086 (PV) on the performance and health of preweaned and immediately postweaned Holstein calves. Holstein calves within 1 d of birth were randomly assigned to one of four dietary treatments (N = 80). The study was conducted in two consecutive periods with 40 calves/period (n = 10 calves/treatment/period). Dietary treatments were given in both the milk replacer and calf starter. Treatments were arranged and analyzed as a 2 × 2 factorial with OG and PV as the main fixed effects. Diets were formulated to supply approximately 10 g/d of OG and 2 billion CFU/d of PV if calves were consuming milk only or 1.36 kg of calf starter only. Calves were housed in an enclosed barn and fed 275 g of a 22% CP and 20% fat milk replacer daily at 0730 and 1630. Calves had ad libitum access to calf starter and water. The quantity of water and starter offered as well as refused was recorded and adjusted daily for approximately 10% orts. Calves were individually housed until they were weaned at 56 d when they were grouped within treatment with four calves/pen for an additional 28 d. There were no treatment or treatment × time differences on starter intake during either the preweaned ($P \geq 0.111$) or postweaned ($P \geq 0.297$) periods. Additionally, there were no treatment or treatment × time differences ($P \geq 0.500$) in ADG during either the preweaned (0.593 ± 0.096 kg/d) or postweaned (0.845 ± 0.096 kg/d) periods. The surface expression of CD14 on peripheral blood monocytes decreased ($P \leq 0.001$) with increased calf age; however, there were no treatment or treatment × time differences ($P \geq 0.339$). Similarly, there were no treatment or treatment × time differences ($P \geq 0.316$) on the surface expression of CD62L on peripheral blood neutrophils. There was an OG × PV × time interaction (P = 0.018) in peripheral blood neutrophil counts, whereas there was a tendency (P = 0.089) for the Control and OG + PV to have reduced neutrophils when compared with OG and PV calves at 21 d. Lastly, there were no treatment × time differences ($P \geq 0.430$) on hematocrit percentages; however, there was a significant time effect (P = 0.001), whereas hematocrits were elevated at 14 and 21 d. These data indicate that OmniGen-AF and Provia 6086 supplementation during the preweaned and immediate postweaned periods did not influence growth performance, leukocyte, or hematological measures in these Holstein calves.

Key Words: calf, growth, immunity

0110 Health status of dairy feeder calves arriving to a veal facility. D. L. Renaud*, T. F. Duffield, D. F. Kelton, S. J. LeBlanc, and D. B. Haley, *Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

There are approximately 959,600 dairy cows producing 479,800 male dairy calves every year in Canada. Based on information gathered from the 2015 Canadian National Dairy Study, less than 7% of male calves are euthanized at birth, leaving a significant number of male calves to enter the red meat industry. In Ontario and Quebec, the majority of male dairy calves flow into the veal industry. In 2015, 213,659 veal cattle from approximately 551 producers were slaughtered in Ontario and Quebec. Currently, there is little information about the fitness of dairy feeder calves (traditionally referred to as veal calves) entering the veal industry in Canada. The objective of this descriptive study was to evaluate the health status of calves arriving at a large veal farm. Using a scoring program (Calf Health Scorer App) developed by McGuirk et al. (2014) and supplemental scoring adapted from Wilson et al. (2000), Holstein and crossbred calves (n = 1356; 1335 male and 14 female) of unknown age were evaluated immediately on arrival at the commercial milk-fed veal facility in Southwestern Ontario. The results from the period of November 2015 until March 2016 were tabulated and confidence intervals (CI) were calculated (Wald's test) using Stata 14 (StataCorp College Station, Texas). Enlarged navels with at least heat or pain or moisture were found in 25.6% (95% CI: 23.3–27.9%) of calves, diarrhea was present in 16.7% (95% CI: 14.7–18.7%), fever (defined as greater than 39.5°C or 103.1°F) was present in 15.1% (95% CI: 13.2–17.0%), lack of subcutaneous fat or emaciated appearance was present in 22.4% (95% CI: 20.1–24.6%), depression or dullness was present in 26.8% (95% CI: 24.6–29.3%), signs of clinical dehydration (defined as >5% dehydration based on skin tent, attitude, presence or absence of suckle reflex and eye recession) were present in 26.5% (95% CI: 24.2–28.9%), and respiratory disease (defined as a combination of abnormal nasal and ocular discharge, ear and head position, cough and temperature) was present in 9.2% (95% CI: 7.6–10.7%). Based on the results gathered thus far, a significant proportion of calves (42.7% [95% CI: 40.0–45.3%]) are entering the facility with at least one identifiable health abnormality. This represents a significant welfare concern and the causes of the abnormalities need to be further understood to motivate a change in the way dairy feeder calves are treated.

Key Words: dairy calf health, health screening, veal production

0111 Acute immunological responses to a combined viral-bacterial respiratory disease challenge in feedlot heifers supplemented with yeast. A. B. Word*[1], P. R. Broadway[2], N. C. Burdick Sanchez[2], Y. L. Liang[3], K. P. Sharon[3], S. L. Roberts[4], J. T. Richeson[4], P. J. Defoor[5], M. D. Cravey[6], J. R. Corley[7], M. A. Ballou[1], and J. A. Carroll[2], *[1]Texas Tech University, Lubbock, [2]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [3]Texas Tech University, Department of Animal and Food Sciences, Lubbock, [4]Department of Agricultural Sciences, West Texas A&M University, Canyon, [5]Cactus Feeders, Canyon, TX, [6]Phileo Lesaffre Animal Care, Milwaukee, WI, [7]Phileo Lesaffre Animal Care, Cedar Rapids, IA.*

Two treatments were evaluated in commercial feedlot heifers to determine the effects of a yeast supplement on immune responses to a combined viral-bacterial respiratory challenge. Thirty-two beef heifers (325 ± 19.2 kg BW) were selected and randomly assigned to one of two treatments, and fed for 31 d: (1) Control (CON), receiving a standard feedlot ration without a yeast supplement, or (2) Yeast (YEAST), control ration plus a combination live yeast (2.5 $g \cdot hd^{-1} \cdot d^{-1}$) and yeast cell wall (2.5 $g \cdot hd^{-1} \cdot d^{-1}$) supplement (Phileo-Lesaffre Animal Care, Milwaukee, WI). All cattle were challenged intra-nasally with 1×10^8 PFU bovine herpesvirus-1 (BHV-1) on d –3 and then allowed to rest in outdoor pens for 3 d. On study d 0, each animal was challenged intratracheally with an average dose of 3×10^7 CFU *Mannheimia haemolytica*, was fitted with an indwelling jugular catheter and an indwelling vaginal temperature recording device, and was moved into individual stanchions in an environmentally-controlled barn. Whole blood samples were collected at the time of BHV-1 challenge at 1-h (serum) or 2-h (complete blood cell counts) intervals from 0 to 8 h, and at 12, 24, 36, 48, 60, and 72 h relative to *M. haemolytica* challenge. Data were analyzed using the mixed procedure of SAS specific for repeated measures with fixed effects of treatment, time, and their interaction. Water intake per hour tended (P = 0.06) to be greater in the YEAST group compared with CON. Nasal lesion scores tended (P = 0.07) to be decreased in the YEAST group compared with CON (2.50 ± 0.26 vs. 3.19 ± 0.26, respectively). There was no difference in cortisol concentrations or vaginal temperature between treatment groups ($P \geq 0.37$). There was no treatment difference (P = 0.21) in total white blood cell counts following BHV-1 challenge. There was a trend (P = 0.13) for serum haptoglobin concentration to be reduced in the YEAST (11,757.3 ± 1631.7 μg/dL) group compared with CON (15,396.174 ± 1631.7 μg/dL). Cattle in the CON group tended (P = 0.07) to have greater neutrophils than YEAST (6.39 ± 0.39 vs. 5.37 ± 0.37 K/μL, respectively). In summary, feeding a combination live yeast and cell wall yeast supplement tended to reduce nasal lesion score, inflammatory response, and neutrophil count with no

effect on febrile response in beef heifers. Further research is warranted to determine if other measures of the inflammatory response were influenced by yeast supplementation in this model of respiratory disease challenge.

Key Words: feedlot health, respiratory disease challenge, yeast

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0112 SafmannanTM and ActiSafTM supplementation in milk replacer modulates health and performance in high-risk, preweaned Holstein calves.

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The objectives of the study were to determine if supplementing milk replacer with Safmannan (SM) and ActiSaf (AS) would affect calf growth and health throughout the preweaned and immediate postweaned periods. The study was performed over 67 d, with 39 Holstein bull calves. Calves were housed in individual pens in an environmentally controlled barn, and were provided ad libitum access to a texturized calf starter and water, as well as offered 350 g of milk replacer solids, 22% CP and 20% fat, at 0700 and 1600 from d 0 to 56. Calf starter and water refusals were recorded daily and intakes calculated. Calves were randomly assigned to treatments that included CON, milk replacer with no added supplements; SM, milk replacer with 5 g SM/calf/d; and SM + AS, milk replacer with 2 g SM/calf/d and 3 g AS/calf/d. Individual BW was measured on d 0, 21, 42, 56, and 67. Blood samples were collected and analyzed for hematology on d 10, 28, and 56; while plasma and whole blood were collected and analyzed for plasma haptoglobin concentrations, neutrophil surface expression of CD62L, and neutrophil phagocytosis and oxidative burst capacity to an environmental *E. coli* on d 0, 10, 28, and 56. All data were reported as CON, SM, and SM + AS, respectively. The LSMeans with various superscripts differ ($P \leq 0.05$). Individual calf starter intake did not differ over the entire study, although from 0 d to 21 d, calves receiving the SM + AS supplement consumed more starter (0.025^{a}, 0.034^{ab}, $0.074^{b} \pm 0.018$ kg/d; $P < 0.05$). Neither ADG (0.63, 0.68, 0.69 ± 0.054 kg/d; $P = 0.699$), nor feed:gain (1.74, 1.74, 1.73 ± 0.070 kg/kg; $P = 0.990$) differed among treatments. Total leukocyte counts were greater in the CON calves on d 10 than the other treatments (14.2^{a}, 9.2^{b}, $11.1^{b} \pm 1.2$ 10^{6}/mL; $P < 0.05$) and was lower in the SM calves on d 28 than the CON and SM + AS treatments (10.4^{a}, 7.9^{b}, $10.6^{a} \pm 0.87$ 10^{6}/mL; $P < 0.037$). Neutrophil surface expression of CD62L was greatest in SM calves when compared with CON calves ($92{,}772^{a}$, $110{,}441^{b}$, $94{,}526^{ab} \pm 5334$ mean fluorescence intensity; $P = 0.052$). Additionally, there were treatment × time interactions on neutrophil phagocytosis and oxidative burst $P \leq 0.024$), whereas SM calves had greater percentages of neutrophils phagocytizing and producing an oxidative burst on d 28. These data suggest that both yeast supplementation strategies may influence the health of high-risk, preweaned Holstein calves.

Key Words: calf, growth, yeast

0113 Evaluation of horn bud wound healing following cautery disbudding of preweaned dairy calves treated with aluminum-based aerosol bandage.

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Dehorning pain management has been extensively studied, though few studies have evaluated the effects of cautery disbudding on wound healing. Inflammation and delayed healing are common postdisbudding, with undocumented significance for health. Our objective was to determine healing following disbudding with or without treatment using topical aluminum-based aerosol bandage (ALU). In a prospective study, Holstein heifer calves raised at three commercial dairy farms were disbudded within 3 wk of life. Local anesthesia and analgesia were performed before disbudding, and ALU treatment was randomly allocated to the right or left bud within each calf. Disbudding site (DS) healing was evaluated thereafter on a weekly basis for 3 wk and lesion score (LS) was categorized as: (1) no scab or discharge; (2) dried scabs; and (3) purulent discharge. LS was dichotomized (normal: LS = 1; and abnormal: LS > 2) to facilitate analysis and interpretation of results and logistic regression was used for statistical analysis. Results were considered statistically significant when $P < 0.05$ and tendency was considered when $0.05 < P < 0.10$. In total, 220 calves were enrolled. There was no difference in LS between groups during the first 2 wk postdisbudding, but at the third week postdisbudding, the proportion of LS = 3 was greater for control DS compared with ALU (16.8 vs. 8.1%, respectively; $P = 0.02$). Similarly, the odds of having LS > 2 were only different during the third week postdisbudding with control DS being 1.42 times more likely to have LS > 2 than ALU treated DS (95% CI = 0.964– 2.10; $P = 0.07$). Abnormal healing during week 1 increased the odds of having abnormal healing in week 2 (OR = 5.36; 95% CI = 2.96–9.69; $P < 0.01$). Likewise, abnormal healing during week 2 increased the odds of abnormal healing during week 3 (OR = 4.22; 95% CI = 2.72– 6.54; $P < 0.01$). A reduction in LS at the third week postdisbudding was observed when using ALU. Once abnormal healing started, it increased the likelihood of abnormal healing later. Discharge and/or scabs may be a part of the normal healing process; however, in this study, it was considered abnormal since no cultures were performed to rule out DS infection. Our data indicates that use of ALU may benefit healing after cautery disbudding in preweaned dairy calves.

Key Words: dairy calves, dehorning, wound healing, well-being

0114 Automated milking systems: using productivity and behavioral data to detect illness in dairy cows. M. T. King*[1], E. A. Pajor[2], S. J. LeBlanc[3], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*University of Calgary, Calgary, AB, Canada,* [3]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

To develop better ways of using milking activity, productivity, and behavioral data to detect illness, we collected longitudinal data throughout the lactation of 57 Holstein dairy cows (19 PP, 38 MP; 3.1 ± 1.1 lactations) housed in a free-stall barn equipped with an automated milking system (AMS). Cases of illness were recorded, including subclinical ketosis (SCK; n = 23), calving-related disease (CRD; n = 14), hoof disorders and severe lameness (n = 16), pneumonia (n = 8), and gastrointestinal issues and displaced abomasum (DA; n = 7). We collected continuous milking activity data from the AMS. Lying, rumination, and activity data were recorded by electronic data loggers. Data were analyzed in repeated measures mixed linear regression models. Days relative to the day of diagnosis/treatment (d 0) were analyzed as a fixed effect for each illness separately, with data extending back to d –14. Analyses were performed between: (a) the day from which each outcome variable deviated significantly from baseline production/behavior (Tukey's tests were used to make day-by-day comparisons), and (b) d –1, since recovery had begun following treatment on d 0. Outcome variables tested were milk yield (3-d rolling average), daily rumination time, activity (unit-less measure of head and neck motion), and lying behavior (lying time, bout frequency, bout length). Mean milk production declined by 4.3 and 4.1 kg/d from d –4 to diagnosis of DA ($P < 0.001$) and pneumonia ($P = 0.01$), respectively. From d –14 to diagnosis of hoof disorders, production steadily declined by 0.6 kg/d ($P < 0.001$). Mean rumination time declined by 54 and 55 min/d from d –5 to diagnosis of DA ($P < 0.001$) and pneumonia ($P = 0.03$), respectively. Before SCK diagnosis (2 tests/fresh cow ~1 wk apart), rumination decreased by 13 min/d from d –6 to diagnosis ($P = 0.05$); this was most drastic d –3 to –1 (–34 min/d; $P = 0.001$). Activity levels declined by 40 units/d from d –4 to diagnosis of DA ($P < 0.001$), but decreased gradually from d –14 to diagnosis of SCK (–15 units/d; $P < 0.001$) and CRD (–23 units/d; $P < 0.001$). Lying behavior was less predictive of illness, as it did not vary until the day of diagnosis of any illness. These results suggest that the effects of illness on rumination, activity, and productivity are apparent several days before diagnosis and could be used to earlier identify illness in AMS herds. Since behavior and productivity appear to respond differently to various types of illness, it is possible that certain parameters may be illness-specific.

Key Words: automated milking, dairy cow behavior, illness

0115 Occurrence of mycotoxins in the 2015 U.S. corn crop. P. N. Gott*[1], B. G. Miller[1], R. Beltran[2], and G. R. Murugesan, *BIOMIN America Inc., San Antonio, TX.*

Mycotoxins are toxic metabolites produced by filamentous fungi which commonly contaminate feedstuffs harvested for both human and livestock consumption. Although the different types of mycotoxins have variable effects on different livestock species, exposure to mycotoxins can impair health and adversely affect animal performance. The objective of the current study was to determine the occurrence of mycotoxins in the 2015 corn crop in the United States and to assess the potential risk to livestock species. From September 2015 to January 2016, 381 corn samples were collected from 20 states as part of the annual Biomin Mycotoxin Survey. Samples were analyzed either by high performance liquid chromatography (HPLC) or liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques, which are highly sensitive in detecting very low mycotoxin concentrations. The major mycotoxin groups analyzed were aflatoxins (Afla), zearalenone (ZEN), trichothecenes including deoxynivalenol (DON) and T-2 toxin (T-2), fumonisins (FUM), and ochratoxin A (OTA). Mycotoxins were detected in 94% of the corn samples tested and 50% of the positive samples contained more than one mycotoxin. Co-occurrence of mycotoxins may lead to synergism and enhanced toxicity in animals which consume contaminated feed. The percentage of positive samples, mean of positives (ppb), maximum of positives (ppb), and risk threshold (ppb) for the six major mycotoxins are presented in Table 1. The occurrence of Afla, T-2, and OTA were minimal in relation to ZEN, DON, and FUM in these samples. The highest threat in these corn samples was posed by DON due to its high prevalence and number of samples above the FDA recommended level. As a result of their common co-occurrence, ZEN also presents a major threat. In terms of occurrence, FUM ranks second among the six major mycotoxins analyzed in these samples. With the increased occurrence and co-occurrence levels in 2015 compared with the previous year, DON, FUM, and ZEN pose a higher risk to livestock production in 2016.

Key Words: mycotoxins, deoxynivalenol, fumonisin

0116 Associations of hygiene and lying behavior with the risk of elevated somatic cell count and lameness. I. Robles*[1], D. F. Kelton[2], H. Barkema[3], G. P. Keefe[4], J. P. Roy[5], M. A. von Keyserlingk[6], and T. J. DeVries[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]University of Calgary, Calgary, AL, Canada, [4]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada, [5]Faculté de Médecine Vétérinaire, University of Montreal, St. Hyacinthe, QC, Canada, [6]Animal Welfare Program–University of British Columbia, Vancouver, BC, Canada.*

The objective of this study was to identify how cow-level factors and housing management affect the risk of elevated SCC (eSCC) and lameness in lactating dairy cows. Cows from six commercial free-stall dairy herds in Ontario, Canada, were enrolled in a longitudinal study. Ten Holstein cows/herd were randomly selected based on days in milk (DIM; <120 d), absence of mastitis treatment in the last 3 mo, and somatic cell count (SCC < 100,000 cells/mL). Data on SCC were collected through DHI testing (~5 wk intervals). The study began within 7 d after a DHI-milk test, continued until three tests were completed (~105d), for a total of three observation periods/cow. Elevated SCC was used to indicate subclinical mastitis. An incident of eSCC was defined as a cow having a SCC > 200,000 cells/mL at the end of a period when SCC was <100,000 cells/mL at the beginning of that period. Lying behavior was recorded for 6 d after each milk sampling using data loggers. On d 1 of each recording period, a trained observer scored cows for lameness (5-point numerical rating scale, NRS $\geq$ 3 = lame). Hygiene scoring (four-point scale), also done by a trained observer, occurred on each visit. Cows were categorized as clean (1, 2) or dirty (3, 4). Stall cleanliness was assessed with a 1 m^2 metal grid, containing 88 squares, centered between stall partitions of every 10th stall, and then counting the squares containing visible urine and/or fecal matter. Data were analyzed using multivariable logistic regression models. Cows averaged (mean ± SD) 627 ± 107.7min/d lying, 9 ± 2.8 lying bouts/d, and 72 ± 19.9 min/bout. Over the study period, 13 eSCC were detected, resulting in an incidence rate of 0.73 eSCC/cow-year at risk. The risk of experiencing an eSCC increased 1.4× ($P < 0.01$) with every 20,000 cells/mL SCC increment at the beginning of the study. Mean proportion of soiled squares/stall was 27%. Each SD (18.8%) increment in proportion of dirty squares/stall was associated with lameness (NRS $\geq$ 3; OR = 1.5; $P = 0.05$) and increased the odds of having a dirty udder (DU; OR = 2.4; $P = 0.02$). Each SD (108min/d) increment in lying time/d increased the risk of having dirty upper legs and flank (DULF; OR = 2.1; $P < 0.01$), and tended to increase the risk of having a DU (OR = 1.44; $P < 0.08$). For each 9.6% increase above mean (100%) cow/stall stocking density, the risk of having DULF increased by 1.7× ($P = 0.03$). These results indicate that lower stocking density and management practices that improved stall hygiene and should be encouraged to reduce the risk of poor hygiene and clinical lameness in dairy cows housed in free-stall barns.

Key Words: subclinical mastitis, lameness, cleanliness

0117 Using milk fat-to-protein ratio to evaluate dairy cows energy balance status. T. Schcolnik*, *Afimilk, Afikim, Israel.*

The objective of the present study was to establish the association of negative energy balance status of the calving cow with the duration of high daily fat-to-protein ratio (FPR). Such an association could be utilized to evaluate fresh cow's energy balance status. A dairy cow's physiological condition is reflected in the composition of its milk. AfiLab™ is a real-time milk analyzer measuring individual cows' milk fat, protein and lactose contents during each milking. Fat-to-protein ratio is a combination of milk protein production rate with body fat mobilization rate; that is, it reflects energy availability to a cow's body needs of maintenance and production. Negative energy balance (NEB), ketosis, and body condition score losses are related to reduced conception rate and decreased milk yield. Utilizing FPR for evaluation of cows energy status provides valuable indications, which enable prompt intervention and increase economic profit. Daily milk component data, extracted from the ICBA Israeli HerdBook, included 117,846 observations (days in milk [DIM] periods) of 23,192 cows at first lactation or more, calving through 2014, in 44 Israeli Holstein herds using Afilab. Analysis was done by SAS®PROC GLIMMIX. Means of FPR up to 50 DIM were calculated for 3 periods: 1–15, 16–35, and 36–50 DIM. Four categories were defined by FPR threshold of 1.4: (1) means of all periods < 1.4, (19,181 cows); (2) mean of any one period $\geq$ 1.4 (2979 cows); (3) means of any two periods $\geq$ 1.4 (779 cows); and (4) means of all three periods $\geq$ 1.4 (253 cows). The results establish valid associations between production and fertility traits and the duration of NEB after calving. Least squares means of conception to first AI service were 35.34, 31.62, 30.17, and 33.0% for groups 1, 2, 3, and 4, respectively. Differences between groups 2 and 3 vs. group 1 were statistically significant. The least squares means of days open for the same groups were 122, 125, 129, and 128 d, respectively. The least squares means for 180 d milk yield were 7621, 7685, 7636, and 7468 kg, respectively. Differences between groups 1, 2, and 3 vs. group 4 were statistically significant. We concluded that the intensity of the negative effects of NEB on fertility and production variables examined, relates to the duration of NEB after calving. Therefore, real-time detection of NEB per individual cow allows for specific and prompt treatment.

Key Words: energy status, fat-to-protein ratio, production

0118 Evaluation of three lameness detection strategies on the odds of cure in dairy cows. E. M. Wynands*, D. Moe, and G. Cramer, *Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul.*

The high prevalence of lameness in U.S. freestall dairy herds is both an animal welfare and an economic concern. To achieve a low prevalence of lameness, strategies to lower incidence need to be combined with methods to decrease duration. This requires methods to detect and treat lameness. The objective of this study was to evaluate the impact of three lameness detection strategies on the odds of cure. A trial was conducted from June to August 2015 on a dairy farm in Minnesota. Three pens of cows were randomized to different lameness detection strategies. The strategies were: (1) locomotion scoring using a 3-point scale (VLS), (2) headlock scoring by observing leg posture and weight-bearing while cows were restrained (HS), and (3) casual observation at unspecified times (farm's current strategy), serving as the control group (C). Cows newly detected as lame by the different strategies were evaluated in a hoof trimming chute and treated for the cause of lameness. All groups were locomotion scored for lameness once per week (LS) as cows exited the parlor. The weekly LS were used as the outcome measure to assess odds of cure. Cows that began nonlame as defined by LS and who were subsequently diagnosed as lame by LS were included in the analysis. The scores from the LS system showed a high degree of week-to-week variability. Logistic regression models were constructed for the odds of cure at 3 wk (n = 486) and 6 wk (n = 290) following lame diagnosis. At the 3 wk follow-up, 176 individuals (36.2%) remained lame. Primiparous cows had higher odds of recovering than multiparous cows (OR, 1.79; CI, 1.20–2.67). Days in milk at enrollment were negatively associated with odds of cure (P = 0.03). There was no significant association between detection strategy and odds of cure at 3 wk. At the 6 wk follow-up, 125 individuals (43.1%) remained lame. Primiparous cows had higher odds of recovering than multiparous cows (OR, 2.71; CI, 1.62–4.53). The odds of cure were higher in the VLS group compared with the C group (OR, 2.15; CI, 1.12–4.12). The week-to-week variability in individual cow LS identified a limitation of the LS system, as this variation is inconsistent with the pathology of lameness. These results show that the odds of cure improved when the VLS active detection protocol was implemented.

Key Words: dairy, lameness, locomotion scoring

0119 Risk factors for subclinical ketosis in grazing dairy herds in Brazil. R. R. Daros*[1], M. J. Hötzel[2], S. J. LeBlanc[3], J. A. Bran[2], A. J. Thompson[1], and M. A. von Keyserlingk[1], *[1]Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada, [2]Universidade Federal de Santa Catarina, Florianopolis, Brazil, [3]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

Minimizing disease, including subclinical ketosis (SCK), continues to be a challenge for the dairy industry. Work on SCK has focused on confinement systems, with little work on pasture-based dairies. The aim of this study was to determine the prevalence and herd-level risk factors for SCK in cows housed on pasture. We visited 48 pasture-based farms in southern Brazil between February and September 2015. All farms used a rotational grazing system (2 to 3 paddocks per day). Cows (n = 13 per farm) between 3 and 21 d in milk were assessed for SCK based on blood β hydroxybutyrate ≥ 1.2 mmol/L. Data regarding number of recumbent cows up to 3 d after partum (a crude measure of milk fever) over the course of 1 yr, supplemental feeding, and transition cow management were collected using a questionnaire by interview and environmental inspection. Herds were categorized either as Holstein or crossbred (crossbred Holstein and Jersey, or a mix of Holstein and Jersey). Herd-level prevalence of down cows was categorized into low (0–5%), medium (5–10%), and high (>10%). Herd prevalence of SCK was log transformed. Univariable linear regression models were used to select variables associated with SCK (P < 0.2). Variables from the final multivariable model were back transformed for interpretation. The overall prevalence of ketosis was 21%. Breed, down cow prevalence, and access to water (free versus limited access), were retained in the final model. Referent herds had 8% (95% confidence interval [CI]: 5–11%) SCK prevalence and consisted of Holsteins with free access to water and low prevalence of down cows. Compared with referent herds: Crossbred herds had 1.7 times higher predicted prevalence of SCK (95% CI: 1.14–2.55; P = 0.01); limited access to water increased herd level predicted prevalence of SCK by 1.5 times (95% CI: 1.05– 2.31; P = 0.03) and herds with high predicted prevalence of down cows had three times higher prevalence of SCK (95% CI: 1.75–5.09; P < 0.01). Pasture-based dairies appear to have similar point prevalence of SCK to confinement systems, but the risk factors are different. This work indicates that crossbred herds have higher levels of SCK, so prevention methods for SCK in these herds is especially important. Improved feeding and management that would prevent down cows and allowing cows to have free access to water should decrease the prevalence of SCK in grazing dairy herds.

Key Words: health, hyperketonemia, transition period

0120 Mortality risk factors for calves entering a multilocation white veal farm in Ontario.
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Mortality in preweaned dairy breed calves of both sexes represents a potential welfare issue and a source of economic loss for the industries involved. While morbidity and mortality in veal production has been described, this work reflects a wide range of management practices and requirements throughout the world. In preweaned dairy heifers, rates of morbidity and mortality can also range dramatically, due in large part to differing management strategies. It has been over two decades since mortality in veal calves in Ontario was last described. The objective of this retrospective cohort study was to determine if recorded on-arrival data collected from a large white veal farm could be used as predictors of mortality. Data was collected from 10,910 calves entering seven barns of a single white veal farm, all locations of barns within Ontario, from 1 Jan. to 31 Dec. 2014. Calves were followed until death or marketing; no calves were culled during the year. Three logistic regression models were used to determine the effects of weight on arrival, season of arrival, supplier, sex, barn, and standardized purchase price on the risk of overall mortality, mortality in the first 21 d after arrival, and mortality after the first 21 d. In the overall mortality model, significant associations ($P < 0.05$) were seen with season, barn, supplier and weight, with lighter weight calves arriving in winter being at increased odds of mortality. The early mortality model contained significant ($P < 0.05$) associations with weight, season, barn, supplier and tended ($P < 0.10$) to have an association with standardized price; lighter weight calves arriving in winter at lower prices were at increased odds of mortality. The late mortality model had significant ($P < 0.05$) associations with season of arrival, barn and supplier. While not a proxy for body condition, increased weight on arrival being protective for early mortality may have somewhat reflected this, as the distribution of weights was fairly tight and likely represented calves at a week of age or less. Although failure of passive transfer is a significant risk factor for mortality, the seasonal association we saw could reflect early life nutrition stress as opposed to seasonal variation in passive transfer. A further exploration of dairy farm of origin risk factors for veal calf mortality may serve to improve the productivity and welfare of dairy calves of both sexes.

Key Words: calf, mortality, veal

0121 Assessment of tubal patency by hysterosalpingo-contrast sonography in cow.
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Recently, several studies have reported the use of ultrasound contrast media to assess tubal patency during transvaginal ultrasound in the imaging of infertile women. However, in the field of veterinary medicine, there is little information regarding the use of hysterosalpingo-contrast sonography to test for tubal patency. The present study aimed to estimate the clinical usefulness of hysterosalpingo-contract sonography for tubal patency in cows. Five nonlactating Holstein cows were used, and four of them were treated twice. Sixteen microliters of perflubutane (Sonazoid® for injection, Daiichi-Sankyo) was diluted with 60 mL of saline as echo-contrast medium. At the luteal phase (around 10 d after ovulation), a 16 Fr balloon catheter was gently inserted into one uterine horn, the balloon was placed approximately 5 cm cranial to the uterine bifurcation. The linear probe of the ultrasound (5.0 MHz) was inserted rectally and 30 mL of echo-contrast medium was injected slowly into the uterine horn through the catheter. After the injection, the flow of contrast medium in the uterus, uterotubal junction, and oviduct was monitored with the ultrasound for 20 min. The opposite oviduct was tested in the same way. Tubal patency was diagnosed if the contrast medium was visualized at the infundibulum of the oviduct. Twelve of 18 cases were diagnosed as tubal patency. Contrast medium was first observed within the uterus and then immediately moved toward the oviduct. In the case of tubal patency, it was visualized as a funnel-like appearance adjacent to the ovary at the infundibulum of the oviduct. The transit time of the contrast medium to reach the infundibulum of the oviduct after injection was 6.5 ± 3.3 min ($n = 12$). Two cases were diagnosed as tubal obstruction. Although the contrast medium was clearly visualized in the uterus and the isthmus of the oviduct, an accumulation of contrast medium was found at the position of the ampulla of the oviduct in one of them. The other four cases were inconclusive because of poor image quality due to posterior echo enhancement and technical artifact. The present study demonstrated that the flow of the contrast medium injected to the uterus was visualized at the infundibulum of the oviduct, suggesting that hysterosalpingo-contrast sonography is useful as a diagnostic tool for tubal patency in cows. However, further technical improvement is required to reduce inconclusive cases for accurate diagnosis.

Key Words: tubal patency, hysterosalpingo-contrast sonography, cow

0122 Retained placenta and subclinical endometritis: Prevalence and relation with reproductive performance in crossbred dairy cows. R. R. Buso, C. C. Campos, T. R. Santos, J. P. E. Saut, and R. M. Santos*, *FAMEV-UFU, Uberlândia, Brazil.*

Our objective was to determine the effects of type of calving (eutocic vs. dystocic) and season of the year (rainy vs. dry) on retained placenta (RP) and subclinical endometritis (SE) prevalences verify the relationship between these two diseases, and effects of RP and SE on culling rate, calving to conception interval, and number of AI/conception in crossbred dairy cows. The study was conducted in nine different dairy farms located in Minas Gerais state, Brazil. Retention of fetal membranes was recorded on the first day postpartum. Endometrial cytology was performed between 30 and 80 d in milk (DIM) and the threshold used for SE diagnosis was ≥5% neutrophils. Data were analyzed by logistic regression and ANOVA on Minitab program. The prevalence of RP was 14.93% (69/462) and of SE was 27.49% (127/462). A tendency of effect of RP on SE prevalence was detected (35.82 vs. 26.07%; $P = 0.10$). Dystocia increased RP prevalence (68.42 vs. 12.19%; $P < 0.05$). Cows, which calved during rainy season, had greater SE prevalence (35.48 vs. 20.41%; $P < 0.05$). RP increased culling rate (19.40 vs. 6.08%; $P < 0.05$), calving to conception interval (177.46 vs. 131.19 d; $P < 0.05$) and number of AI/conception (3.30 vs. 2.46; $P < 0.05$), although SE occurrence did not affect these variables ($P > 0.05$). In conclusion, RP showed to be a risk factor for SE, dystocia is a predisposing factor for RP and cows calving during the rainy season had an increase in SE prevalence. There is a negative impact of retained placenta on reproductive efficiency of crossbred dairy cows. Supported by FAPEMIG e CnPQ.

Key Words: cytological endometritis, reproductive efficiency, dairy cows

0123 Association of rumination time and health status with milk production in early lactation dairy cows. V. H. Asselstine[1], E. I. Kaufman[1], S. J. LeBlanc[2], B. W. McBride[1], T. F. Duffield[2], and T. J. DeVries*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to characterize the associations of rumination time (RT) and health status with milk yield (MY) and milk composition in early lactation dairy cows. A total of 339 dairy cows (first lactation, $n = 107$; second lactation, $n = 112$; ≥ third lactation, $n = 120$) on 4 commercial dairy farms in Ontario, Canada, were monitored from 1 to 28 d in milk (DIM) for rumination behavior (24h/d using an automated system), milk composition (fat, protein, somatic cell count, and milk urea N 1×/wk), and hyperketonemia (HYK; blood β hydroxybutyrate ≥ 1.2 mmol/L, measured 1×/wk). Cows were milked 3 times/d at each farm; Two farms recorded milk weights at each milking to determine daily MY ($n = 170$). Cases of retained placenta, metritis, milk fever, or mastitis during the study period were recorded. Cows were categorized into 1 of 3 groups: healthy (HLT) cows had no HYK or any other recorded disease ($n = 139$); HYK cows with no other health problems during transition ($n = 97$); or hyperketonemic plus (HYK+) cows that had HYK and ≥1 other health problems ($n = 53$). Data were summarized by week and analyzed in repeated measures general linear mixed models. A positive association was found between weekly summarized RT and MY in first (+0.006 ± 0.003 kg milk/min RT, $P = 0.04$) and second lactation (+0.01 ± 0.004 kg milk/min RT, $P < 0.01$) cows from 4 to 28 DIM. A positive association was also seen in parity 3+ cows, however, the relationship between RT and MY differed ($P < 0.01$) across weeks (wk +1: +0.05 ± 0.007 kg/min RT; wk +2: +0.06 ± 0.008 kg/min RT; wk +3: +0.05 ± 0.011 kg/min RT; wk +4: +0.04 ± 0.014 kg/min RT). During wk +2 and +3, second lactation HYK cows had lower milk protein percentage compared with HLT cows (0.08 ± 0.043w/w% and 0.12 ± 0.041w/w%, respectively; $P \leq 0.05$). Hyperketonemia+, second lactation cows also had lower milk protein content compared with HLT cows in wk +1, and +2, (0.14 ± 0.068w/w% and 0.20 ± 0.089w/w%, respectively; $P \leq 0.05$). Over the 4 wk observation period, first lactation HYK+ cows tended to have lower protein compared with HLT cows ($P = 0.1$) and ≥ third lactation HYK and HYK+ cows produced less protein than HLT cows ($P \leq 0.03$). Second lactation cows in HYK+ produced less milk than HLT cows during wk +1 (7.1 ± 2.9 kg/d, $P = 0.02$), +2 (14.2 ± 4.0 kg/d, $P < 0.001$), +3 (13.8 ± 4.5 kg/d, $P = 0.003$), and +4 (10.6 ± 5.3 kg/d, $P = 0.05$). Ketosis decreased MY and protein percentage variably with parity. Rumination time was shown to have a positive association with MY in early lactation dairy cows.

Key Words: hyperketonemia, milk production, rumination behavior

0124 Associations of cow-level factors with the risk of poor hygiene. I. Robles[*1], D. F. Kelton[2], H. Barkema[3], G. P. Keefe[4], J. P. Roy[5], M. A. von Keyserlingk[6], and T. J. DeVries[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]University of Calgary, Calgary, AL, Canada, [4]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada, [5]Faculté de médecine vétérinaire, University of Montreal, St. Hyacinthe, QC, Canada, [6]Animal Welfare Program, University of British Columbia, Vancouver, Canada.*

The objective of this study was to identify how cow-level factors affect the risk of having poor hygiene. Cows from 68 commercial dairy herds in Ontario, Canada, were enrolled in a cross-sectional study. Cows were housed in free-stall (FS, n = 43) or tie-stall (TS, n = 25) barns. Twenty-five percent of the cows in each lactating pen (FS) or row of stalls (TS) in herds with > 160 cows, or a minimum of 40 cows per herds with <160 cows were randomly selected for hygiene scoring (n = 2594 cows). Cows were scored for cleanliness on each of three visits (7d apart), on a scale of 1 (clean) to 4 (dirty), in each of three zones (lower leg, udder, and upper leg and flank); scores were categorized as: clean $\leq$ 2 or dirty $\geq$ 3. DHI data from the test closest to the first visit and another test closest to the last visit were obtained. Data were analyzed using multivariable logistic regression models. DIM (mean ± SD) was 171.6 ± 106.1 for cows housed in FS barns and 177.4 ± 114.0 for those in TS barns. In FS barns, first and second parity cows were at 1.5× and 1.4×, respectively, greater risk ($P < 0.01$) of having dirty lower legs (DLL) compared with $\geq$ 3 parity cows. Also in FS barns, first parity cows had a lower risk of having a dirty udder (OR = 0.40; $P < 0.05$), as well as dirty upper legs and flank (DULF; OR = 0.84; $P = 0.01$), when compared with $\geq$3 parity cows. In TS barns, first lactation cows had a 2.3× greater risk ($P < 0.01$) of having DLL compared with $\geq$3 parity cows. First and second parity cows in TS barns had greater risk (OR = 1.9 and 1.3, respectively) of having DULF ($P < 0.01$). Each SD (114.0) increment in DIM was associated with lower risk of having DLL (OR = 0.41; $P < 0.01$) and dirty upper legs and flank (DULF; OR = 0.60; $P < 0.01$) for cows in TS barns. Each SD (106.1) increment in DIM was associated with lower risk of having a dirty udder (OR = 0.90; $P = 0.03$) and DULF (OR = 0.84; $P < 0.01$) for cows housed in FS barns. The results suggest that cow hygiene varies by parity and stage of lactation in both free-stall and tie-stall barns.

Key Words: hygiene, housing, parity

0125 Genomic markers associated with hyperketonemia in Jersey cows. R. S. Pralle[*1], H. A. Adams[2], T. L. Chandler[1], and H. M. White[1], *[1]Department of Dairy Science University of Wisconsin, Madison, [2]CRI International Center for Biotechnology, Mount Horeb, WI.*

Hyperketonemia is a metabolic disorder in dairy cattle commonly attributed to a parturition-induced negative energy balance. Prevalence of this disorder is variable among dairy operations with similar management strategies, as well as among dairy breeds. This suggests differences in genetic selection practices among farms and breeds that may alter a cow's predisposition to hyperketonemia. Identification of genomic markers for hyperketonemia could assist dairy producers in identifying transition cows requiring intensive management. The objective of our study was to identify genetic markers associated with the hyperketonemia phenotype. Genomic marker association was performed on 387 Jersey cows genotyped with the *GeneSeek* Genomic Profiler LD v3 chip. Blood and hair samples were collected at a single time-point from Wisconsin (5–21 d in milk [DIM], five herds) and New England (2–30 DIM, five herds) Jersey cows. Serum β-hydroxybutyrate (BHBA) concentration was determined by a colorimetric assay. Samples were diagnosed into categorical phenotypes for threshold and gap analysis, generating two case-control phenotype sets based on serum BHBA concentrations. Threshold hyperketonemia cases were defined as BHBA concentration $\geq$ 1.2 mM, and remaining cows were controls; gap cases were BHBA concentrations $\geq$ 1.2 mM, and controls were BHBA concentrations $\leq$ 1.0 mM. A log-additive model that accounted for parity group was applied to the data, using the SNPassoc package in R, to identify markers significantly associated with hyperketonemia status. Means ± standard error are reported. Markers were considered significant at a false discovery rate-corrected $P \leq 1 \times 10^{-6}$. The mean DIM at sample collection was 15.0 ± 0.73. The prevalence of hyperketonemia within the dataset was 22.0%. Blood BHBA was 0.68 ± 0.01 mM for controls and 2.08 ± 0.13 mM for cases. Thirteen markers were found to be significant in the gap and/or threshold analyses. Six markers were significant across both analyses. Of the significant markers identified in threshold or gap comparisons, markers were identified within exonic regions of eight genes: periphilin 1, multimerin 2, alkB homolog 1 histone H2A dioxygenase, Parkinson disease protein 2 co-regulated-like, ataxin 1, protein phosphatase 1, β-γ crystallin domain containing 3, and 2,3-cyclic nucleotide 3 phosphodiesterase. Although gene functions are not exclusive to energy metabolism, the research objective was to identify markers consistently associated with hyperketonemia, which may serve as valuable genetic markers. Identification of these markers can aid in establishing a marker-assisted management program for Jersey dairy producers striving to effectively

manage hyperketonemia.

Key Words: Jersey, hyperketonemia, genome association study

0126 Meta-analysis of factors influencing new intramammary infection rate in experimental challenge teat dip efficacy trials. B. D. Enger[*1], R. R. White[1], S. C. Nickerson[2], L. K. Fox[3], *[1]Virginia Tech, Blacksburg, [2]University of Georgia, Athens, [3]Washington State University, Pullman.*

Using an effective teat dip before and after milking reduces the incidence of new intramammary infection (IMI) on dairies. Many factors influence a teat dip's efficacy, and this is why all teat dips should be confirmed efficacious before commercial circulation. To date, many teat dip efficacy trials have been conducted and are published in peer-reviewed journals. The objective of the present study was to conduct a meta-analysis of data from peer-reviewed teat dip efficacy trials that used an experimental challenge study design to identify factors influencing the new IMI rate. A dataset of 21 studies (148 observations) was created. The new IMI rate, based on percentage of new quarter infections/month (PNQI/mo), was calculated for each recorded observation and used as the dependent variable for model derivation. A linear, mixed-effects model with a random study effect, weighted for the standard error of the measurement was derived in a stepwise manner where parameters were sequentially eliminated for nonsignificance. The final mixed model included the terms for the causative mastitis pathogen ($n = 2$; $P = 0.55$), postmilking treatment ($n = 4$; $P < 0.01$), geographic region where the trial was conducted ($n = 3$; $P = 0.02$), and interaction between study region and pathogen group ($P < 0.01$) and postmilking treatment and pathogen group ($P < 0.01$). Overall, the new IMI rate between the causative mastitis pathogens, *Staphylococcus aureus*, 0.0409 ± 0.0097 PNQI/mo and *Streptococcus agalactiae*, 0.0344 ± 0.0096 PNQI/mo, were similar. Quarters not dipped with a postmilking teat dip, 0.0859 ± 0.0087 PNQI/mo, had a greater new IMI rate than those dipped with a postmilking teat dip containing either iodine, 0.0127 ± 0.0099 PNQI/mo, a chlorine compound, 0.0258 ± 0.0095 PNQI/mo, or an "other" active ingredient, 0.0263 ± 0.0106 PNQI/mo ($P < 0.05$). Quarters dipped with postmilking teat dips had similar new IMI rates ($P > 0.05$). Studies conducted in the southern United States, 0.0531 ± 0.0040 PNQI/mo, had a higher new IMI rate than those conducted in the Pacific Northwest, 0.0072 ± 0.0141 PNQI/mo ($P < 0.05$), but not those in the eastern United States, 0.0527 ± 0.0206 PNQI/mo ($P > 0.05$). The results of this meta-analysis indicate that using an efficacious postmilking teat dip has a greater impact on the new *Staphylococcus aureus* and *Streptococcus agalactiae* IMI rate than the active germicidal ingredients present in the postmilking teat dip itself.

Key Words: active ingredient; mastitis; teat disinfectant

0127 The effects of short-term feeding of tocopherol mix (α-, β-, γ-, and δ) on blood neutrophil function and immunometabolic-related gene expression in lactating dairy cows. Y. Qu[*1], T. H. Elsasser[2], M. Garcia[1], C. M. Scholte[1], E. E. Connor[3], J. R. Newbold[4], and K. M. Moyes[1], *[1]Department of Animal and Avian Sciences, University of Maryland, College Park, [2]USDA-ARS, Animal Biosciences and Biotechnology Laboratory, Beltsville, MD, [3]USDA-ARS, Animal Genomics and Improvement Laboratory, Beltsville, MD, [4]Cargill Innovation Center, Velddriel, the Netherlands.*

Alpha-tocopherol has been well-studied regarding improving neutrophil function, especially its involvement in respiratory burst. However, no studies have identified the effect of feeding a tocopherol mix, which contains additional isoforms, on immune cell function. The objective of this study was to investigate how short-term feeding of tocopherol mix alters bovine blood neutrophil (BBN) function and immunometabolic-related gene expression. Twelve healthy, multiparous Holstein cows (DIM: 179 ± 17d) were fed a vegetable-derived tocopherol oil supplement (Tmix) enriched with γ- and δ-isoforms (9% α-, 1% β-, 24% δ-, and 62% γ-tocopherol) at ~620 g Tmix·cow^{-1}·d^{-1} top dressed for seven consecutive days. Jugular blood (~200 mL) was collected from all cows on d 0 before feeding and on d 7 postfeeding of Tmix. Whole blood was then used to measure respiratory burst response via chemiluminescence analysis. Isolated BBN (3×10^6 cells/mL) were used for chemotaxis and immunometabolic-regulated gene expression analysis by quantitative real-time PCR. For gene expression analysis, cells were incubated with lipopolysaccharide (LPS) at a final concentration either of 0.0 or 1.5µg/mL for 2 h at 37°C, 95% humidity, and 5% CO_2. Data were analyzed as a complete randomized design. Significance was declared at $P \leq 0.05$. With regard to function, Tmix improved ($P = 0.04$) BBN chemotaxis function but did not alter ($P = 0.9$) the respiratory burst response in whole blood. For gene expression analysis, LPS challenge increased the expression of proinflammatory genes tumor necrosis factor-α and interleukin-6. However, Tmix did not alter the expression of genes associated with the immune or metabolic response. In conclusion, short-term feeding of Tmix did not impair BBN function of respiratory burst but improved chemotaxis, and Tmix did not alter the immunometabolic response of genes in BBN. Additional evaluations of the effect of individual tocopherol isoforms will offer valuable information regarding their specific roles on bovine immune cell function and gene expression.

Key Words: bovine, neutrophil, tocopherol

0128 Predicting hyperketonemia prevalence in Jersey herds from milk composition and cow test-day information using multiple linear regression.

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Multiple linear regression models have been validated to predict hyperketonemia in Holstein herds; however, potential differences in milk composition and hyperketonemia prevalence warranted further sampling and distinct models for Jersey herds. The objective of this study was to validate the use of multiple linear regression models to predict β-hydroxybutyrate (BHBA) from milk composition and continuous test-day variables in Jersey cows to serve as a diagnostic tool for determining herd-level ketosis prevalence. Blood samples were collected on the same day as milk sampling from 468 Jersey cows 5 to 20 DIM on six dairy farms. Serum BHBA concentration was quantified by colorimetric assay (Stanbio, Boerne, TX). Milk samples were analyzed for concentrations of milk BHBA, acetone, and fatty acid (FA) groups (saturated, unsaturated, trans, short, medium, and long chain FA) by fourier transform infrared (FTIR) spectrometry from MilkoScan FT+ (FOSS Analytical A/S, Hillerød, Denmark), in addition to standard milk analysis variables. Continuous test-day variables were exported from DairyComp305 (Valley Ag Software, Tulare, CA) records. Models were built in the REG procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) using forward stepwise selection by excluding variables with a P-value > 0.15 and using selection criterion of sequential sums of squares, error sums of squares, and Akaike's information criterion. Statistical parameters (R^2, adjusted R^2, root mean square error) were calculated to evaluate model performance. Hyperketonemia, defined as a serum BHBA $\geq$ 1.2 mM, prevalence within the sample set was 20%. Data interrogation justified development of separate models for primiparous and multiparous groups, as well as 5 to 11 and 12 to 20 DIM groups. Significant variables were BHBA, acetone, fat %, protein %, somatic cell count, FA groups, pervious days carried calf, age at calving, previous ME305 milk production, test-day DIM and milk production. Overall, model accuracies were 91% for multiparous cows 5 to 11 DIM (R^2 = 0.85), 86% for multiparous cows 12 to 20 DIM (R^2 = 0.64), 90% for primiparous cows 5 to 11 DIM (R^2 = 0.64), and 90% for primiparous cows 12 to 20 DIM (R^2 = 0.83). Collectively, models predicted animals with hyperketonemia at the 1.2 mM threshold with 86% accuracy. Results suggest that modeling blood BHBA based on milk composition data and cow-test day information provides a practical tool for monitoring hyperketonemia prevalence in Jersey herds.

Key Words: *ketosis, linear regression, Jersey*

0129 Liver transcriptome modifications by nutrient restriction in early lactation Holstein cows challenged with intramammary lipopolysaccharide.

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The objective was to test effects of nutrient restriction on liver transcriptome 24 h after an intramammary lipopolysaccharide (LPS) challenge in early lactation cows. At 24 ± 3 d in milk, multiparous cows were either allowed to continue ad libitum intake of a lactation diet (CON, n = 6), or the ration was diluted with barley straw (48% DM) for 4 d (RES, n = 6). On d 3, one healthy rear mammary quarter was infused with 50 µg of LPS (*E. coli* 0111:B4). Blood and liver biopsies were collected on d 4, corresponding to 24 h after LPS challenge. Liver transcriptome was analyzed with 44K bovine microarrays (Agilent Technologies). Blood and transcriptomic data were analyzed using SAS mixed models and GeneSpring (moderated t test with Westfall-Young correction, $P < 0.05$), respectively, and data mining was performed using Panther and Pathway Studio software. Energy balance did not differ before diet change. By experimental design, energy intake was limited to 41 and 97 ± 15% of NE_L requirements in RES and CON, respectively (mean ± SD; $P < 0.001$). Plasma NEFA and BHBA were greater, and glucose was lower for RES compared with CON (1221 vs. 382 µM, 2.67 vs. 0.70 mM, 56 vs. 69 mg/dL respectively, $P \leq 0.05$, before biopsy), which is consistent with 4 d of nutrient deficit in REST. We detected 77 differently expressed genes (DEG) between CON and RES, with 29 down-regulated and 48 up-regulated in RES. Genes involved in fatty acid synthesis (*ACAT2*, *FASN*, *SCD*), lactate metabolism (*LDHC*), and cortisol binding (*SERPINA6*) were down-regulated in RES, whereas those involved in fatty acid oxidation, detoxification, cholesterol synthesis, lipoprotein lipid secretion, and gluconeogenesis (*ACADVL*, *CPT1A*, *CPT1B*, *ANGPTL4*, *CYP4A11*, *HMGCSA*, *APOA1*, *APOA4*, *GK*, *PC*, and *PCK2*) were up-regulated in RES. Overall, DEG were in agreement with negative energy balance and plasma metabolite profile, and reflect a state intense lipomobilization, glucose deficit and ketogenesis in RES. Preliminary results suggest that nutrient restriction did not change liver expression of genes directly involved in immune function 24 h after

an intramammary LPS challenge.

Key Words: inflammation, liver transcriptome, undernutrition

0130 Growth and transcriptional profile analysis following oral probiotic supplementation in dairy cows.

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The objective of this study was to assess the impact of probiotic administration on growth and global gene expression profile in dairy cow. Use of probiotic supplements is a nonchemical approach to promote animal health. Understanding the mechanism of action of probiotics in cows may aid in sustainable dairy production. Lactating Holstein-Friesian cows (n = 10) received daily oral doses (50ml) of a commercial probiotic FASTtrak microbial pack (Conklin Company, Kansas City, MO) (containing *Lactobacillus acidophilus, Saccharomyces cerevisiae, Enterococcus faecium*, *Aspergillus oryza*, and *Bacillus subtilis*) over a 60-d period. Body weight was recorded weekly. Whole blood was collected at the beginning (d 0) and end of the study (d 60). Blood samples were analyzed for total and viable cell count, packed cell volume (PCV), white blood cell differential counts (WBC), and total protein concentration in plasma. Daily supplementation of probiotics had no effect on BW, PCV, and total protein concentration in plasma at the end of the study ($P > 0.05$). Percentage lymphocyte count increased ($P < 0.05$), and percentage neutrophil count ($P < 0.05$) decreased in probiotic-treated animals. Gene expression analysis identified 10,859 differentially expressed genes, 1168 up-regulated and 9691 down-regulated genes respectively following probiotic administration. Pathway analysis identified 87 bovine pathways impacted by probiotic treatment. These pathways included the Toll-like receptor signaling pathway, inflammation response and Wnt signaling pathways. Oral administration of probiotic to dairy cows has a systemic effect on global gene expression, including genes involved in immunity and homeostasis (Wnt). The results of this study show that the utilization of probiotics in animal agriculture impacts genes important to dairy cow health and production. Further definition of the interaction between the pathways involved may aid in the design of the most effective probiotics for optimum dairy production and health.

Key Words: dairy cows, innate immunity, microarray, probiotic

0131 Mammary gland transcriptome and proteome modifications by nutrient restriction in early lactation Holstein cows challenged with intramammary lipopolysaccharide.

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The objective was to evaluate the effect of nutrient restriction and intramammary lipopolysaccharide (LPS) challenge on mammary gland (MG) gene expression in early lactation cows. At 24 ± 3 d in milk, multiparous cows were either allowed to continue ad libitum intake of a lactation diet (CON, n = 6), or the ration was diluted with barley straw (48% DM) for 4 d (RES, n = 6). On d 3, one healthy rear mammary quarter was infused with 50 µg of LPS. Mammary biopsies were performed 24 h after LPS challenge. RNA and proteins analyzed using bovine 44 K microarrays (Agilent Technologies) and micro-LC-MS/MS, respectively. Transcriptomic data were analyzed using GeneSpring (moderated-t test with Westfall-Young correction, $P < 0.05$). Proteins were analyzed with Progenesis LC-MS software v.4.1 (Nonlinear Dynamics). Production and energy balance did not differ before diet change. Negative energy balance was aggravated in RES (41 vs. 97 ± 15% of requirements, mean ± SD; $P < 0.001$). A total of 87 differentially expressed genes (DEG) were highlighted through the comparison of RES vs. CON group. Among the 33 DEG identified in the transcriptomic analyses, 11 and 22 were down- and up-regulated by restriction, respectively. Among the up-regulated DEG, there were *PDK4* and *CPT1A* which are involved in the regulation of fatty acid, ketone, and glucose metabolism. *CPT1A* is the key enzyme in the carnitine dependent fatty acid transport, promoting fatty acid oxidation. Genes involved in immune response such as *PGLYRP3* and *TRIB2* were up-regulated, suggesting a higher inflammatory response in RES than CON. Proteomic analysis identified 54 proteins with 14 up- and 40 down-regulated in RES cows. Up-regulated proteins were mostly involved in gene expression mechanisms such as translation, RNA splicing and cellular protein modification. The down-regulated proteins (*e.g.*, EIF3H, RS27A, RS15) take part in protein metabolism. This is coherent with transcriptomic results, namely the down-regulation of *RPL 37A*, a component of ribosomal complex, which catalyzes protein synthesis and may partially explain the lower milk protein yield in RES (834 vs. 1163 g/d; P = 0.02). Proteins involved in antigen processing and presentation were down-regulated in RES compared with CON, suggesting an impaired ability to counteract inflammation in RES MG. Preliminary transcriptomics and proteomics analyses show that undernutrition may influence the MG response

to inflammation at each level of gene expression.

Key Words: mammary omics, undernutrition, inflammation

0132 Methionine supplementation modulates the inflammatory response of dairy cow blood neutrophils in response to lipopolysaccharide.

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Methionine (Met) is among the two most-limiting amino acids for milk production in dairy cow diets. The accepted optimal ratio when formulating diets is a Lys:Met of 3:1. However, blood from cows fed corn silage-based diets without supplemental rumen-protected Met averages ~3.6:1. Our recent in vivo research on immunonutrition revealed the immune system could benefit from additional Met. To study the effect of different Lys:Met ratios, blood neutrophils were isolated from five mid-lactating pluriparous Holstein cows (238 ± 20 DIM, 33.8 ± 3.9 kg/d average milk production) to obtain a homogenous pool. Neutrophils were then incubated at a concentration of 6 × 10^6 cells/mL for 2 h in a sterile incubator at 37°C and 5% atmospheric CO_2. A 3 × 2 factorial arrangement of treatments including three Lys:Met ratios (3.6:1, 2.9:1, 2.4:1) and two levels of lipopolysaccharide (LPS, 0 and 50 μg/mL) were evaluated in triplicate. After incubation, cellular RNA was used to measure expression of genes related to immune function and oxidative stress. Data were $\log_2$ normalized and subjected to ANOVA using the Proc MIXED procedure of SAS. As expected, LPS increased ($P < 0.05$) the expression of pro- and noninflammatory cytokines (*IL1B*, *IL10*, *IL6*, *TNF*) and immune-related nuclear receptors (*NFKB1*, *NR3C1*). However, LPS decreased ($P < 0.05$) the expression of chemokine *CXCR1* and antimicrobial enzyme *LYZ*, the latter only when cells were incubated with higher Met (2.9:1 or 2.4:1), and had no effect ($P < 0.05$) on other pathogen killing mechanisms (*MPO, SOD1*). Among genes related to Met metabolism, LPS increased ($P < 0.05$) expression of *MAT1A*, while reducing expression of *GPX1* and *GSR*, suggesting a greater use of Met and a reduced antioxidant system during the inflammatory response. Compared with the lowest level of supplemental Met (3.6:1 Lys:Met) the highest level (2.4:1 Lys:Met) decreased ($P < 0.05$) expression of *NFKB1*, *NR3C1*, and *GSR*, while it increased ($P < 0.05$) *IL6* independently of the LPS level. Furthermore, expression of the noninflammatory cytokine *IL10* was greatest ($P < 0.05$) at 2.4:1 Lys:Met and in non-LPS challenged cells, indicating that supplemental Met improved the oxidative status and the noninflammatory conditions of neutrophils. Overall, data support the idea that Met supplementation could improve the inflammatory and oxidative status of bovine neutrophils.

Key Words: LPS, methionine, neutrophils

0133 Feasibility and safety of nitric oxide releasing solution as a treatment for bovine mastitis.

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Nitric oxide releasing solution (NORS) is a liquid formulation that releases nitric oxide (NO). NO is a broad, nonspecies-specific nonmicrobial nanomolecule, endogenously produced during the innate response in mammals. The objective of this study was to explore the feasibility of NORS as a potential treatment for bovine mastitis (BM). Two common pathogens found in BM (10^6 CFU/mL of *Escherichia coli* and *Staphylococcus aureus*) were added to raw milk from healthy cows, as an in vitro model, to determine the antimicrobial efficacy of NORS at different concentrations (100–400 mM) and at two different NORS:milk ratios (2:1 and 1:1). Next, 10 ex vivo samples of milk from dairy cows presenting with clinical mastitis were obtained and treated with NORS to confirm efficacy. A dose escalating safety study was then performed using three dairy cows, where 40 mL of increasing concentrations of NORS (50–400 mM) were infused into the teat. Nitrite and methemoglobin levels were measured 5, 30, and 460 min posttreatment and nitrites in milk were measured 8 and 24 h posttreatment. Nitrite was measured by chemiluminescence, while methemoglobin was measured using a CO-Oximeter. Results show that NORS could eradicate, in vitro, both bacteria, and was dilution and time dependent. In the 2:1 ratio, NORS significantly ($P < 0.05$) reduced bacterial concentration in milk both in vitro and ex vivo within 2 min, and had no detectable bacteria after 5 min of exposure. In the 1:1 ratio, a significant bacterial load reduction ($P < 0.01$) occurred within 10 min and no detectable bacteria within 20 to 30 min. In the safety study we found an increase in blood nitrites ($P < 0.05$) within 5 min of the NORS treatment at all concentrations. After 8 h, the blood values at all concentrations returned to baseline ($P = 0.27$). Blood methemoglobin changes were nominally increased in the 5 and 30 min samples post 400 mM NORS and no detectable change was seen after 8 h. Eight hours posttreatment, before the evening milking, milk nitrites were 18 times higher than baseline, while 24 h posttreatment nitrites returned to baseline level ($P = 0.36$). NORS was found to eradicate bacteria in milk and, clinically, the treatment was well tolerated. This suggests that NORS has a potential to be a safe and effective nonmicrobial treatment for BM and may allow salable milk during antimicrobial treatment of mastitis. Further, it would provide an alternative to antibiotics, thus contributing to the reduction of antibiotic drug resistance. Further studies are justified.

Key Words: mastitis, nitric oxide, safety study, treatment

0134 Methionine coupled with choline supplementation alters inflammation and oxidative stress gene network expression of dairy cow blood neutrophils. M. Vailati Riboni[*1], A. Bellingeri[2], I. Khan[3], and J. J. Loor[1], [1]*University of Illinois, Urbana,* [2]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [3]*University of Agriculture, Peshawar, Pakistan.*

The nutritional status of dairy cows is tightly-correlated to the maintenance of proper immune function and health. Methionine (Met), besides being one of the first-limiting amino acids, has stimulatory effects on immune cells both directly and indirectly as a source of antioxidants. The objective of this study was to investigate the effect of supplementing Met or choline as its potential precursor on neutrophil gene expression. Blood neutrophils were isolated from five lactating multiparous Holstein cows (153 ± 5 DIM, 34.6 ± 2.7 kg/d average milk production) to obtain a homogenous pool. Cells were then incubated at a concentration of 6×10^6 cells/mL for 4 h in a sterile incubator at 37°C and 5% atmospheric CO_2. A 3 × 3 factorial arrangement of treatments, including three Lys:Met ratios (3.6:1, 2.9:1, 2.4:1) and three levels of choline chloride (3, 400, 800 μg/mL), were evaluated in triplicate cultures. Cellular RNA was used to measure expression of genes related to inflammation, antioxidant status, and the Met cycle. Data were $\log_2$ normalized and subjected to ANOVA using the Proc MIXED procedure of SAS. The greater expression ($P < 0.05$) of *MTR* at 2.9:1 and 2.4:1 Lys:Met indicated greater flux through the Met cycle compared with 3.6:1 Lys:Met. Both *BHMT* and *CHDH* were undetectable, indicating that neutrophils cannot generate Met from choline through the betaine pathway. Compared with the lowest level of supplementation (3 μg/mL), at the highest level of choline (800 μg/mL) there was lower expression ($P < 0.05$) of pro- (*IL6*, *IL1B*) and noninflammatory (*IL10*) cytokines, and antimicrobial mechanisms (*MPO*, *SOD1*), coupled with lower expression ($P < 0.05$) of genes related to the antioxidant system (*CDO1*, *CSAD*, *CTH*, *GSR*, *GSS*). These indicated a degree of inflammation, together with oxidative stress in neutrophils at the low choline level. In contrast, the interaction among treatments revealed that at higher (2.9:1 and 2.4:1 Lys:Met) Met and low choline (3 μg/mL) supplementation level the expression of *GSS*, *GSR*, *IL1B*, and *IL6* was lower ($P < 0.05$), hence, limiting the negative effect of a low choline level. Furthermore, higher Met supplementation increased ($P < 0.05$) neutrophil recognition capacity (*TLR4*, *SELL*) when incubated together with 400 μg/mL of choline. Overall, data indicate a choline requirement in bovine neutrophils that could potentially be overcome by Met supplementation. Despite this, neutrophil function appeared to be enhanced at high Met together with adequate choline supplementation.

Key Words: methionine, choline, neutrophils

0135 Impact of a BRDC vaccine with a MLV or KV IBR component on the innate inflammatory profile of nulliparous heifers. C. L. Widener*, D. J. Hurley, W. M. Graves, A. H. Nelson, D. A. L. Lourenco, and J. F. Bohlen, *University of Georgia, Athens.*

To investigate the difference in the inflammatory response between bovine respiratory disease complex (BRDC) vaccines containing either a modified live vaccine (MLV) or a killed component for infectious bovine rhinotracheitis (IBR), 28 Holstein heifers (mean ± SD; 12.4 ± 0.5 mo) in two replicates (spring $n = 12$ and fall $n = 16$) were synchronized for estrus using a 7-d CIDR protocol. This protocol included two injections of $PGF_{2\alpha}$, one at CIDR removal and a follow-up injection 16 h later. All animals were calf-hood vaccinated with an available BRDC vaccine with a modified live IBR component. At approximately Heat 2, heifers were vaccinated with either the calf-hood MLV ($n = 14$) or a BRDC vaccine with a killed ($n = 14$) IBR component. Heifers were vaccination blocked according to prevaccination bovine viral diarrhea virus (BVDV) serum neutralizing (SN) titers. On d –7, relative to vaccination, a complete blood count (CBC) and an assay to measure neutrophil activity, as indicated by the relative presence of reactive oxygen species (ROS) were performed to establish a baseline immune profile. Two heifers were removed from the trial for preexisting immunological challenge. These assays were repeated on d 1 postvaccination, d 3 postvaccination, and then weekly until the heifer was bred (Heat 4). Data were analyzed with the PROC MIXED procedure of SAS. The fixed effects for the model were: season, vaccine type, and week relative to vaccination. There was no difference ($P > 0.05$) in postvaccination SN titers. Vaccine type had no significant effect ($P > 0.05$) on any of the cell types measured by the CBC. Season did have a significant effect ($P = 0.0008$) on the circulating lymphocytes, with the fall heifers exhibiting higher average lymphocytes on d 1 and wk 2, 3, 4, 5, and 6 postvaccination. The ROS response ratio was not impacted by vaccine ($P > 0.05$) but was influenced ($P < 0.0001$) by both season and the season × week interaction. When comparing seasons, spring heifers maintained a higher average response ratio when compared with the fall heifers on d 3 and wk 1, 2, 3, 5, and 6 postvaccination, which is substantiated by their higher average circulating granulocytes ($P < 0.05$). These seasonal differences may be a consequence the severe immunological challenge experienced by the fall group shortly after vaccination, which may have obscured their true vaccine response.

Key Words: neutrophil, ROS, IBR MLV

0136 Association between bovine milk infrared temperature and bacteriological results from CHROMagar Mastitis Plates and PathoProof Mastitis Complete-16 Kit. M. G. Marrero-Pérez*, J. Curbelo-Rodríguez[2], G. Ortiz-Colón, H. L. Sánchez-Rodríguez, and Y. R. Vélez-Robles, *University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico.*

Pathogen identification is an important tool for dairy farmers to treat mastitis infections properly. Alternative tools for early identification of mastitis cases should help farmers to increase milk quality and animal health. Infrared thermography (IRT) was used to determine the milk's temperature profile through the short milking tube, and its association with CHROMagar Mastitis plates (CHROM) and the PathoProof Mastitis Complete-16 Kit (PtoPrf-16) bacteriological results were evaluated. Individual mammary quarters (n = 23) with subclinical mastitis (determined by the California Mastitis Test) from two dairy herds in Puerto Rico were evaluated. Milk samples (10 mL in duplicates) were collected and stored in ice for subsequent evaluation. During the morning milking (from 0300 to 0600 h), IRT images were collected 2 min postmilking unit attachment in 30s intervals using an IRT camera (FLIR-E8). Temperature and relative humidity were also recorded using HOBO-U23Prov2Data Loggers. Somatic cell count (SCC) and bacterial identification were determined using a DeLaval Cell counter and CHROM, respectively. Additional samples were sent to the Dairy Herd Improvement Association (Manheim, PA) for bacterial identification using the PtoPrf-16, to be compared with CHROM results. A PROC GLIMMIX in SAS (University Edition version) was used to determine differences in IRT and logSCC by bacteriological results (CHROM vs. PtoPrf-16). The two herds had no difference in logSCC and IRT (P = 0.10); therefore, quarters were analyzed collectively. No differences in IRT or logSCC were found when mastitis pathogens were or not isolated in quarter milk samples using the PtoPrf-16 (P = 0.29 and P = 0.07) and CHROM (P = 0.80 and P = 0.67), respectively. No differences in IRT were observed when PtoPrf-16 (P = 0.69) and CHROM results (P = 0.91) were further categorized by Gram-positive, Gram-negative, mixed isolation, and no-detection, with mean IRT and standard error values of 33.61 ± 0.05 and 33.11 ± 0.36, 33.26 ± 0.62 and 33.75 ± 0.45, 33.86 ± 0.55 and 32.67 ± 0.57, and 32.98 ± 0.43 and 33.36 ± 0.72, respectively. The CHROM and PtoPrf-16 tests can identify 10 and 15 different mastitis pathogens, respectively. However, only 34.78% of bacteriological results concurred among them. The IRT was affected by relative humidity (P < 0.05) but not by ambient temperature (P = 0.16). More data is required to characterize the use of IRT as a tool to discriminate quarters with mastitis. The lack of association among IRT and mastitis pathogens could be attributed to environmental factors such as relative humidity. The discrepancies among bacteriological results among CHROM vs. PtoPrf-16 suggest that additional studies are required to further characterize these differences.

Key Words: CHROMagar, infrared thermography, PathoProof Mastitis

0137 The endometrial microbiome in transition cows fed an energy-restricted diet. G. Esposito*[1,2], J. J. Lim[3], T. Tasara[4], P. C. Irons[2,5], E. C. Webb[2], and A. Chapwanya[3], *[1]Department of Production Animal Studies, Faculty of Veterinary Sciences, University of Pretoria, Pretoria, South Africa, [2]Institute of Food, Nutrition and Well-being, University of Pretoria, Pretoria, South Africa, [3]Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, [4]3Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland, [5]Department of Production Animal Studies, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, South Africa.*

The objective of this study was to evaluate the effect of negative energy balance (NEB) on the endometrial microbiome of transition cows. Ten Holstein cows blocked by parity, BW, and BCS were randomly assigned to two groups: control (CTL) and NEB (80% of the net energy required). Endometrial cytobrush samples were collected at 14 and 35 d postpartum (DPP) and DNA was extracted using a QIAamp DNA Micro Kit. Based on next generation sequencing of 16S rRNA genes, 403 operational taxonomic units (OTUs) were detected. All continuous variables were analyzed by ANOVA. A comparison of the alpha diversity, based on the number of OTUs present, revealed no significant differences in uterine microbiome diversity between 14 and 35 DPP in the CTL group, but significantly (P < 0.05) lower diversity 14 and 35 DPP within the NEB group. The CTL group microbiome on 14 DPP showed significantly (P < 0.05) higher diversity compared with the NEB group. At genus level, the majority of OTUs detected were shared between 14 and 35 DPP (CTL, 47/68 [69%]; NEB, 40/65 [61.5%]) within both groups. However, in the CTL group, there were 16 OTUs detected at 14 DPP that were not present at 35 DPP, while 5 OTUs found at 35 DPP were not detected at 14 DPP; in the NEB group, 4 OTUs at 14 DPP were not detected at 35 DPP, whereas 21 OTUs detected at 35 DPP were not present on 14 DPP. At phylum level, on 14 DPP, Proteobacteria (39.2 vs. 0.46%) and Firmicutes (44.3 vs. 33.7%) were higher in CTL compared with the NEB uterine microbiome; Bacteroidetes (29.9 vs. 8.9%), Fusobacteria (14.3 vs. 3.4%), and unassigned (20.1 vs. 1.4%) phyla predominated in NEB compared with CTL. On 35 DPP, the CTL microbiome was predominated by Cyanobacteria and Proteobacteria sequences, while the NEB microbiome was predominated by Firmicutes and Bacteroidetes. Differences in Proteobacteria and Bacteroidetes levels at 14 and 35 DPP, respectively, were statistically significant (P < 0.05). Relative

abundances of Actinobacteria, Cyanobacteria, and Proteobacteria were significantly higher at 35 DPP compared with 14 in NEB cows. No significant differences were detected in CTL. Our preliminary data although inconclusive due to small sample size and individual animal microbiome variations suggest that there might be some microbiome composition differences between the CTL and NEB at 14 and 35 dpp.

Key Words: NEB, endometrial microbiome, next generation sequencing

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0138 Fecal microbial shifts of the German Holstein dairy cows with left-sided displacement of the abomasum.

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One of the most common diseases in high-performance German Holstein dairy cows is left-sided displacement of the abomasum (LDA). Hypomotility of the abomasum is detrimental during the pathogenesis of LDA. Also, it is known that the improper interactions between the gut microbiota and the enteric nervous system contribute to dysfunctions of gastrointestinal motility. Therefore, we hypothesized that the gut microbial composition will be different between German Holstein dairy cows with and without LDA. We compared the fecal microbiota between cows with and without LDA using 16S ribosomal RNA (rRNA) gene analysis. A total of 20 German Holstein dairy cows at one dairy farm in South Korea, including eight cows without LDA (control group) and 12 cows with LDA (LDA Group), were enrolled in this study. All cows were housed under the same conditions and were fed the same feed without any antibiotics or supplementary additives. Right after LDA was diagnosed, fecal samples were collected immediately from the rectum. Total DNA representing the fecal microbial communities was extracted from individual fecal samples using the stool DNA extraction kit, and the 16S universal primers 27F (5′ GAGTTTGATCMTGGCTCAG 3′) and 800R (5′ TACCAGGGTATCTAATCC 3′) were used to amplify 16S rRNA genes (V1-V4 hyper variable regions). The composition and relative abundance of each member of the microbiota in feces from the control group were different from the LDA group. The proportion of Spirochaetes was significantly different between groups at the phylum level ($P <$ 0.001). An average of 1.5% of the microbiota was members of Spirochaetes in the feces of the control group. On the other hand, there were no Spirochaetes detected in the feces of the LDA group. At the genus level, relative abundance of five genera was significantly different between groups. The proportion of the genus *Enterohabdus* (a member of Actinobacteria), the proportions of members of Firmicutes including *Cellulosilyticum*, *Streptococcus*, and *Turicibacter*, and the proportion of *Treponema* (a member of Spirochaetes) were all significantly higher in the control group than in the LDA group. However, further studies will be needed to elucidate the roles of these genera in the pathogenesis of LDA. Overall, results from this study show that the fecal microbial compositions of German Holstein dairy cows with LDA shifted and were less diverse than those in normal cows.

Key Words: German Holstein dairy cow, microbiome, LDA

0139 Genetic parameters and impact of postpartum diseases on lactation curves in dairy cattle.

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Genetic improvement for milk yield in dairy cattle has impacted the shape of the lactation curve, in addition to the total production. Likewise, incidence of diseases early in the lactation can affect the lactation curve. The objective of this study was to investigate simultaneously health and genetic factors influencing the lactation curve. Test-day milk records on more than 6000 Holstein cows across four states (California, Florida, Minnesota, Texas) and nine herds were evaluated. The trajectory of the lactation curve was modeled using nonlinear mixed effects models including Wood's and Wilmink's functions. The effects of environmental and health indicators on the level of milk yield, increase in milk production early in lactation (Wood's) or milk yield at peak (Wilmink's), and persistency thereafter were evaluated. These effects included: season (summer or winter), state, parity, vaginal mucus score at 7 d postpartum, metritis at 7 d postpartum, mastitis cases within the first 60 d postpartum, blood β-hydroxybutyrate (BHBA) indicating subclinical ketosis, body condition score at 35 d (BCS35), displaced abomasum (DA) by 60 d postpartum, respiratory illness by 60 d postpartum (Resp), and lameness at 35 d. Sire of cow was included in the model as random effect. Estimates from the Wood's model indicated that multiparous cows have significantly higher levels of milk yield immediately after calving and lower persistency than primiparous cows. Lactation curves in winter had higher yield immediately after calving and lower persistency than in summer. Metritis had a negative effect on milk yield level immediately

after calving as well as on persistency. Mucus score and DA had a negative impact on milk yield immediately after calving. Consistent with Wood's estimates, Wilmink's estimates indicated that multiparous cows have higher milk production and lower persistency than primiparous cows. Number of mastitis cases and DA were associated with lower overall milk production and higher persistency. Beta hydroxybutyrate was associated with a higher level of milk yield and lower persistency. The ratio of sire to residual variance estimates from Wood's and Wilmink's functions were consistent and approximately 0.4. Wood's model offered a better fit for the lactation curves considered. Our findings demonstrate the need to incorporate disease indicators on the assessment of the genetic component influencing the trajectory of the lactation curve. These findings contribute to a long-term multistate project database (USDA-NIFA-AFRI-003542) for direct measures of fertility.

Key Words: lactation curves, metritis, nonlinear mixed models

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0140 Genetic and environmental components of disease traits in dairy cattle.

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Diseases in U.S. Holstein are responsible for losses of approximately $ 1 billion annually in dairy production due to reduced milk production and increased costs. The objective was to assess the impact of environmental factors and magnitude of genetic parameters on the incidence of diseases in dairy cows early (<10 d) and late (35 to 60 d) postpartum. Binary and multinomial disease records on approximately 6000 Holstein cows from farms in Texas, Minnesota, California, and Florida were evaluated using mixed effects logistic and Poisson models. Early postpartum binary diseases included: dystocia, retained placenta, subclinical ketosis (blood β-hydroxybutyrate BHBA > 1), and metritis. Late postpartum binary diseases included: displacement of abomasum, mastitis, respiratory problems, and clinical endometritis. Mucus score at 7 d, number of mastitis cases up to 60 d, and lameness at 35 d (five levels) were analyzed assuming a Poisson model. Fixed effects in all models included: lactation number (3 levels), season (summer and winter), U.S. region, and farm. Other fixed effects evaluated depending on the disease included: twins, body condition score, BHBA level, calf gender, stillbirth, first test-day milk production record, and other diseases. The cow's sire was included as a random effect in the models. Overall lactation, region, and season had a significant effect on the incidence of all diseases, except for lactation on respiratory problems, and season on mastitis and displacement of abomasum. First lactation cows exhibited the highest incidence of dystocia, metritis, and clinical endometritis and lowest incidence of mastitis, retained placenta, lameness, and displacement of abomasum. Clinical endometritis, metritis, lameness, and respiratory problems were lower in summer than winter. Dystocia, retained placenta, and subclinical ketosis were positively and significantly associated with clinical endometritis and metritis. Subclinical ketosis and dystocia were positively and significantly associated with displacement of abomasum. Mastitis was negatively and significantly associated with milk yield at first test-day. Heritability estimates for the diseases ranged from 0.06 (retained placenta) to 0.4 (respiratory problems). The differences in genetic parameter estimates among alternative disease descriptors offer insights into effective approaches to lower the incidence of disease through genetic selection. These findings contribute to a long-term multistate project database (USDA-NIFA-AFRI-003542) for direct measures of fertility.

Key Words: metritis, postpartum, production

0141 Undernutrition alters metabolic responses to acute inflammation in early lactation cows.

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The objective was to test effects of nutrient restriction on responses to an intramammary lipopolysaccharide (LPS) challenge in early lactation cows. Multiparous Holstein cows were either allowed ad libitum intake of a lactation diet throughout the study (CON, n = 9, 7.1 MJ/kg DM NE_L, 17.4% CP), or the ration was diluted with barley straw (48% DM) for 4 d (RES, n = 8, 5.2 MJ/kg DM NE_L, 12.2% CP) starting at 24 ± 3 d in milk. After 72 h, one healthy rear mammary quarter was infused with 50 µg of LPS (*E. coli* 0111:B4). Blood samples were collected at –1.5, –0.5, 1, 2, 4, 6, and 10 h relative to LPS. Data were analyzed using SAS mixed models. Intake, milk, and protein yields and NE_L balance did not differ before diet change (21.8, 39.0, 1.15 kg/d, and –5.6 MJ/d, respectively, on d –1), but were significantly affected in RES (9.8, 28.3, 0.79 kg/d and –74 MJ/d, respectively, on d 3 of restriction and before LPS), as were plasma indicators (Table 1). Insulin response (area under the curve, AUC) to LPS was lower

Table 0141.

Table 1. Plasma insulin and metabolite concentration at 72 h of dietary treatments and response to LPS challenge. $P < 0.01$ for all variables.

	Treatments	
	CON	RES
Insulin (μIU/mL)		
72 h	17	11
AUC_{10h} [1]	174	42
NEFA (μM)		
72 h	370	1672
AUC_{10h}	-1,957	-9,047
BHBA (mM)		
72 h	0.69	2.98
AUC_{10h}	3.68	-6.05
Glucose (mg/dL)		
72 h	69	50
AUC_{10h}	-17	64

[1] Incremental area under the curve during 10 h post-LPS, concentration units per 10 h.

in RES compared with CON, but it was greater for NEFA, BHBA, and glucose. The NEFA nadir post LPS was 599 and 101 μM at 4 h for RES and CON ($P \leq 0.001$), respectively, and it preceded insulin change in RES. The BHBA decrease in RES was consistent with NEFA response to LPS, but BHBA increased from a low baseline in CON (treatment × time interactions, $P \leq 0.05$). The negative glucose AUC in CON could be related to the insulin increase post LPS. Rectal temperature increase did not differ between treatments (+2.1 ± 0.15°C at 6 h). Nutrient restriction altered peripheral metabolic responses to an intramammary LPS challenge.

Key Words: inflammation, undernutrition, dairy cow

0142 Potential modulation of the toxic effects of *Escherichia coli* in bovine endometrium by lactic acid bacteria. S. Genís[*1], A. Sánchez-Chardi[2], A. Bach[3,4], and A. Arís[1]. [1]*Department of Ruminant Production, IRTA, Caldes de Montbui, Spain,* [2]*Servei de Microscopia, UAB, Cerdanyola del Valles, Spain,* [3]*ICREA, Barcelona, Spain,* [4]*IRTA, Caldes de Montbui, Spain.*

The ultrastructural assessment of toxic effects using field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) can provide important information to elucidate the mechanisms of infection and to develop preventive strategies. The aim of this study was to evaluate the effects of a lactic acid bacteria (LAB) combination, based on *L. rhamnosus* MOI 25, *P. acidilactici* MOI 25, and *L. reuteri* MOI 2, at preventing *Escherichia coli* infection and maintaining bovine endometrial tissue health. Triplicate samples of epithelial cell cultures were studied in a 2 × 2 factorial design in the presence or absence of an *E. coli* infection and with or without LAB. Samples were mounted in FESEM stubs and observed without coating in a Zeiss Merlin microscope. A qualitative assessment of general structure of the epithelium (size and shape of cells, ultrastructure, and amounts of ultrastructure of microvilli), presence of *E. coli* and LAB in cell surface, cell debris, presence of mucus in the cell surface, mitochondrial damage, and cell death was performed by the analysis of 10 random selected areas for each treatment. For TEM, contrasted ultrathin sections were observed in a Jeol 1400 operating at 80kV. A semiquantitative approach was performed by the analysis of 10 random selected sections in three areas for each treatment and data were analyzed using a Fisher exact test. *Escherichia coli* alone or with LAB appeared in low numbers in epithelial cells surface and in no case formed biofilms or interactions between each other. *Escherichia coli* abundance was lower ($P < 0.05$) in samples treated with LAB than in those infected with *E. coli* alone. Healthy epithelium was observed in cells treated with LAB (epithelial cells with normal size and shape and normal aspect of microvilli), whereas in cultures infected with *E. coli*, abundant areas with cell debris and bacilli in epithelial cell surface were observed. The incidence of necrosis (as assessed by TEM) in *E. coli* samples tended ($P = 0.07$) to be greater than in noninfected cultures. Control or LAB preincubated cells showed less mitochondrial damage ($P = 0.01$) than nontreated cells, a parameter strongly related to cell death. Overall, LAB appear to offer protection against *E. coli*, by mechanism different than the formation of biofilms, and thus, LAB combinations could be used as a preventive strategy for metritis.

Key Words: endometrium, FESEM, TEM

0143 **Meta-analysis of factors influencing new intramammary infection rate in natural exposure teat dip efficacy trials.**

0144 Effects of lactic acid bacteria on metritis prevalence and endometrium inflammation in dairy cows. S. Genís*[1], R. L. A. Cerri[2], A. Bach[3,4], B. F. Silper[2], J. Denis-Robichaud[5], and A. Arís[1], *[1]Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, [2]Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, [3]ICREA, Barcelona, Spain, [4]IRTA, Caldes de Montbui, Spain, [5]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

The aim of this study was to evaluate the effects of a treatment with *Lactobacillus rhamnosus*, *Pediococcus acidilactici*, and *Lactobacillus reuteri* (LAB) on the prevalence of metritis and the modulation of endometrial inflammation in dairy cows. In Experiment 1, 135 cows were enrolled 3 wk before calving and randomly assigned to treatments to ensure similar frequencies for parity and previous illness in all treatment groups. The treatment groups were: (1) two intravaginal doses of lactic acid bacteria (LAB) per wk during 3 wk precalving (vaginal); (2) 1 intrauterine dose 1 d after calving (endometrial); and (3) no intervention (control). Metritis was diagnosed at d 6 when body temperature > 39.5°C and purulent vaginal discharge (>50% pus or worse) was observed. Data were analyzed using a chi-square. Vaginal treatment reduced ($P < 0.05$) metritis prevalence up to 62% compared with the Control group. However, prevalence of metritis did not differ between the endometrial and control groups. In Experiment 2, a combination of in vivo and ex vivo assays to evaluate whether LAB exerted some effects on the endometrium was designed. Twenty healthy do-not-breed cows were enrolled in Experiment 2, and 3 wk before culling were randomly distributed into two treatment groups: (1) 2 doses of vaginal LAB per wk during 3 wk (LAB); and (2) two vaginal doses of carrier (sterile sodium chloride 0.9%) per wk during 3 wk (CTRL). Endometrium was recovered at slaughterhouse and cut in the laboratory in 0.8 cm^2 explants that were incubated by sixtiplicate in 24 well-plates and either infected with *Escherichia coli* or maintained in medium for evaluating the basal expression of proinflammatory genes in the endometrium. Supernatant was collected for IL-8, IL-1β, and IL-6 analysis by ELISA. Explants were recovered for the quantification of proinflammatory gene expression by qPCR. Data were analyzed using an ANOVA, considering treatment and infection as fixed effects and animal as a random effect. Neither the expression of proinflammatory genes nor the direct quantification of IL-8, IL-1b, or Il-6 differed between infected and noninfected explants. In conclusion, when an intravaginal treatment of LAB is applied there is an important reduction on metritis prevalence although this reduction is not mediated by a direct effect the probiotic on the endometrium neither by increasing the protection against *E. coli* nor by reducing basal inflammation.

Key Words: *Escherichia coli*, LAB, metritis.

0145 Metritis severity score misclassification underpredicts consequence cost of disease. M. M. McCarthy* and M. W. Overton, *Elanco Animal Health, Greenfield, IN.*

The objective of this research was to determine the impact of disease misclassification on the estimated impact of metritis on milk production and time to pregnancy. Differential misclassification introduces bias that usually results in underestimating the true association. A convenience sample of DairyComp305 data representing 1 yr of calvings ($n = 3485$) from one midwestern Holstein herd was used. This herd was chosen because it had good recorded incidence of both mild and severe metritis cases. The original dataset included metritis recorded as mild or severe, or not recorded (NR) where no metritis was observed, and was considered to contain the metritis true severity (TS). First, to evaluate the impact of misclassification bias, we retrospectively randomized 40% of mild metritis to be classified as NR to represent inconsistent disease recording (IR); then, all mild metritis cases were changed to NR to represent poor disease recording (PR). The TS, IR, and PR datasets were analyzed separately in JMP. ANOVA was conducted for second test 305 d mature equivalent (2nd305ME), and a multivariate Cox proportional hazards model was conducted for time to pregnancy, censoring at 300 d in milk. Nonsignificant variables were removed when $P > 0.10$, but the variable metritis was forced into all models. Based on the TS model, adjusting for effects of lactation group, month fresh, early lactation mastitis and displaced abomasum, a case of mild metritis was associated with 405 kg less 2nd305ME and a case of severe metritis was associated with 1106 kg less 2nd305ME compared with no metritis. For the IR model, a case of mild metritis was associated with 376 kg less 2nd305ME and a case of severe metritis was associated with 1050 kg less 2nd305ME compared with no metritis. For the PR model, severe metritis was associated with 990 kg less 2nd305ME compared with NR. The IR and PR models underestimated 2nd305ME loss for severe metritis cases by 56 and 116 kg/cow, resulting in 8721 and 18,007 kg of milk loss unaccounted for at the herd level, respectively, compared with TS. For the TS model, cows that did not have metritis were 1.31 times more likely to get pregnant than cows with severe metritis ($P = 0.01$). The risk ratio difference in IR and PR models were 0.03 and 0.08, respectively. Overall, misclassification of metritis cases results in greater bias and largely underestimates the true association between metritis

and consequence costs of the disease.

Key Words: disease consequence, metritis severity, misclassification bias

0146 Subacute ruminal acidosis negatively affects conception rate in Holstein heifers. H. Khalouei*[1], A. A. Alamouti[2], A. Mohammadi-Sangcheshmeh[2], N. Farzaneh[3], J. C. Plaizier[1], and E. Khafipour[1], *[1]Department of Animal Science, University of Manitoba, Winnipeg, Canada, [2]Department of Animal and Poultry Sciences, Aburaihan Campus, University of Tehran, Pakdasht, Tehran, Iran, [3]Faculty of Veterinary Medicine, Ferdowsi University, Mashhad, Iran.*

Symptoms of subacute ruminal acidosis (SARA) have been studied at length, but its effects on reproductive performance are not fully understood. Our objective was, therefore, to determine if experimentally induced SARA reduces conception rates of Holstein heifers. One hundred and ten heifers were synchronized for artificial insemination by two injections of $PGF_{2\alpha}$ in a 13 d interval, and assigned randomly to two treatments. The control heifers received a diet containing 32% (DM basis) barley-based concentrate, while the SARA challenge group received a diet containing 68% of this concentrate. The remainder of the diet consisted of corn silage, alfalfa hay, wheat straw, soybean meal, wheat bran, and a vitamin-mineral supplement. Diets were fed ad libitum. The SARA challenge diet started 3 d after the second $PGF_{2\alpha}$ injection, and continued for 7 d. Forty one heifers from the SARA group and 39 heifers from the control group showed visible signs of heat and were inseminated. Heifers in SARA group had higher DMI (10.4 vs. 9.0 kg d^{-1}, $P < 0.01$) and lower rumen pH (6.02 vs. 6.45, $P < 0.01$) and fecal pH (6.71 vs. 6.97, $P < 0.01$) at 6 h post feeding compared with control heifers. The SARA challenge increased rumen concentrations of ruminal lactate, propionate, and valerate, but did not affect the concentrations of acetate, butyrate, and isovalerate. The challenge did not affect glucose, urea nitrogen, aspartate aminotransferase, calcium, and cortisol concentrations in blood, but it lowered blood β-hydroxybutyrate ($P < 0.01$). Induction of SARA markedly reduced first service conception rate tested by ultrasonography 28 d after insemination (53.7 vs. 71.8%, $P < 0.05$). Additionally, 100% of control heifers that were confirmed as pregnant in the 28 d test were also pregnant at 60 d test, whereas this ratio was only 73.9% ($P < 0.01$) in SARA-challenged heifers, suggesting that SARA had a persistent effect on reproduction that lasted at least 60 d after insemination. Results suggest a negative effect of SARA on fertility of dairy heifers. Further studies are required to investigate the possible effects of lipopolysaccharide translocation and systemic immune response that is associated with SARA on embryo survivability to fully elucidate the mode of action of SARA on reproductive performance.

Key Words: conception rate, fertility, subacute ruminal acidosis

0147 Evaluating milk fat to protein ratio and milk fat to lactose ratio as indicators for early lactation disease. S. Paudyal*[1,2], F. P. Maunsell[3], C. A. Risco[3], A. Donovan[3], A. De Vries[4], D. Manriquez[1], and P. J. Pinedo[1,5], *[1]Department of Animal Sciences, Colorado State University, Fort Collins, [2]West Texas A&M, Canyon, [3]College of Veterinary Medicine, University of Florida, Gainesville, [4]Department of Animal Sciences, University of Florida, Gainesville, [5]Texas A&M AgriLife Research, Amarillo.*

The objective was to evaluate the potential of milk fat to protein (FP) and milk fat to lactose (FL) ratios for detection of clinical disease before evident clinical signs. Milk component data from 198 Holstein cows were recorded until 60 days in milk (DIM), using the AfiLab® milk analysis system at the University of Florida (UF) Dairy Unit. Milk components were recorded as an average of AM and PM milkings. Occurrence of health disorders (mastitis [MAS], metritis [MET], clinical hypocalcemia [HYC], digestive disorders [DIG], lameness [LAM], and ketosis [KET]) were assessed by UF veterinarians and farm personnel. Two indices were developed: (i) Cow index (CI) = measurement on the day of diagnosis (d 0) minus –3 to –5 d average relative to d 0, divided by the –3 to –5 d average; and (ii) mates index (MI) = (–3 to –5 d average minus pen mates –3 to –5 d average value)/pen mates d 0 value. Cow alert value (CAV) and mates alert value (MAV) were set when the respective index value was less than –0.1 or more than +0.1.The correlation between FP and FL was intermediate for both sick and healthy cows ($r = 0.50$ and 0.56, respectively). The odds (95% CI) of MAS multiplied by 1.16 (1.01–1.34) and 1.36 (1.26–1.47), for each decimal unit increment in FP and FL, respectively. For each decimal unit increment in FP and FL, the odds of MET multiplied by 1.38 (1.25–1.54) and 1.36 (1.25–1.47), respectively; the odds of KET multiplied by 1.43 (1.31–1.57) and 1.34 (1.24–1.44); the odds of HYC multiplied by 0.40 (0.22–0.73) and 1.39 (1.14–1.69); and the odds of DIG multiplied by 1.31 (1.22–1.39) and 1.35 (1.24–1.47). The odds of LAM were only significant for changes in FL [1.28 (1.12–1.45)]. Sensitivity and specificity calculations (Table 1) suggested that changes in both FP and FL may be used as indicators of disease; MAS and KET were better detected using FL, whereas FP was more effective for HYC detection. Overall, MAV was more effective than CAV on disease detection.

Key Words: disease, fat/lactose, fat/protein

Table 0147.

Table 1: Sensitivity and specificity of alarms by disease condition

	CAV[1]		MAV[2]	
Disease[3]	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Fat/Protein ratio				
MAS	54.1	65.9	59.5	66.9
MET	54.8	65.9	54.8	66.8
HYC	100	65.9	100	66.8
DIG	44.1	65.9	55.9	66.9
LAM	52.6	65.9	42.1	66.8
KET	48.8	65.9	53.5	66.8
Fat/Lactose ratio				
MAS	65.7	67.2	74.3	68.6
MET	54.8	67.1	74.2	68.6
HYC	50.0	67.1	50.0	68.5
DIG	53.5	67.2	56.9	68.6
LAM	50.0	67.1	50.0	68.5
KET	57.1	67.1	66.7	68.6

[1]Cow alert value; [2]Mates alert value; [3]MAS = mastitis; MET = metritis; HYC = clinical hypocalcemia; DIG = digestive disorders; LAM = lameness; KET = ketosis

0148 Associations between multiple activity and physiological parameters around the time of disease diagnosis and calving in Holstein cows.

D. Manriquez*[1], F. P. Maunsell[2], S. Paudyal[1], A. Donovan[2], A. De Vries[3], and P. J. Pinedo[4], *[1]Department of Animal Sciences, Colorado State University, Fort Collins, [2]College of Veterinary Medicine, University of Florida, Gainesville, [3]Department of Animal Sciences, University of Florida, Gainesville, [4]Texas A&M AgriLife Research, Amarillo.*

Our objective was to describe the associations between multiple activity and physiological parameters around the time of disease diagnosis and calving in Holstein cows. Health disorders included mastitis (MAS), metritis (MET), milk fever (MF), depression-dehydration-fever (DDF), digestive problems (DIG), lameness (LAM), and clinical ketosis (KET). Behavioral activity included general activity index (HEAD, activity units), rumination time (RUM, min/d), steps (STEP, *n*/d), steps per hour (SH, *n*/h), and lying bouts (LB; *n*/d) from –15 to 15 d relative to disease diagnosis and calving. Data were collected from 198 Holstein cows from –15d from due date to 60 d in milk, at the University of Florida Dairy Unit. HEAD and RUM data were recorded using a neck collar containing rumination loggers (Hr-Tag®, SCR Engineers Ltd., Netanya, Israel), and STEP, SH, and LB data were recorded by a device attached on one hind leg (Pedometer plus®, Afikim, Israel). Data were log transformed and analyzed using MIXED procedures of SAS. To assess associations between activity variables, Spearman's *P* correlations and *P*-values were calculated using JMP12 (Table 1). The only significant negative correlation was found between SH and LB (–0.60) in cows diagnosed with MAS, showing reduced SH from –9d to –4d and an increased LB activity from –8 to –2 d. Correlations between HEAD and RUM were positive and significant for all the diseases, showing a marked decrease from –6 to –5 d to the time of diagnosis with a subsequent increase until 10 d after diagnosis. In addition, significant positive correlations were determined in MAS cows (RUM/STEP, $r = 0.41$); MET cows [RUM/SH ($r = 0.46$); RUM/LB ($r = 0.53$); and SH/LB ($r = 0.59$)]; MF cows [RUM/STEP ($r = 0.63$); and HEAD/SH ($r = 0.54$)]; DDF cows [RUM/LB ($r = 0.52$); and STEP/LB ($r = 0.43$)]; DIG cows (HEAD/RUM, $r = 0.65$); LAM cows [HEAD/STEP ($r = 0.44$); HEAD/LB ($r = 0.40$); and RUM/LB ($r = 0.62$); KET cows [HEAD/STEP ($r = 0.40$); HEAD/SH ($r = 0.57$); HEAD/LB ($r = 0.51$); RUM/SH ($r = 0.52$); and RUM/LB ($r = 0.39$)]. Significant positive correlations at calving included HEAD/SH ($r = 0.93$), RUM/STEP ($r = 0.91$), RUM/LB ($r = 0.93$) and STEP/SH ($r = 0.94$). Correlation patterns between activity and physiological parameters were dependent on specific diseases, suggesting differential potentials as indicators for early disease that could be used in dairy health monitoring programs.

Key Words: activity, rumination, disease

Table 0148.

Table 1. Spearman's p Coefficients by disease and Activity and Physiological Parameters.

		Mastitis (n=31)		Metritis (n=31)		Milk Fever (n=6)	
Variable	by Variable	Spearman *p*	*P-value*	Spearman *p*	*P-value*	Spearman *p*	*P-value*
HEAD	RUM	0.57	<0.01*	0.80	<0.0001*	0.66	<0.0001*
HEAD	STEP	0.17	0.38	0.21	0.29	0.09	0.73
HEAD	SH	0.20	0.31	0.38	0.053	0.54	0.04*
HEAD	LB	-0.01	0.94	0.28	0.16	-0.01	0.97
RUM	STEP	0.41	0.03*	0.22	0.27	0.63	<0.01*
RUM	SH	0.21	0.28	0.46	0.02*	0.03	0.9
RUM	LB	0.14	0.46	0.53	<0.005*	-0.17	0.53
STEP	SH	0.23	0.22	0.20	0.33	0.45	0.1
STEP	LB	-0.10	0.62	0.03	0.87	0.13	0.64
SH	LB	-0.60	<0.001*	0.59	<0.01*	0.26	0.35

		DDF (n=27)		Digestive (n=51)		Lameness (n=15)	
Variable	by Variable	Spearman *p*	*P-value*	Spearman *p*	*P-value*	Spearman *p*	*P-value*
HEAD	RUM	0.41	0.03*	0.65	0.0001*	0.57	<0.01*
HEAD	STEP	-0.04	0.84	-0.07	0.74	0.44	0.02*
HEAD	SH	0.17	0.39	0.00	0.99	0.05	0.78
HEAD	LB	0.07	0.74	0.04	0.82	0.40	0.03*
RUM	STEP	0.25	0.19	-0.05	0.79	0.27	0.16
RUM	SH	-0.01	0.94	0.09	0.65	-0.13	0.49
RUM	LB	0.52	<0.01*	0.26	0.17	0.62	<0.001*
STEP	SH	-0.13	0.50	-0.01	0.97	0.17	0.37
STEP	LB	0.43	0.02*	-0.17	0.37	-0.02	0.9
SH	LB	-0.20	0.30	0.25	0.2	0.11	0.57

		Ketosis (n=37)		Calving (n=190)	
Variable	by Variable	Spearman *p*	*P-value*	Spearman *p*	*P-value*
HEAD	RUM	0.78	<0.0001*	0.04	0.85
HEAD	STEP	0.40	0.03*	-0.39	0.16
HEAD	SH	0.57	0.001*	0.93	<0.0001*
HEAD	LB	0.51	0.004*	-0.35	0.21
RUM	STEP	0.36	0.054	0.91	<0.0001*
RUM	SH	0.52	0.004*	-0.45	0.1
RUM	LB	0.39	0.03*	0.93	<0.0001*
STEP	SH	0.25	0.19	-0.44	0.1
STEP	LB	0.35	0.06	0.94	<0.0001*
SH	LB	0.25	0.19	-0.44	0.1

* Significant correlations at 0.05 alpha level. HEAD: Activity units/d; RUM: Rumination min/d; STEP: Daily steps; SH: steps per hour/d; LB: Lying bouts/d.

0149 DI/LC-MS/MS-based metabolomics identifies early predictive serum biomarkers for ketosis in dairy cows. B. N. Ametaj*[1], G. Zhang[1], E. Dervishi[1], S. M. Dunn[1], R. Mandal[2], D. S. Wishart[2], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [2]*University of Alberta, Edmonton, Canada.*

Subclinical ketosis is a prevalent metabolic disease in transition dairy cows that affects 30 to 40% of the cows during early lactation. Cows with ketosis have lower milk yield and reproductive performance, greater risk of other periparturient diseases, and higher culling rate. The objectives of this study were to retrospectively evaluate alterations of metabolites in the serum of dairy cows with ketosis before, during, and after

the diagnosis of disease and identify monitoring and diagnostic serum metabolite biomarkers for ketosis. One hundred transition dairy cows, 20 healthy cows (CON), and six cows with ketosis were sampled during d –8, –4, at disease diagnosis, and wk +4 and +8 relative to parturition. One hundred and twenty-eight serum metabolites were quantitatively profiled in CON and ketosis cows using a targeted metabolomics approach based on DI/LC-MS/MS at all time points. Univariate and multivariate data analyses were conducted at each time point to examine alterations of serum metabolites throughout the progress of ketosis. Significant changes were detected in the concentrations of several molecular species of amino acids, glycerophospholipids, sphingolipids, acylcarnitines, biogenic amines, and hexose in the serum of cows with ketosis during the entire experimental period. Multivariate analysis (i.e., PCA and PLS-DA) also showed clear distinctions between the two groups on the basis of the measured 128 serum metabolites at five time points. Furthermore, several metabolic pathways including Lys degradation, biotin metabolism, Try metabolism, urea cycle, Arg-Pro metabolism, protein biosynthesis, Met metabolism, phospholipid biosynthesis, Val-Leu-Ile degradation, betaine metabolism, Asp metabolism, His metabolism, and β-Ala metabolism were perturbed in cows with ketosis during the onset and progression of disease. These new findings give insights into further understanding of the pathobiology of ketosis in dairy cows. Biomarker analysis showed that AUCs for ROC curves were 0.996 (95% CI, 0.969–1) at –8 wks, 0.995 (95% CI, 0.938–1) at –4 wks, 0.99 (95% CI, 0.882–1) at disease wk, 1 (95% CI: 1–1) at +4 wks and 0.985 (95% CI: 0.806–1) at +8 wks, respectively, which suggest that serum biomarkers identified have pretty accurate predictive, diagnostic, and prognostic abilities for ketosis in transition dairy cows.

Key Words: amino acid, biomarkers, dairy cows, ketosis, lipid profiles

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0150 Targeted metabolomics reveals multiple metabolite alterations in the urine of transition dairy cows preceding the incidence of lameness.

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Lameness (Lam) is a major issue of transition dairy cows consuming high grain diets affecting 25–35% of the herd. It is associated with decreased milk production, fertility problems, and high culling rates and treatment costs. Various hypotheses have been forwarded during the years with regards to the causes of lameness including rumen histamine, endotoxin, or biogenic amines. Although much is known about non-mechanical lameness, the precise pathobiology is not known. The objectives of this study were to evaluate weekly metabolite composition of urine in dairy cows starting from the beginning of dry off until 8 wks postpartum. Urine samples were collected from 100 cows at −8, −4, disease week, +4, and +8 wk around calving and stored at −80 C until analyzes. DI/LC-MS/MS analyzes were conducted on samples collected from 20 healthy control cows (CON) and 6 cows diagnosed only with lameness (no other periparturient diseases). A total of 154 metabolites including 41 carnitines, 9 lysophosphatidylcholines, 74 phosphatidylcholines, 15 sphingomyelines, 11 amino acids, 2 biogenic amines, hexose, and carnosine were identified and quantified. Data were processed statistically by MetaboAnalyst and univariate analyses. Results showed that 41, 29, 59, 26, and 40 metabolites were identified and measured to be different between the two groups on −8, −4, disease week, +4, and +8 wks around calving, respectively. The highest number of altered metabolites was identified during the week of diagnosis of Lam. Several metabolic pathways were found to be associated with the disease including amino acid metabolism, catecholamine biosynthesis, protein biosynthesis and urea cycle at −8 wks precalving; cysteine, glutamate, tyrosine, and glutathione metabolism at −4 wks precalving; and β-alanine metabolism during the week of disease. ROC analyses, for determination of specificity and sensitivity of potential biomarkers, identified several metabolites with AUCs for ROC curves 0.96 (95% CI, 075–1.0) at −8 wks; 0.971 (95% CI, 0.905–1.0) at −4wks; 1.0 (95% CI, 1.0–1.0) at disease week; 1.0 (95% CI, 1.0–1.0) at +4wks; and 1.0 (95% CI, 1.0–1.0) at +8wks postpartum. PLS-DA analysis also showed clear separation between the two groups of cows with regards to altered metabolites. In conclusion, targeted metabolomics can be used to identify metabolic alterations in the urine of dairy cows before, during, and after diagnosis of Lam; it can also help to better understand the pathobiology of disease, identify cows more susceptible to Lam, and develop new preventive strategies.

Key Words: dairy cows, DI-/LC-MS/MS, lameness, targeted metabolomics, urine

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0151 Elevated serum amyloid A concentrations in the first days after calving are an early disease indicator in dairy cows.

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Early disease detection is critical for maintaining cow health and productivity. Serum amyloid A (SAA) is an acute phase protein that is primarily produced in the liver and is elevated in response to infections and tissue damage in dairy cows. To evaluate whether serum concentrations of SAA may assist in early disease detection, blood samples were taken from 57 Holstein cows at d −21, −14, −7, −3, −1, 0, 1, 3, 7, 14, 21, and 28 relative to calving and analyzed for SAA concentrations. Cows were grouped based on severity of diseases

(no disease, subclinical, mild clinical, and moderate clinical), class of diseases (no disease, metabolic, infectious, both types), time of diagnosis (no disease, 0–3 d, 4–7 d, 8–28 d after calving), and birth complications (yes, no) in early lactation and examined for group differences. Serum amyloid A concentrations in the first days after calving were higher and elevated longer in cows that developed diseases in early lactation. Observed group differences reflected the severity of disease and preceded clinical disease diagnosis irrespective of disease class. Group differences were strongest 1 d after calving, when 0% (healthy), 43% (subclinical disease), 57% (mild clinical disease), and 72% (moderate clinical disease) of cows had SAA above 125 mg/L (sensitivity for any disease: 66%; specificity: 100%). Cows with birth complications had higher SAA concentrations 2 wk before calving than cows without birth complications. Our results support our hypothesis that greater tissue damage and disproportionate inflammatory responses after calving are gateway disorders that increase disease risk in early lactation. Serum amyloid A can detect those risk factors 1 d after calving and thereby opens opportunities for prevention and early treatment.

Key Words: biomarker, dairy cows, diseases, serum amyloid A

0152 The effect of dry period length and antibiotic treatment at drying off on somatic cell counts across the dry period. R. J. Vanhoeij[*1], A. van Knegsel[2], B. Kemp[2], and T. J. G. M. Lam[3,4], *[1]Wageningen University, Netherlands, [2]Adaptation Physiology Group, Wageningen University, [3]Animal Health Service, Deventer, Netherlands, [4]University of Utrecht, Department of Farm Animal Health, Netherlands.*

Management measures to reduce the risk of new intramammary infections (IMI) during the precalving period include use of dry cow antibiotics. Blanket dry cow therapy is not allowed in several European countries, among which is the Netherlands. Moreover, shorter dry periods are of interest because of beneficial effects on the energy balance and metabolic status in the subsequent lactation. The aim was to study the effect of dry period (DP) length on SCC in the subsequent lactation and occurrence of IMI, based on SCC, across the dry period. This aim was approached in 2 separate experiments: Experiment 1 was conducted with use of dry cow antibiotics and experiment 2 without use of dry cow antibiotics. In experiment 1 Holstein-Friesian cows (n = 167) were randomly assigned to three DP lengths (0, 30, 60 d). Cows with a 30-d or 60-d DP were treated with dry cow antibiotics (Supermastidol®, Virbac Animal Health, Barneveld, Netherlands) at drying off. In experiment 2, Holstein-Friesian cows (n = 127) were randomly assigned to two DP lengths (0 or 30 d) and were not treated with dry cow antibiotics. Data were analyzed using a logistic regression model (SAS Institute Inc., 2011), including DP length as fixed effect. Somatic cell count was log transformed before statistical analysis (LnSCC). Data are expressed as LMEANS ± SE. In experiment 1, cows with a 0-d DP had a greater average SCC in the subsequent lactation (LnSCC 5.01 ± 0.06) than cows with a 30-d (LnSCC 4.68 ± 0.06) or 60-d DP (LnSCC 4.52 ± 0.06) ($P < 0.01$). The proportion of cows with a chronic IMI (SCC ≥ 200,000 both pre- and postpartum) was greater in cows with a 0-d DP (5/10), than in cows with a 30-d DP (1/13) or a 60-d DP (1/12) ($P = 0.04$). In experiment 2, cows with a 0-d DP had a greater postpartum average SCC (LnSCC 4.51 ± 0.04), than cows with a 30-d DP (LnSCC 4.24 ± 0.04) ($P < 0.01$). The proportion of cows with a chronic IMI during the precalving period was not different between cows with a 0-d DP (6/11) or a 30-d DP (2/6) ($P = 0.47$). Postpartum average SCC for lactation is greater in cows with a 0-d DP, than in cows with a 30-d DP, regardless of use of dry cow antibiotics. Studies are ongoing to evaluate whether the greater SCC in early lactation in cows with a 0-d DP is actually correlated with intramammary bacterial infections.

Key Words: continuous milking, mastitis, somatic cell count

0153 Enhancement of the dry-off process by intramammary infusion of metalloproteinase 9 nanoparticles. S. Parés[*1], O. Cano-Garrido[2], E. Garcia-Fruitós[1], F. Fàbregas[1], A. Bach[3,4], N. Ferrer-Miralles[2], M. Terré[3], A. Villaverde[2], and A. Arís[1], *[1]Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, [2]Departament de Genetica i de Microbiologia, UAB, Cerdanyola del Valles, Spain, [3]IRTA, Caldes de Montbui, Spain, [4]ICREA, Barcelona, Spain.*

The dry-off of dairy cows is associated to welfare and high risk of intramammary infections that could be improved by fostering involution and immune system during early dry period. Metalloproteinase 9 (MMP-9) is a tissue-remodeling enzyme physiologically released in the mammary gland during the dry period. The objective of this study was to explore the role of the infusion of MMP-9 at dry-off. MMP-9 was produced in *Lactococcus lactis* as a soluble protein and nanoparticulated (NP) format. Twelve cows with Somatic Cell Count (SCC) < 200.000 and > 15 kg of milk/d at dry-off were enrolled in the study. Treatments were randomly assigned to the front or rear quarters, thus 24 quarters were distributed in 2 treatments: 1 mg of soluble MMP-9 and 100 mg of NP MMP-9, providing both the equivalent metalloproteinase activity measured by zymography. Saline solution was infused in the contralateral quarters as a negative control for each treatment. Samples of mammary secretion were collected at 0, 1, 2, 3, and 7 d relative to dry-off and processed for SCC determination or kept frozen for subsequent determination of MMP-9 activity, and bovine serum albumin (BSA), lactoferrin, sodium, and potassium concentrations. SCC was analyzed with a Scepter

cell counter, MMP-9 activity by zymography, lactoferrin by ELISA, BSA using a colorimetric assay, and sodium and potassium by ICP–OES. All data were analyzed by ANOVA. As expected, both the soluble and NP forms increased ($P < 0.0001$) the metalloproteinase activity in mammary gland compared with controls. However, only the NP form was able to modulate some involution and immune markers. The NP form increased immunity markers including SCC up to 400 fold ($P < 0.001$) at d 1–7, and lactoferrin concentration up to 1.8-folds ($P < 0.05$) at d 1 and 2 after dry-off, compared with saline controls. Also, there was an increase ($P < 0001$) in involution markers. Concentration of BSA in mammary secretion raised up to eightfold at 1, 2, and 3 d and the sodium/potassium ratio ($P < 0.001$) by 4.5-fold at D1 after dry-off, compared with controls. In conclusion, infusions of either soluble or NP forms of MMP-9 at dry-off, increased the metalloproteinase activity in mammary gland, but only the NP form enhanced the of the involution process and immune system.

Key Words: dry period, nanoparticles, metalloproteinase 9

0154 Effects of inhibiting prolactin production with cabergoline on the physiology of the cow-dry period. S. Parés[1], A. Arís*[1], M. Terré[2], F. Fàbregas[1], E. Garcia-Fruitós[1], J. Ruberte[3], V. Nacher[3], A. De-Prado[4], and A. Bach[2,5], *[1]Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, [2]IRTA, Caldes de Montbui, Spain, [3]CBATEG Universitat Autònoma de Barcelona, Bellaterra, Spain, [4]Ceva Santé Animale, Libourne, France, [5]ICREA, Barcelona, Spain.*

Cabergoline is an ergot derivative with a high affinity for the D2 dopamine receptor whose dopaminergic effects cause inhibition of prolactin (PRL) secretion, and has been recently released as a dry-off facilitator (Velactis®, Ceva, France). A deep study of its effects along the dry period can help to understand the physiology of the mammary involution. Twenty-four Holstein cows (6 primiparous and 18 multiparous) were distributed in 2 treatments: i.m. injection of either 5 mL providing 5.6 mg of cabergolin (CAB) or 5 mL of saline solution (CTR) at the moment of dry-off. Mammary gland biopsies of posterior quarters and tail blood samples were taken at −10, 9, and 23 d relative to dry-off and −9 and 21 d relative to calving to evaluate the effects of PRL inhibition on hormonal and tissue involution markers along the dry period and onset of the subsequent lactation. Blood concentrations of insulin, PRL, IGF1, IGFBP5, GH, and progesterone were determined using immunoassays. Expression of genes coding for *p16, ki67, igf, igfbp5, upa, mmp9, prlr, occludin, and caspase 3* was determined by qPRC. Lastly, immunohistochemical detection of Ki67, SIRT1 and P16 was performed and quantified by light microscopy. As expected, cows on CAB tended ($P = 0.07$) to have lower serum PLR concentrations (23.5 ± 0.29 ng/mL) than cows on CTRL (36.4 ± 0.25 ng/mL), and had a lesser ($P < 0.001$) expression of PRL receptor (*prlr*) in the mammary tissue (2.09 ± 0.455 vs. 1.77 ± 0.423 relative expression units, respectively). Cabergolin increased ($P < 0.05$) blood insulin concentration (14.3 ± 1.28 μU/mL) compared with CTR (10.7 ± 1.14 μU/mL), but blood concentration of the remaining hormones were not affected although decreased ($P < 0.05$) as the dry period progressed. The expression of *igf1* in the mammary gland increased ($P < 0.05$) in CAB cows in the dry period and decreased at the beginning of next lactation. Also, CAB cows had greater ($P < 0.05$) expression of *mmp9* and *occludin*, which indicates a greater tissue involution and remodeling ($P < 0.05$). Also, immunohistochemical analyses showed an increase ($P < 0.05$) in sirt1 (a gene related with cell proliferation and insulin sensitivity) in CAB animals (75.9 ± 12.02 relative units) compared with CTR (56.9 ± 12.31 relative units) at the onset of the subsequent lactation. In conclusion, this study demonstrates that PRL inhibition by cabergoline at dry-off induces an increased proliferation and tissue remodeling of the mammary gland.

Key Words: dry period, prolactin, tissue remodeling

Table 0155.

Table 1. Antibiotic use, clinical and bacteriological outcomes for two clinical mastitis treatment programs.

Outcome	Culture Based	Positive Control	Treatment Effect		
			Estimate	95% CI	*P*-val
Primary IMM Therapy [% (n)]	28.21 (117)	100 (159)	OR_{PC}=<0.01	<0.01 to 0.02	<0.01
Primary or Secondary IMM Therapy [% (n)]	35.90 (117)	100 (159)	OR_{PC}=<0.01	<0.01 to 0.02	<0.01
IMM Tubes per Quarter Case [mean (n)]	1.13 (117)	3.29 (159)	$Diff_{PC}$=-2.16	-2.49 to -1.83	<0.01
Days of Non-Saleable Milk [mean (n)]	5.72 (98)	6.73 (125)	$Diff_{PC}$=-1.02	-1.44 to -0.56	<0.01
Days to Clinical Cure (Normal Milk) [mean (n)]	3.45 (117)	3.12 (159)	$Diff_{PC}$=0.32	-0.17 to 0.82	0.21
Bacteriological Cure [% (n)]	76.92 (52)	77.08 (48)	OR_{PC}=1.00	0.49 to 2.06	0.98
New Intramammary Infection Risk [% (n)]	20.20 (99)	24.58 (118)	OR_{PC}=0.78	0.41 to 1.48	0.74
Recurrence (14 to 60 Days after CM) [% (n)]	12.82 (117)	25.16 (159)	OR_{PC}=0.44	0.23 to 0.84	0.01
Removal from Herd (Within 21 Days after CM) [% (n)]	7.14 (98)	14.40 (125)	OR_{PC}=0.48	0.18 to 1.14	0.09

0155 The treatment of only environmental Streptococci clinical mastitis cases reduced antibiotic use, days out of the tank, recurrence of clinical mastitis and a tendency to reduce culling. A. Lago*, C. Tovar, J. Zaragoza, D. Luiz, and D. Pearce, *DairyExperts Inc., Tulare, CA.*

This study objective was to compare antibiotic use, clinical and bacteriological outcomes for selective treatment of only clinical cases where environmental streptococci were isolated versus blanket therapy. Cows with mild or moderate clinical mastitis (CM) from a California Central Valley dairy herd were assigned to either a) a positive-control treatment group (PC) or b) a laboratory-culture-based treatment group (CB). Quarter cases assigned to PC received immediate intramammary (IMM) treatment with ceftiofur (Spectramast LC; Zoetis Inc., New York, NY) and repeated once a day for a total of 3 d. Quarters assigned to CB underwent culture over a 24 h period at DairyExperts Laboratory (DairyExperts Inc., Tulare, CA). Only quarters showing environmental streptococci growth were treated the next day with the same therapy as cases assigned to PC. Mixed Models were used with cow included as a random effect. A total of 276 quarter cases of clinical mastitis from 223 cows were enrolled into the study. Results are summarized on Table 1. The selective treatment of only CM cases from which environmental streptococci were isolated resulted in about a two-thirds reduction both in the number of cases treated and in the number of IMM tubes used, as well as a reduction of 1 d out of the tank. Interestingly, CM recurrence was significantly lower and removal from herd tended to be lower when only environmental streptococci were treated with IMM antibiotics.

Key Words: clinical mastitis, selective treatment, streptococcus

0156 Effect of the selective treatment of gram-positive clinical mastitis cases versus blanket therapy. A. Lago*, D. Luiz, D. Pearce, C. Tovar, and J. Zaragoza, *DairyExperts Inc., Tulare, CA*.

This study objective was to compare antibiotic use, clinical and bacteriological outcomes for selective treatment of only Gram-positive clinical cases versus blanket therapy. Cows with mild or moderate clinical mastitis (CM) from a California Central Valley dairy herd were assigned to either a) a positive-control treatment group (PC) or b) a laboratory-culture-based treatment group (CB). Quarter cases assigned to PC received immediate intramammary (IMM) treatment with ceftiofur (Spectramast LC; Zoetis Inc., New York, NY) and repeated once a day for a total of 3 d. Quarters assigned to CB underwent culture over a 24 h period at DairyExperts Laboratory (DairyExperts Inc., Tulare, CA). Only quarters showing Gram-positive growth were treated the next day with the same therapy as cases assigned to PC. Mixed Models were used with cow included as a random effect. A total of 473 quarter cases of clinical mastitis from 425 cows were enrolled into the study. Results are summarized on Table 1. The selective treatment of only CM cases from which Gram-positive bacteria was isolated resulted in about half reduction both in the number of cases treated and in the number of IMM tubes used. Furthermore, the withholding of antibiotic treatment did not have any deleterious effect on time for milk to return visibly normal, bacteriological cure, new infection risk, CM recurrence or removal from the herd.

Key Words: clinical mastitis, selective treatment, gram-positives

0157 Comparison of PCR and culture methods for detecting mastitis causing mycoplasma in bulk tank milk from commercial dairy herds. A. M. Britten, E. D. Tretter*, and M. Gurajala, *Udder Health Systems Inc., Meridian, ID*,

In this study we compared the mycoplasma detection of direct culture, broth enhanced culture and PCR from 1299 bulk tank

Table 0156.

Table 1. Antibiotic use, clinical and bacteriological outcomes for two clinical mastitis treatment programs.

Outcome	Culture Based	Positive Control	Treatment Effect		
			Estimate	95% CI	*P*-val
Primary IMM Therapy [% (n)]	46.45 (211)	100 (262)	OR_{PC}=0.02	<0.00 to 0.04	<0.01
Primary or Secondary IMM Therapy [% (n)]	56.87 (211)	100 (262)	OR_{PC}=0.03	0.01 to 0.06	<0.01
IMM Tubes per Quarter Case [mean (n)]	1.52 (211)	3.33 (262)	$Diff_{PC}$=-1.80	-2.06 to -1.54	<0.01
Days of Non-Saleable Milk [mean (n)]	7.08 (191)	6.70 (234)	$Diff_{PC}$=0.37	-0.12 to 0.85	0.14
Days to Clinical Cure (Normal Milk) [mean (n)]	3.97 (211)	3.56 (262)	$Diff_{PC}$=0.38	0.32 to 0.74	0.06
Bacteriological Cure [% (n)]	68.57 (105)	66.42 (134)	OR_{PC}=1.10	0.64 to 1.90	0.72
New Intramammary Infection Risk [% (n)]	22.35 (170)	20.93 (215)	OR_{PC}=1.08	0.67 to 1.77	0.74
Recurrence (14 to 60 Days after CM) [% (n)]	19.91 (211)	22.90 (262)	OR_{PC}=0.84	0.54 to 1.30	0.43
Removal from Herd (Within 21 Days after CM) [% (n)]	14.66 (191)	14.53 (234)	OR_{PC}=1.01	0.59 to 1.74	0.97

and composite pen milk samples collected from 62 commercial dairies in 11 states. Culture of bulk tank milk directly onto specialized mycoplasma culture media (such as modified Hayflick agar) has proven effective in detection of mycoplasma mastitis outbreaks. Many laboratory methods also incorporate a mycoplasma broth enhancement protocol to increase sensitivity. This routine, direct and broth enhanced mycoplasma culture protocol, of bulk tank milk samples and pooled pen samples is widely practiced as a primary screening method for detection of mycoplasma infection status of a herd. However disease detection can be delayed up to 10 d due to slow colony formation with culture. Direct mycoplasma detection in enriched broth by the use of PCR may have the advantage of detecting the pathogen much sooner, if it is shown to detect the pathogen in bulk tank milk at least as well as culture. All samples in the study were cultured by plating 10 μL of the milk sample directly onto commercial mycoplasma isolation agar. All samples also were enriched by inoculating a 100-μL aliquot into 3 mL of mycoplasma broth and then incubated for 48 h, before plating a 10-μL aliquot of this enriched broth onto mycoplasma agar. Any sample where one or more colonies were detected by either direct or broth enrichment were deemed culture positive. A 2-μL aliquot of the same enriched broth from the culture method was used for the PCR detection method. The PCR method used was a four-way multiplex real time assay including primers for general *Mycoplasma* spp., *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, and an internal positive control. When an organism was detected by any of the mycoplasma primers, the sample was deemed to be PCR positive. Of the 58 PCR detection events 35 (60.3%) were classified as Mycoplasma *bovis*, 16 (27.6%) as *Mycoplasma bovigenitalium*, and 7 (12.1%) were only detected by the *Mycoplasma* spp. primers. The comparison summary presented below shows a high level (98.6%) of agreement between the two methods. Direct PCR amplification of a broth enriched milk sample can rapidly detect *Mycoplasma* spp. in bulk tank and pen samples, and can give results comparable to conventional culture methods. PCR also offers the possibility of providing species differentiation of positive samples.

Key Words: mastitis, mycoplasma, PCR

0158 Effects of antibiotic dry cow therapy and internal teat sealant (Teatseal) on milk somatic cell counts, clinical, and subclinical mastitis in early lactation.

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A randomized multi-site clinical trial was performed to determine the efficacy of an internal teat sealant (TS; Teatseal; Zoetis Australia, Silverwater, NSW, Australia), when used in combination with antibiotic dry cow therapy (ADCT), on milk individual cow cell count (ICCC), milk production and components, and the incidence of clinical and subclinical mastitis in cows up to 60 d in milk (DIM), and when compared with ADCT only. Multiparous Holstein, Jersey, or Holstein cross cows (n = 2200) from 8 farms in Southern and Eastern Australia were randomly assigned to treatment in all 4 quarters with an ADCT alone or with ADCT + TS at dry-off. Individual milk yield, fat and protein percentage, and ICCC were measured at 14 ± 3 d intervals for the first 60 DIM for cows that calved 40 to 100 d after dry-off. The first measurement occurred between 10 and 24 DIM. Clinical mastitis and health events were recorded from dry-off to 60 DIM. Milk yield, ICCC weighted by milk yield, and fat and protein percentage were not affected by treatment or time or their interaction in a generalized linear model. Treatment with ADCT + TS decreased geometric mean ICCC (P = 0.021), compared with treatment with ADCT alone. Geometric mean ICCC (x1000 cells/mL) was 32.0 (95% CI: 26.8 to 38.3) and 43.5 (95% CI: 36.2 to 52.1), respectively. The odds of at least 1 case of subclinical mastitis (ICCC $\geq$ 250,000 cells/mL) were 1.9 times higher (95% CI: 1.4 to 2.6) with ADCT alone, compared with ADCT + TS. Four cows had a first case of clinical mastitis in the dry period; while, 5% of cows had a first case of clinical mastitis between 0 and 60 DIM. Of the 1528 cows included in this analysis, 43 cases (5.7%) and 33 (4.3%) were from the ADCT and ADCT + TS groups, respectively (P = 0.194). Proportional hazards estimates of survival showed no difference in the number of d post-calving to detection of first cases of clinical mastitis between treatments over the first 60 DIM (P = 0.153). The estimated hazard ratio for clinical mastitis over this period in the ADCT + TS cows (relative to ADCT alone) was 0.70 (95% CI: 0.43 to 1.14). The combination of ADCT and TS provides benefits over ADCT alone through improved prevention of subclinical mastitis and reduced ICCC in the first 60 DIM.

Key Words: intra-mammary infection, survival analysis

0159 A new protocol for the isolation of key recombinant proteins in livestock production using lactic acid bacteria as a cell factory.

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Escherichia coli is one of the most widely used expression hosts for the production of recombinant proteins. However,

obtaining pure and active proteins is not an easy task, especially considering difficult-to-express proteins, such as membrane or aggregation-prone proteins. Besides, *E. coli* contains lipopolysaccharide (LPS) that must be removed, which involves costly and time-consuming purification processes. Interestingly, *Lactococcus lactis*, which does not produce LPS, is able to form protein nanoclusters (aggregates) rich in the recombinant protein produced. The objective of this study was to develop an economically-affordable protocol to extract functional proteins from protein nanoclusters of *L. lactis*. For that, interleukin-8 (IL-8) stimulating protein (IL8SP), a difficult-to-express protein, and metalloproteinase 9 (MMP-9), an aggregation-prone protein, were used as model proteins. These proteins, that play important roles during the dry period of cows and have important economical potential, were recombinantly produced in *L. lactis* in the form nanoclusters. Next, IL8SP and MMP-9 nanoclusters were isolated and solubilized followed by some washing steps. Solubilized proteins were further purified following standard procedures for His-tagged proteins. Purified IL8SP and MMP-9 were quantified by Bradford assay and Western blot. The biological activity of IL8SP was measured in vitro by determining the expression of *interleukin-8 (IL-8*) in bovine mammary gland epithelial cell cultures. Specifically, cells were treated with 2 doses of IL8SP (9 and 90 μg). MMP-9 activity was determined by zymography. Data were analyzed using ANOVA. High aggregation ratios in nanoclusters were obtained for MMP-9 (99.24 ± 0.02%), whereas lower ratios were observed for IL8SP (37.32 ± 0.34%). Concerning biological activity, purified IL8SP showed a 1.6 and threefold-increase ($P < 0.0001$) of *IL-8* expression, compared with the control cells, using 9 and 90 μg, respectively. Protein MMP-9 obtained with this protocol was also fully active when tested by zymography. In summary, these results show that it is possible to obtain soluble, pure and fully-active proteins from *L. lactis* protein-rich nanoclusters through a novel, cost-effective, and easy protocol.

Key Words: nanoclusters, protocol, recombinant protein

0160 The negative effects of electromagnetic field exposure in male New Zealand White rabbits.

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The objective of the current study was to understand the possible effects of EMF on liver enzymes and organs of male New Zealand White rabbits under ecological conditions. Rabbits were assigned randomly to 2 treatment groups of 8 each; treatment group (T) was housed directly beneath high voltage power lines (21 μT, 380 kV), whereas controls (C) kept 500 m away from the power lines (0.21 μT) for 7 wk. At the end of 7 wk, blood samples were taken from each rabbit and 4 rabbits per treatment were euthanized. Remaining rabbits were kept in normal laboratory condition for another 7 wk for recovery. The liver enzymes and organ weights were analyzed by Proc T-Test procedure. The exposure to EMF significantly increased the serum γ glutamyltransferase (C = 12.1 ± 1.7 vs. T = 17.2 ± 2.9 IU/l, $P < 0.05$), alkaline phosphatase (C = 71.7 ± 4.9 vs. T = 84.3 ± 6.2 IU/l, $P < 0.05$), aspartate aminotransferase (C = 23.5 ± 2.6 vs. T = 31.4 I ± 3.1 IU/l, $P < 0.05$) and alanine transaminase (C = 35.3 ± 3.8 vs. T = 45.5 ± 5.2 IU/l, $P < 0.05$) levels. Although liver, kidney, heart, testis and brain weights between groups did not differ, significant histopathological alterations were apparent in the treated animals. Histopathological examination of the liver showed dilated sinusoids, degenerative changes in hepatocytes, moderate fatty vacuolation and infiltration of a small amount of inflammatory cells in the portal area. Hyalinated cylinders in tubule lumens, vacuolated renal tubules and infiltration of leukocytes were seen in the kidney. The decrease in seminiferous tubule diameters in testis, and vacuolation of neurons and focal gliosis in the brain was noted in treated rabbits. However, serum liver enzymes and histopathological lesions were transient and returned to normal by wk 7 of recovery. Therefore, EMF exposure may have an adverse effect on the male rabbits, but this effect was not permanent.

Key Words: liver enzymes, histopathology, EMF

0161 Embracing innovation in the animal drug approval process.

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The mission of the Center for Veterinary Medicine (CVM) in the U.S. Food and Drug Administration (FDA) is to protect human and animal health. CVM's Office of New Animal Drug Evaluation encourages the development of new innovative technologies and non-traditional therapeutic indications as one means of facilitating this mission and to meet the growing needs in the animal care and food and fiber production sectors. CVM is committed to maximizing use of all forms of available information to meet this challenge. Current tools being implemented and/or investigated include use of scientific focus groups/technology teams, evaluation of "Early Information," pre-development meetings with drug sponsors, and CVM Outreach to academic bench scientists through, webinars and industry meeting presentations. These tools allow CVM to gather information, identify data gaps, and implement benefit-risk analysis decisions early in the drug approval process. Drug application review also affords the opportunity for continued communication between CVM, research scientists, and sponsors, as well as providing a channel for end-user feedback to CVM. Additional tools include expanded use of literature to support data requirements, use of electronic data capture, systematic reviews and meta-analyses, and collaborative data sharing within CVM and across international regulatory groups. Many of these new tools and approaches rely heavily on the academic research community

through consultations, published peer-reviewed information, and as study investigators. Scientists should consider and identify the applicability of their research and, just as importantly, foster productive partnerships with drug sponsors. An understanding of the regulatory processes underlying animal drug development and the tools used by CVM to evaluate data to support drug development is critical to the growth of the animal drug industry and the animal sciences industries. The benefit of innovative approaches to drug sponsors includes but is not limited to early and continual feedback from CVM, increased predictability of drug development requirements, and consistent application of requirements leading to global approval and use of an animal drug. The benefit to the researcher is continued partnerships with the animal drug industry and increased communication on how their scientific program could support drug development. The benefit to the public is increased availability of safe and effective drugs for use in production animals.

Key Words: animal health, drug, FDA, food, innovation, livestock

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0162 Regulation of animal drugs and foods in the 21st century: Enhancing communication among industry, academics, regulators, and the public.

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The mission of the Center for Veterinary Medicine (CVM) in the Food and Drug Administration (FDA) is to protect human and animal health. One aspect of CVM's role is to ensure that animal drugs and foods are safe, have proper manufacturing processes and labeling, and that any food products from treated animals are safe. Scientists at CVM rely on communication with researchers, veterinarians, and producers to stay informed of common industry practices and any scientific information that may influence the conduct of safety and the effectiveness of studies for approval. CVM also conducts outreach to educate a variety of stakeholders on the wide array of information publicly available on the CVM website and in partnership with professional scientific and industry societies. These resources are valuable to individuals involved with drug and food additive development, including basic research, clinical and laboratory studies, and post-market evaluations. By understanding and implementing the appropriate regulatory requirements and guidance for animal drug or food approvals, researchers are more likely to generate high-quality data and improve CVM's ability to use that information as part of the approval process. The CVM website contains information on important initiatives needing further research, such as antiparasitic resistance, antimicrobial resistance, and unapproved drugs, and provides information for the Center's internal research efforts. Special initiatives to increase the availability of approved drugs for minor uses and minor species include conditional approval, expanded exclusivity, available funding for some designated product studies, and assistance with outside programs intended to support minor species drug approval. The CVM website also serves as an authoritative resource for important information on the use of approved drugs, labeling, and descriptions of the safety and effectiveness of studies supporting those approvals. CVM portals also serve a critical role in allowing end-users of CVM-approved products to report adverse events and complaints for animal drugs and food for pets, and livestock, allowing CVM to monitor for possible safety, effectiveness, and/or manufacturing concerns and take appropriate regulatory action. Continued education and outreach to researchers, veterinarians, and producers allows CVM to continue to make scientifically sound decisions and achieve our mission of protecting human and animal health.

Key Words: animal health, communication, FDA0

0163 Exploring a new presentation form of recombinant proteins for animal production.

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Bacterial cell factories are widely used for the biofabrication of recombinant enzymes, vaccines, and hormones. However, the use of treatments based on recombinant proteins in animal science is limited, mainly because the production of soluble proteins has two important drawbacks: low stability and high costs. In this scenario, we have developed an alternative based on protein nanoparticles produced in lactic acid bacteria (LAB) that can potentially revolutionize livestock production. These nanoparticles are a low-cost source of releasable, highly stable, and functional protein that can be easily produced through a fully-scalable process. The objective of this study was to demonstrate the potential of this new protein format by exploring the composition and the biological effect of nanoparticles composed of bovine metalloproteinase 9 (MMP-9) and 2 (MMP-2), which have critical roles during the dry period of cows. Because understanding the architecture of these protein nanoparticles is pivotal to tune and exploit their activity, stability, and slow-release properties, in a first

experiment, the particle size of MMP-9 and MMP-2 produced by 2 different strains was determined from 474 micrographs obtained by high-resolution microscopy techniques. Next, in Experiment 2, metalloproteinase activity was measured in vitro by zymography. Lastly, in a third experiment involving 12 Holstein cows at dry-off, we assessed the potential use of MMP nanoparticles in vivo. Briefly, 12 quarters were infused with MMP-9 nanoparticles and 12 with saline solution (control) and samples of mammary secretion were obtained during 7 d post-dry off. Data were analyzed using ANOVA. In Experiment 1, particle size was affected ($P < 0.0001$) by the combination of the specific protein and the bacterial strain used for their production (353.2 and 431.6 ± 8.47 nm for MMP-9 and 391.8 and 387.6 ± 8.47 nm for MMP-2 produced in strain A and strain B, respectively). In Experiment 2, MMPs tested in vitro showed activity in a strain- and protein-dependent manner (1.38 and 2.33 ± 0.16 AU for MMP-9 and 1.97 and 1.52 ± 0.16 AU for MMP-2 produced in strain A and strain B, respectively; $P = 0.0028$). Mammary secretions from the in vivo study (Experiment 3) indicated a clear increase ($P < 0.001$) in metalloproteinase activity in comparison with the control at d 1 and 3. In summary, this study shows that it is possible to produce tunable and fully functional MMP nanoparticles in LAB, proving an important combined effect of the strain and the protein used to define its final characteristics.

Key Words: metalloproteinase, nanoparticles, proteins

0164 Reduced severity of histological lesions in mink selected for tolerance to Aleutian mink disease virus infection- A field survey. A. H. Farid*[1] and L. E. Ferns[2], [1]*Department of Animal Science, Dalhousie University Faculty of Agriculture, Truro, NS, Canada,* [2]*Pathology Laboratory, Veterinary Services, Nova Scotia Department of Agriculture, Truro, Canada.*

Aleutian mink disease virus (AMDV) causes Aleutian disease (AD), which is a major problem for the mink industry worldwide. Chronically infected adult mink exhibit persistent antiviral antibody production, hypergammaglobulinemia, generalized plasmocytosis and immune complex-mediated glomerulonephritis and arthritis. The disease has no vaccine or treatment, and many years of testing for anti-viral antibodies by the counter-immunoelectrophoresis (CIEP) and eliminating seropositive animals has not been effective for virus eradication, encouraging some ranchers to select their herds for tolerance to the disease. The objective of this study was to assess the effect of selection for tolerance on the severity of AD histological symptoms. Carcasses of 680 sero-positive (CIEP-P) black mink from 28 ranches in Nova Scotia, Canada, and 132 sero-negative (CIEP-N) mink from 15 of these ranches were collected at pelting time. Animals on three of the ranches have been selected for tolerance to AMDV based on health, with or without the iodine agglutination test which identifies animals with high serum globulins. The severity of the AD lesions was assessed by histological examination of kidneys, lungs, heart, brain and liver. Only six unselected CIEP-P mink showed clinical symptoms of AD at necropsy, whereas histology confirmed the presence of AD-related microscopic lesions in at least one of the five organs in 89.5%, 68.1% and 66.7% of unselected CIEP-P, tolerant CIEP-P and unselected CIEP-N mink, respectively. The maximum intensity of lesion scores on any of the five organs showed that severe and very severe lesions in the unselected CIEP-P group (44.7%) was 5.7 and 29.8 times greater than those in the tolerant CIEP-P (7.8%) and unselected CIEP-N group (1.5%). A greater percentage of tolerant CIEP-P mink did not show any AD lesions on any of the organs (31.9%) or showed trace or minor lesions (44.8%) compared with unselected CIEP-P mink (10.5% and 24.7%). The GENMOD procedure of SAS with the cumulative logit model showed significant differences among the three groups for the maximum lesion score. The results implied that selection for AMDV tolerance was manifested as milder disease symptoms in infected mink.

Key Words: Aleutian disease symptoms, counter-immunoelectrophoresis, mink

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0165 Type of blood tube affects haptoglobin concentration when analyzed with a colorimetric assay. M. A. Campbell*[1,2], J. W. Darrah[1], and H. M. Dann[1], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*University of Vermont, Burlington, VT.*

Haptoglobin, an acute phase protein, serves as a biomarker for stress and inflammation in dairy cows. Consequently, obtaining an accurate value for haptoglobin is vital for research and management decisions on-farm. Blood collection methods reported in peer-reviewed articles differ greatly when similar assays are performed. The objective of this study was to determine the effect of type of blood collection tube on haptoglobin concentration using a commonly used, commercially available colorimetric assay. Coccygeal blood was obtained from 21 early lactation, 9 sick, and 30 late lactation dairy cows from three farms to obtain a range in haptoglobin concentrations. For each cow, blood was collected into four separate 10-mL BD Vacutainer tubes: serum separator, lithium heparin, sodium heparin, and K_2–EDTA. Blood was then processed according to tube type. Plasma and serum were analyzed for haptoglobin concentration using a colorimetric assay (Tri-Delta Development Ltd; Maynooth, Ireland). Inter-assay and intra-assay CV were 3.2% and 4.3%, respectively. Data were separated into two categories; low haptoglobin (< 0.2 mg/mL; $n = 35$) or high haptoglobin (≥ 0.2 mg/mL; $n = 25$). Data were logarithmically transformed and analyzed using the MIXED procedure in SAS with cow as the experimental unit. Plasma samples from lithium heparin, sodium heparin, and K_2–EDTA tubes appeared cloudier than serum samples

following addition of assay reagents and interfered with the optical density reading. Haptoglobin concentrations were lower ($P < 0.01$) for serum separator tubes (0.09 mg/mL, 0.76 mg/mL; SEM = 0.02, SEM = 0.06) compared with lithium heparin (0.57 mg/mL, 1.60 mg/mL), sodium heparin (0.55 mg/mL, 1.57 mg/mL), or K_2–EDTA (0.62 mg/mL, 1.60 mg/mL) tubes for the low and high haptoglobin categories, respectively. To assess bias, data were analyzed for agreement between tubes using the Bland-Altman method with the serum separator tube serving as the gold-standard. A maximum allowable difference was determined to exceed the inter- and intra-assay variations set by the manufacturer at 0.15 mg/mL. Compared with serum, there was a significant lack of agreement ($P < 0.01$) with lithium heparin, sodium heparin, and K_2–EDTA (mean biases of 0.66, 0.64, and 0.69 mg/mL, respectively). In addition, lithium heparin, sodium heparin, and K_2–EDTA demonstrated slope biases of 0.42, 0.40, and 0.40, respectively, compared with serum. These results indicate greater disagreement among tubes at higher haptoglobin concentrations. The use of lithium heparin, sodium heparin, and K_2–EDTA tubes before haptoglobin analysis using the Tri-Delta colorimetric assay overestimates haptoglobin concentrations due to interference with assay reagents and is not recommended.

Key Words: haptoglobin, inflammation, vacutainer

0166 Health and production benefits of feeding cowpeas to goats. S. Adjei-Fremah*, A. Everett, R. Franco, K. Moultone, E. Asiamah, K. Ekwemalor, L. E. Jackai, N. Whitley, K. Schimmel, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro.*

The effect of grazing on cowpea forage on growth, parasite egg counts and markers of immunity was evaluated in goats. Spanish ($n = 24$) and Savannah goats ($n = 24$) were stratified by initial body weight (BW) (42.0 ± 7.0 kg) and fecal egg counts (FEC), and randomly assigned to 1 of 12 grazing plots (4 animals/plot) for 4 wks. Plots contained either of two varieties of cowpea commonly used in the Southern U.S., Mississippi silver (MS) and Iron and Clay (IC) or pearl millet (PM) grass as control. Body condition scores (BCS), BW, *FAMACHA* scores, and FEC were measured weekly. Initial and end of study blood samples were collected and analyzed for PCV, total and viable cells, and white blood cell differential counts. The concentration of total proteins, pro-inflammatory cytokines, prostaglandin E2 (PGE2) and total antioxidant capacity (TAC) were evaluated in serum. Body weight, BCS and *FAMACHA* score data were analyzed by repeated measures analysis using the PROC MIXED model procedure of SAS. The model included treatment, time (sampling day), breed, and the treatment x time x breed interaction. The FEC data were log-transformed before statistical analysis. Two-way ANOVA was performed on all other data. Goats fed cowpea forage, BW ($P = 0.01$), percent lymphocyte ($P = 0.008$), and percent neutrophils ($P = 0.013$) increased, and FEC decreased ($P = 0.03$) compared with goats fed control PM forage. A significant interaction ($P = 0.01$) was observed between goat breeds, cowpea varieties and measured parameters such as BW, percent lymphocyte, percent neutrophil and percent viable cells. The MS cowpea forage was associated with greater BW and neutrophil counts in the Savannah breed and with increased lymphocyte counts in Spanish goats. Although feed did not affect serum protein concentration ($P > 0.05$), a decrease in PGE2, TNF-α, IL-8, and IP10, and an increase in TAC, G-CSF, Rantes and IFNγ was observed over time ($P < 0.05$). Results from the study suggest potential benefits and impact of cowpea forage grazing, particularly MS variety on growth, internal parasites burden, and markers of immunity in goats. Feeding a cowpea diet to goats may stimulate and prime innate immune responses for defense against gastrointestinal parasites and warrants further study under different management conditions.

Key Words: cowpea forage, goats, gastrointestinal nematode, inflammation cytokines, immunity markers

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0167 Exposure of bovine blood to pathogen associated and non pathogen associated molecular patterns results in transcriptional activation. K. Ekwemalor*, S. Adjei-Fremah, E. Asiamah, H. Ismail, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro.*

The effect of exposure of cow blood to non pathogen associated (probiotics) molecular patterns on the subsequent response to pathogen associated molecular patterns (PAMPS) was evaluated using transcriptional profiling. Probiotic supplements are beneficial for animal health and rumen function and represent non pathogen associated molecular patterns. Lipopolysacharides form gram negative bacteria are associated with inflammatory diseases and represent PAMPS. A global gene expression profile in whole blood collected from probiotics-supplemented cow was investigated in response to stimulation with lipopolysaccharide (LPS) in vitro. The recommended dose of FASTtrak microbial pack (Conklin Company, Kansas City, MO, USA) was administered orally in 50 ml of sterile water to Holstein-Friesian cows ($n = 10$) in mid lactation, for 60 d. Whole blood was collectedly aseptically and treated with 100 ng/ml of LPS and untreated samples served as control. Total RNA was extracted, and samples (0.5ug, RIN > 7) pooled together, were used for the microarray experiment on a bovine (v2) 4 × 44 arrays with 44,000 gene transcripts. A Real-time quantitative PCR was performed to validate the expression of Wnt signaling pathway and innate and adaptive immune response genes using RT-PCR profilers arrays (Qiagen) with 84 test genes each. Global gene expression analysis identified 13,658 differentially expressed genes

(fold change cutoff $\geq$ 2, $P < 0.05$), 3816 upregulated genes and 9842 downregulated genes. Treatment with LPS resulted in increased expression of TLR4 (Fold change (FC) = 3.16), TLR2 (FC = 2.4), TLR7 (FC = 2.13), WNT5A (FC = 2.68), and transcription factor NF-Kb (FC = 5.4). Genes downregulated in expression included WNT 11 (FC = -2.60), TLR1 (FC = -2.54), TLR3 (FC = -2.43), TLR10 (FC = -3.88), NOD2 (FC = -2.4), NOD1 (FC = -2.45) and pro-inflammatory cytokine IL1B (FC = -3.27). Thus, probiotic supplementation had an effect on the response to LPS exposure with specific effects on Toll-like receptor transcription. Exposure of bovine blood to pathogen associated (LPS) and non pathogen associated (probiotics) patterns resulted in transcriptional activation. Thus, probiotic supplementation may modulate the response to gram negative bacteria.

Key Words: probiotic, microarray, lipopolysaccharides, dairy cows

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0168 Prevalence of *Brucella suis* in hunting dogs in Hawai`i.

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This study examined the prevalence of *Brucella suis* in hunting dogs that have had extensive contact with feral swine, and potential risks for domestic livestock. Increasing feral swine populations across the U.S. increase the risk of transmission of swine diseases, such as the *B. suis* bacterium, to other animals and humans. Blood serum was collected from hunting dogs on the islands of O`ahu and Hawai`i by cooperating veterinary clinics. Based on previously reported prevalence of *B. suis* in feral swine on O`ahu and Hawai`i islands of 20.6% and 10.5%, and an estimate by extension agents of 300 to 500 hunting dogs, sample size needed for detection was calculated as 47 dogs. Serum was tested for *B. suis* specific antibodies using the buffered acidified plate antigen test, and positives were confirmed with the rivanol test. Data on potential risk factors was collected in a pre-structured questionnaire. Areas sampled include Royal Summit, Aiea and Kaneohe Bay on O`ahu and Halaula, Hawi, and North Kohala on Hawai`i. On O`ahu 1/7 (14%) samples were positive for *B. suis* and on Hawai`i 2/49 (4%) of samples were positive for *B. suis*. All positive samples had significant antibody titers on the rivanol test (two at 1:200 and one at 1:100) with no presentation of symptoms in the dogs. The positive dogs hunted in the Halawa/Royal Summit area on O`ahu and in the Hawi and North Kohala areas on Hawai`i. Questionnaire results showed that 83% of dogs were not neutered, 74% had never seen a veterinarian, 25% had contact with domestic pigs, and 46% had contact with other livestock. This study concludes that hunting dogs may be a previously unidentified risk factor for transmission of *B. suis* to domestic swine and other livestock.

Key Words: *Brucella suis*, feral swine, hunting dogs

0169 Pulmonary arterial pressure in yearling Angus cattle managed at high altitude: Study of a non-synonymous SNP in the oxygen-dependent degradation domain of the endothelial PAS domain-containing protein 1 gene.

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Bovine pulmonary hypertension (bPH) has a complex pathophysiology and can progress to right heart failure. Mean pulmonary arterial pressure (mPAP) has been used for decades as an indicator of risk of developing hypoxia-related bPH at altitudes above 1800 m. Veterinarians typically describe yearling bulls and heifers as low, moderate, or high risk for developing bPH using mPAP data. The objective of this study was to evaluate the relationship of a non-synonymous SNP in the oxygen-dependent degradation domain (ODDD) of the endothelial PAS domain-containing protein 1 (EPAS1) gene with mPAP in yearling Angus cattle managed at high altitude. The EPAS1 gene, also known as hypoxia-induced factor (HIF2α), is located on chromosome 11 and several sequence variants have been identified in its ODDD including a downstream G/A SNP (rs208684340). The A allele of this polymorphism was previously associated with hypoxemia in Angus cattle. In the current study, records from Angus cattle (n = 5296 of 280 sires) were obtained from the Colorado State University Beef Improvement Center (CSU-BIC; elevation 2150 m). Risk categories were constructed by estimating the heritability of mPAP as a categorical outcome based on veterinary recommendations. The combination of these efforts yielded mPAP risk categories of low (< 41), moderate (41 to 49) and high (> 49 mmHg) with a categorical-trait heritability of 0.26 ± 0.04 in this population. The percentage of cattle in each of these categories was 50.7, 38.0, and 11.3%, respectively. Forty-seven bulls and heifers, progeny of 33 sires, from this population were genotyped for the G/A SNP using a TaqMan assay (ABI-Roche Molecular Systems, Inc.). The minor allele frequency (MAF; A allele) of this SNP was 32.3% and mPAP averaged 45.2 ± 1.5 with a range of 32 to 95 mmHg. The MAF for the low, moderate and high categories were 46.4, 26.1, and 25.0%, respectively. Genotype was not a predictor (P = 0.4) of mPAP using a generalized linear model analysis. Mean PAP has been a useful tool to predict risk of bPH in yearling cattle for decades and is a moderately heritable trait. However, the

genotype results of this particular variant in the ODDD of the EPAS1 gene does not appear in this preliminary study to be useful to designate Angus cattle from the CSU-BIC into mPA-P-bPH risk categories.

Key Words: Angus cattle, pulmonary hypertension, SNP

0170 Subclinical right heart failure may contribute to the development of liver disease in feedlot cattle during the finishing phase. A. K. Gulick*, K. M. Freeman, B. C. Bernhard, J. O. Sarturi, and J. M. Neary, *Texas Tech University, Lubbock.*

The objectives of this study were twofold: first, to evaluate the relationship between mean pulmonary arterial pressure and mean central venous pressure, and determine if they increase through the finishing period; and second, to determine if mean central venous pressure is associated with liver disease. A cohort of crossbred yearling steers (n = 22; initial BW = 364 ± 52 kg) was studied at an altitude of 975m. Steers were fed for 171 d. Vascular pressures were measured twice: 6 and 54 d before slaughter. Serum biochemistry and liver histology were performed on steers that had the greatest (n = 5) and lowest (n = 5) mean central venous pressure at 54 d before slaughter. Biochemistry included total bilirubin, AST, GGT, and albumin; blood samples obtained 6 and 54 d before slaughter were evaluated. Liver samples were collected from the caudate lobe and lesions scored semiquantitatively. Both central venous and pulmonary arterial pressures increased from 54 d to 6 d before slaughter: 24 ± 1 mmHg to 28 ± 1 mmHg (P = 0.03) and 47 ± 2 mmHg to 54 ± 2 (P < 0.01), respectively. There was a positive association between mean pulmonary arterial and central venous pressures at 6 d (P < 0.01) but not 54 d before slaughter (P = 0.41) indicating that increased pulmonary arterial pressure contributed to right heart failure in cattle closest to slaughter weight. Serum biochemistry was within normal limits even though all steers showed histological evidence of liver damage centered on the centrilobular region (zone 3). All steers showed hydropic degeneration and sinusoid dilation. Lesion severity was greatest in the high mean central venous pressure group: One liver had cirrhosis; another had multifocal necrosis. Congestion was moderate to severe and centered on zones 1 and 2. The findings of this study indicate that subclinical right heart failure secondary to pulmonary hypertension may contribute to hepatic congestion and disease in feedlot cattle during the finishing phase. Serum biochemistry analyses may not represent the insidious liver damage of cattle close to slaughter. Right heart failure secondary to pulmonary hypertension, or cor pulmonale, may contribute to the development of liver disease in feedlot cattle during the finishing phase.

Key Words: health, pulmonary hypertension, steer

0171 Evidence of cor pulmonale and liver disease in association with pneumonia in feedlot and dairy cattle at an altitude of 975 m. A. K. Gulick* and J. M. Neary, *Texas Tech University, Lubbock.*

The objective of this observational study was to determine if cor pulmonale is evident in cattle at the moderate altitude of 975 m. Cor pulmonale is defined as right ventricular enlargement and dysfunction due to diseases affecting the lung or pulmonary vasculature. Right ventricular dysfunction can manifest as congestive hepatopathy. A convenience sample of necropsies were performed on one feedlot (n = 16) and one dairy (n = 4) between May 16 and September 4, 2015. A case history was obtained, gross lesions were recorded, and the cardiac ventricles weighed to determine the ratio of right ventricular free wall to total ventricular myocardium (RV:T). Sections of the right diaphragmatic, middle, and cranial lung lobes and liver were collected for histology. Vascular and hepatic lesions were scored semiquantitatively. Of the 16 feedlot cattle necropsied, 2 died from cor pulmonale secondary to bronchointerstitial pneumonia, 8 died from pneumonia, and 6 died from miscellaneous causes. Dairy cattle died from interstitial pneumonia (n = 3) and miscellaneous causes (n = 1). The RV:T ratio varied according to cause of death (P < 0.001): 0.37 ± 0.02 in cattle that died of cor pulmonale, 0.28 ± 0.01 in cattle that died of pneumonia, and 0.25 ± 0.01 in cattle that died of miscellaneous diseases. All cattle showed histological evidence of pulmonary vascular remodeling regardless of the cause of death or degree of right ventricular hypertrophy. The predominant vascular lesions included pulmonary arterial adventitial hyperplasia and pulmonary venous distension. Anatomic reduction of the pulmonary vascular bed was evident in cattle with pneumonia. Liver disease, consisting of sinusoidal dilation, lipidosis, and necrosis, was most severe in cattle that died of cor pulmonale and pneumonia. These findings indicate that cor pulmonale may be more problematic in cattle at modest elevation than is currently appreciated and confirm our previous epidemiological findings that respiratory disease is a risk factor for cor pulmonale in cattle. Venous congestion secondary to cor pulmonale may have contributed to the development of liver disease in cattle with pneumonia. Systemic consequences of right heart failure result from a reduction in cardiac output and venous congestion; consequently, organs with high oxygen requirements, such as the liver, are most at risk of cellular dysfunction and death. Moreover, because respiratory disease is a risk factor for cor pulmonale and the clinical signs overlap, the true incidence of cor pulmonale may be greater than current estimates suggest.

Key Words: heart, hypertension, pulmonary

0172 Porcine intestinal explants as *ex vivo/in vitro* model to study gastrointestinal disease. N. Reisinger*, P. Fuhrmann, C. Emsenhuber, B. Grenier, E. Mayer, and G. Schatzmayr, *BIOMIN Research Center, Tulln, Austria.*

Intestinal diseases play an important role in livestock animals especially in pigs. To gain more knowledge about pathological processes during intestinal disease usually animal experiments are needed. However, alternatives to animal testing are highly recommended. Ex vivo cultivation of explants might provide an alternative tool to investigate intestinal diseases in pigs. We therefore evaluated the cultivation of porcine intestinal explants.

Intestinal tissue from pigs was obtained from a local abattoir. About 10 cm of the jejunum were transported in prewarmed PBS to the lab. Intestinal tissue was flushed with PBS and was cut open longitudinally. Thereafter, tissue was washed again with PBS and cut into small pieces. Explants were placed into 12-well plates (mucosa facing upward) prefilled with 1.5 mL cultivation medium (D-MEM containing antibiotics and 10% FBS) and were incubated for up to 72 h at 39°C and 5% CO_2. Viability was measured with the water soluble tetrazolium (WST) −1 assay after 2, 4, 24, and 72 h (n = 6). In addition, explants were frozen in liquid nitrogen after incubation for 0, 2, and 4 h and stored at −80°C (n = 9). Gene expression of three different pro-inflammatory cytokines (TNF-α, IL-6, and IL-8) was measured via RT-qPCR. Statistical evaluation was performed with IBM SPSS Statistics software. If data were normally distributed ANOVA was performed. If data were not normally distributed, the Kruskal Wallis Test was used as non-parametric test.

Viability was already significantly decreased after 4 h of incubation compared with fresh explants. Furthermore, there was a significant increase of TNF-α expression after 4 h (25-fold) and IL-6 expression after 2 and 4 h (50 and 320-fold) compared with fresh explants. No effect of incubation time was seen for IL-8 expression.

Our study highlights the importance of measuring viability when cultivating intestinal explants. In addition, dissection of the tissue and isolation of explants seem to stimulate expression of certain pro-inflammatory cytokines.

We can therefore conclude that ex vivo cultivation of intestinal explants might be an alternative screening tool. However, optimization of dissection and culture conditions is needed to prolong possible incubation time.

Key Words: pigs, explants, gastrointestinal diseases

0173 Comparison of strategies for combining dynamic linear models with artificial neural networks for detecting diarrhea in slaughter pigs. D. B. Jensen* and A. R. Kristensen, *University of Copenhagen, Department of Large Animal Sciences, Frederiksberg, Denmark.*

The drinking behavior of healthy pigs is known to follow predictable diurnal patterns, and these patterns are further known to change in relation to undesired events such as diarrhea. We therefore expect that automatic monitoring of slaughter pig drinking behavior, combined with machine learning, can provide early and automatic detection of diarrhea. To determine the best approach to achieve this goal, we compared 36 different strategies for combining a multivariate dynamic linear model (DLM) with an artificial neural network (ANN). We used data collected in 16 pens between November 2013 and December 2014 at a commercial Danish pig farm. The pen level water flow (liters/hour/pig) and drinking bouts frequency (bouts/hour/pig) were monitored. Staff registrations of diarrhea were the events of interest. Mean water flow and drinking bouts frequency were each modeled using three harmonic waves in a multivariate DLM. The DLM was optimized using the pen-groups for which no events were observed (n = 26). The forecast errors produced by the DLM were normalized by the forecast variance and used as inputs for the ANN. In addition, the forecast errors were categorized based on the direction (positive or negative) and simga-1, sigma-2, or sigma-3 cutoff thresholds. Furthermore, observations from between 0 and 48 h before the day of the observation, with steps of 6 h, were included in the ANN training window. Thus between 87 and 277 diarrhea-associated observations were included. The diarrhea-associated observations were paired with an equal number of observations from healthy groups, based on the observation date and the age of the pigs. The complete set of diarrhea positive and negative observations was divided into a training set (80%) and a test set (20%). The ANN's consisted of three layers: an input layer corresponding to the number of forecast error categories, a hidden layer with 50 nodes, and an output layer with one node. The various ANN's were applied to all observations in the test set. The observation-level performance of the ANN predictions was evaluated by the error rate, the specificity (SP), and the sensitivity (SE). The best performance was seen when using a training window of 42 h for the numerical forecast errors, which produced an error rate = 0.16, a specificity = 0.88, and a sensitivity = 0.80. For the other tested strategies, the ranges of error rates and the corresponding specificities and sensitivities were 0.55–0.28, 0.43–0.71, and 0.50–0.74, respectively.

Key Words: artificial neural network, diarrhea, dynamic linear model

0174 Heat stress increases gut permeability in pigs–application of a non-invasive assay. N. Reisinger[*1], S. Schaumberger[2], I. Dohnal[1], B. Doupovec[1], E. Mayer[1], and G. Schatzmayr[1], *[1]BIOMIN Research Center, Tulln, Austria, [2]BIOMIN Holding GmbH, Getzersdorf, Austria.*

Heat stress plays an important role in livestock animals. Several studies already described increased gut permeability in pigs during heat stress using invasive technologies e.g., Ussing Chamber. In human studies non-invasive sugar tests are quite often used to measure gut barrier function. We therefore evaluated the influence of heat stress on gut permeability of pigs with the dual sugar assay. Eight pigs were placed into metabolic cages (two pigs per cage) at D35 after weaning. Pigs were allowed to adapt to the cages for 4 d. At d 0 of the trial period pigs were kept at thermoneutral conditions (32°C, 24 h) and on d 1,2 and 3 pigs were exposed to heat stress conditions (6 h 35°C, 18 h 32°C). At d 0 and 2 the dual sugar permeability assay was performed. Therefore, agar containing lactulose (500 mg/kg body weight) and rhamnose (100 mg/kg body weight) was fed to the pigs. Urine was sampled 2, 4, and 6 h after sugar intake. Urine samples were frozen at- 20°C and lactulose and rhamnose concentrations were determined via HPLC-MS/MS. All data obtained in the experiment were analyzed with a nonparametric test. There was no significant difference between the cumulative rhamnose and lactulose excretion between d 0 (2.1%; 0.52%) and d 2 (1.2%; 0.84%). However, the lactulose rhamnose excretion ratio was significantly increased ($p = 0.0286$) of pigs under heat stress conditions (0.66) compared with thermoneutral conditions (0.24). Our study showed that the dual sugar assay can be used to evaluate gut permeability with a non-invasive method. We furthermore could once more highlight the negative impact heat stress can have on the welfare and health of pigs.

Key Words: gut permeability, heat stress, pigs

0175 The effect of various parameters measured at farrowing on subsequent pig performance. A. L. Robinson[*1], J. Colpoys[2], G. Robinson[1], E. A. Hines[1], E. Edwards[1], J. Bundy[1], A. K. Johnson[1], H. D. Tyler[1], *[1]Iowa State University, Ames, [2]Truman State University, Kirksville, MO.*

The objective of this study was to evaluate birth weight, gender, stall conditions at birth, umbilical diameter, ratio of umbilical diameter to birth weight (as a potential indicator of placental efficiency), and umbilical antiseptic treatment as predictors of pre-weaning mortality, incidence of umbilical hernias, and 150 d weight of pigs in a commercial facility. A total of 466 mixed gender commercial piglets from a breed-to-wean sow farm were enrolled. Piglets were alternately assigned by birth order within a litter to four umbilical treatment groups; iodine (2%), Zurex umbilical dip, a dry dip created using an antibacterial peptide (nisin) mixed with talc (formulation concentration = 3.105 g nisin/100 g talc on a wt/wt basis), and no treatment. At birth, stall conditions (wet/dry and clean/dirty) were evaluated on a 3 point scale (3 = most dirty or most wet and 1 = dry or clean). Before treatment, diameter of the umbilical cords were determined using digital calipers. All data were analyzed using mixed model methods. Models included the fixed effects of birth weight, umbilical diameter at birth, gender, stall conditions and treatment. Pre-weaning mortality was significantly affected by umbilical treatment ($p < 0.05$) and by ratio of umbilical diameter to birth weight ($p < 0.001$). Piglets treated with 2% iodine had a higher mortality rate than piglets treated with other antiseptics or those that were untreated. Piglets with the lowest umbilical cord diameter to birth weight ratio had the highest survival rate. Stall conditions at birth ($p < 0.005$) and the ratio of umbilical diameter to birth weight ($p < 0.05$) affected the incidence of umbilical hernias. Piglets born in wet stall conditions or those with a high umbilical cord to birth weight ratio had a higher incidence of umbilical hernias in the growing phase. Final 150 d weight of pigs was affected by the ratio of umbilical diameter to birth weight ($p < 0.0001$) and gender ($p < 0.0001$), and tended to be affected by stall conditions at birth ($p = 0.06$). Male pigs weighed 93.5 kg, while female pigs weighed 86.5 kg. Piglets with the highest ratio of umbilical cord diameter to birth weight and those born in wet stall conditions weighed less. In conclusion, measuring the umbilical cord to birth weight ratio was a much better predictor of pre-weaning mortality, incidence of umbilical hernias, and 150 d weight than birth weight alone.

Key Words: birth weight, piglet, pre-weaning mortality

0176 Environmental persistence of porcine epidemic diarrhea virus, porcine delta corona virus, and transmissible gastroenteritis in feed ingredients. M. P. Trudeau[*1], H. Verma[2], F. Sampedro[2], P. E. Urriola[1], G. C. Shurson[1], and S. M. Goyal[2], *[1]Department of Animal Science, University of Minnesota, St. Paul, [2]Veterinary Population Medicine, University of Minnesota, St. Paul.*

Porcine epidemic diarrhea virus (PEDV), porcine delta corona virus (PDCoV), and transmissible gastroenteritis (TGEV) are major threats to swine production. Investigations of recent outbreaks confirmed that contaminated feed plays a role in virus transmission. This risk makes it necessary to evaluate the survival of such viruses in various feed ingredients. The objective of our experiment was to characterize the inactivation of PEDV, PDCoV, and TGEV in various feed and ingredient matrices. To determine differences in virus survival, 5-g samples of complete feed, spray-dried porcine plasma, meat meal, meat and bone meal, blood meal, corn, soybean meal, and low, medium, and high oil dried distillers grains with solubles were weighed into separate scintillation vials. These samples were inoculated with 1 mL of PEDV, PDCoV,

or TGEV and incubated at room temperature for up to 56 d. At each time point, surviving virus was eluted and the supernatant was inoculated into vero-81 cells for PEDV, or swine testicular cells for PDCoV and TGEV. Cells were observed daily for 10 d for cytopathic effects, and this information was used to calculate a median tissue culture infectious dose ($TCID_{50}$) using the Karber method. Inactivation kinetics were determined using the Weibull model. A delta value was estimated from the model, indicating the time necessary to reduce virus concentration by 1 log. This delta value was then compared across ingredients using the mixed procedure of SAS, and correlations between ingredient proximate analysis data and delta values were determined. Results showed that soybean meal had the greatest delta value (7.50 d) for PEDV compared with other ingredients ($P < 0.06$). Likewise, PDCoV (42.04 d) and TGEV (42.00 d) delta values were highest in soybean meal ($P < 0.001$). There was a moderate positive correlation between moisture and the delta value for PDCoV ($r = 0.49$, $P = 0.01$) and TGEV ($r = 0.41$, $P = 0.02$). There was also a moderate negative correlation between lipid content and the delta value for TGEV ($r = -0.51$, $P = 0.01$), suggesting that TGEV is less stable in ingredients with greater lipid content compared with ingredients with less lipid content. In conclusion, these results indicate that the first log reduction of PDCoV and TGEV takes the greatest amount of time in soybean meal and it appears to be the result of greater moisture content.

Key Words: feed, inactivation kinetics, virus transmission

0177 Bovine macrophage phenotype influences inflammatory response to lipopolysaccharide.

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Severe inflammation during gram negative bacterial disease is common in periparturient dairy cows and increases the severity of diseases such as *Escherichia coli* mastitis. Tissue inflammation is partly orchestrated by macrophage responses to bacterial infection. Studies in monogastric species showed classical phenotype macrophages have proinflammatory responses and alternative phenotype macrophages have protective and restorative responses during disease. However, responses of diverse bovine macrophage phenotypes to lipopolysaccharide are unclear. The objective of this research was to compare the lipopolysaccharide-induced inflammatory response in several phenotypes of bovine primary monocyte-derived macrophages. Peripheral blood mononuclear cells were isolated from whole blood using Ficoll ($n = 8$ cows). Monocytes were identified using mouse anti-bovine CD172α monoclonal antibody and separated from lymphocytes using magnetic assisted cell sorting. Monocytes were cultured with interferon-γ or interleukins (IL) 4 and 13 to induce a classical or alternative macrophage phenotype, respectively, then stimulated with lipopolysaccharide. Macrophage mRNA was quantified in adipose using qPCR. Fold changes in mRNA concentration were calculated by $2^{-\Delta\Delta Ct}$, using the untreated cells as calibrator and three endogenous control mRNA. Treatment differences in mRNA expression were identified using Fisher pairwise comparisons and ANOVA ($P \leq 0.05$). Flow cytometry showed magnetic assisted cell sorting increased CD172α^+ cells from 22.3 ± 1.9 to 81.6 ± 2.8%. After 48 h *in vitro, CD68* expression increased and CD172α^+ was 95.2 ± 0.4%. Lipopolysaccharide increased *IL6*, *IL10*, *TNF,* and *CCL2* expression. Lipopolysaccharide stimulated *IL6* and *IL10* expression was decreased in alternative macrophages, whereas lipopolysaccharide stimulated *TNF* expression was increased in classical macrophages. Lipopolysaccharide stimulated *CCL2* expression was not different between macrophage types. Together these results show an exacerbated proinflammatory cytokine profile in a model of classical bovine macrophages during gram negative bacterial disease. Results suggest that macrophage phenotype could be involved with severe inflammatory responses seen during dairy cow periparturient periods characterized by prolonged and exacerbated lipolysis and increased disease susceptibility. Ongoing research will describe macrophage phenotype during bovine disease and identify factors contributing to phenotype change. Such factors could ultimately be manipulated to control the bovine macrophage inflammatory response.

Key Words: inflammation, macrophage, periparturient

0178 High immune response technology for use in commercial swine herds: A broad based approach to disease resistance.

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Societal concern regarding food safety and animal health are increasing, encompassing issues including the presence of antibiotic residues in meat, antimicrobial resistant organisms and the risk of zoonotic disease. Therefore, effective economic alternatives, with the potential to improve productivity in addition to animal health and robustness, are essential to the industry's continued success. Previous research in pigs has clearly demonstrated favorable responses to breeding pigs for high immune response (HIR). However, this method has not been tested in commercial swine herds. Since the HIR technology identifies animals with increased capacity for immune response (IR) and subsequently increased disease resistance, its implementation and integration into commercial pig breeding programs is expected to bring health and production benefits. The HIR test measures IR to benign and carefully selected test antigens (Ag), one that elicits antibody-mediated IR (AMIR) and another that elicits cell-mediated IR (CMIR). The study objective was to re-establish and refine the HIR test for pigs in a pilot study and then to utilize this test within a commercial

facility. Two groups of weaned piglets, 24 piglets/group were HIR phenotyped. Antibody-mediated IR, as measured by antigen-specific ELISA, was greater in the older versus younger test piglets ($p < 0.0001$, un-paired t test). Cell-mediated IR, was observed by delayed-type hypersensitivity, measured by change in double-skin-fold thickness (DSFT) both 24 and 48 h after intradermal injection of CMIR-associated antigen, and did not differ between test groups. Results indicate it is possible to phenotype and rank pigs for IR using a standardized HIR protocol. Applying this protocol to approximately 3600 weaned F1-barrows from seven different swine genetics companies is now underway as part of a large collaborative Genome Canada project examining associations between IR with swine health, production and genomic information.

Key Words: broad-based disease resistance, immune response phenotype, swine

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0179 Immunomodulatory activities of polyphenol extract from cowpea (*Vigna unguiculata*) on bovine polymorphonuclear neutrophils.

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The response of the bovine neutrophils to bacterial endotoxin and its significance in the inflammatory response has been studied widely. Studies have also shown that a cowpea diet is protein-rich and contains phenolic compounds with antioxidant properties beneficial for animal health and wellbeing. In this study, the effect of a polyphenol extract (CPE) from cowpea *(Vigna unguiculata)* on modulation of the response to endotoxin from gram negative bacteria was evaluated. Secretion of mediators of inflammation, such as cytokines, and prostaglandin E2 (PGE2), and expression of innate immune response genes by LPS stimulated bovine polymorphonuclear neutrophils (PMNs) were measured. Neutrophils were isolated from whole blood collected from Holstein-Friesian cows (n = 10) in mid-lactation. Lipopolysaccharide-stimulated PMNs were treated with 10 μg/ml of CPE. The secretion of cytokines (TNF-α, IFNγ, GM-CSF, G-CSF, IL-1A, IL-8, IP10, and RANTES), and prostaglandin E2 (PGE2) were measured using commercial ELISAs. Real-time qRT-PCR was performed using an innate and adaptive immune response array with 84 test genes. Cowpea polyphenolic extracts decreased PGE2 levels (8.2 ± 2.5 pg/ml) in LPS-stimulated PMNs, relative to control LPS-only treated PMNs (48.7 ± 4.1 pg/ml). Treatment with CPE resulted in decreased concentrations of six of the tested cytokines, except GM-CSF and IP10. Real-time qRT-PCR analysis of LPS-exposed PMNs revealed downregulation of proinflammatory cytokines (TNF-α, IL-1A, IL-1B, IL-8) and transcription factor NF-kB, and up-regulation of anti-inflammatory cytokine IL13(8.4-folds) by the cowpea phenolic extract. Thus cowpea polyphenolic extract may exert their anti-inflammatory activities and modulate innate immune response through modulation of the neutrophil under pathogen- induced conditions in cows. Therefore, integrating and utilizing cowpea into animal production system may enhance nutrition and improved health and needs further study.

Key Words: bovine, cowpea, cytokines, phenolic compounds, polymorphonuclear neutrophils, prostaglandin e2

0180 Prevalence of digital dermatitis in Canadian Holsteins classified as high, average, or low antibody and cell-mediated immune responders.

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Lameness is one of the major issues affecting production and animal welfare in the Canadian dairy industry, with digital dermatitis being the most common lesion. Studies have shown dairy cattle classified as high immune responders have lower incidence of disease, therefore it may be possible the immune response (IR) plays a role in preventing hoof lesions. The objective of this study was to compare the prevalence of digital dermatitis in Canadian dairy cattle that were classified for antibody (AMIR) and cell-mediated immune response (CMIR). Cattle (n = 341) from five commercial dairy farms in Ontario were evaluated for IR using a protocol that captures both AMIR and CMIR. They were classified as high, average and low responders based on standardized residuals for AMIR and CMIR. Residuals were calculated using a SAS general linear model that included the effects of herd, parity, stage of lactation and stage of pregnancy. Hoof health data was collected in 2012 by the farm's hoof trimmer using Hoof Supervisor software. Only the first trim date for each animal was included and multiple lesions per cow were considered. Trimmers scored each lesion for severity with 1 = least, 2 = moderate, 3 = most. Hoof health data was analyzed using a SAS general linear model which included the effects of herd, stage of lactation (at trim date), parity (at trim date) and IR category (high, average and low). Data is presented as prevalence within IR category. Preliminary results showed that high (17% of highs) AMIR cattle had a trend (P = 0.098) toward lower prevalence of digital dermatitis then average (28% of averages) AMIR cattle. It was observed that high (17% of highs) CMIR cattle had a trend (P = 0.081) toward lower prevalence of digital dermatitis compared with average (27% of averages) CMIR cattle and significantly (P = 0.04) lower digital dermatitis compared with low (30% of lows). Similarly high CMIR

cows also had significantly lower ($P = 0.03$) prevalence of the most severe type of digital dermatitis lesion compared with low CMIR cows. Since digital dermatitis is primarily caused by extracellular bacteria which is typically associated with AMIR these results still indicate that having a more robust or high IR is associated with lower prevalence of infectious hoof lesions. Therefore by breeding animals for high IR it is likely that improvements in hoof health can be made.

Key Words: digital dermatitis, immune response, dairy cattle

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181 MiRNaseq of neutrophils during the transition period in cows with divergent metabolic phenotypes. M. A. Crookenden*[1], C. G. Walker[1], A. Heiser[2], J. J. Loor[3], K. M. Moyes[4], J. K. Kay[1], S. Meier[1], A. Murray[5], V. S. R. Dukkipati[5], M. D. Mitchell[6], and J. R. Roche[1], *[1]DairyNZ, Hamilton, New Zealand, [2]AgResearch, Palmerston North, New Zealand, [3]University of Illinois, Urbana, [4]Department of Animal and Avian Sciences, University of Maryland, College Park, [5]Massey University, Palmerston North, New Zealand, [6]University of Queensland, Australia.*

Several adaptations in leukocytes, i.e., neutrophils, are required for a successful transition to lactation in dairy cows. Micro RNA (miRNA) molecules are small non-coding RNAs that regulate gene expression; their importance in immune cell function has been well documented. We characterized the miRNA of neutrophils isolated from dairy cows divergent in their risk of infection or metabolic dysfunction, at three time points over the transition period: day of calving, 1 wk, and 4 wk post-calving. From a total of 150 cows, 10 cows were selected with high ($n = 5$; High Risk) and low ($n = 5$; Low Risk) concentrations of non-esterified fatty acids, β-hydroxybutyrate, and liver triacylglycerol during wk 1 and 2 post-calving. Neutrophils were isolated from whole blood using differential centrifugation. Flow cytometric analysis of these isolates revealed a median of 75% ± 2% neutrophils (± SEM). Total RNA was extracted from neutrophils using TRIzol®, size selected for miRNA, and sequenced on the Illumina HiSeq. The miRNA reads were mapped to the *Bos taurus* 6 genome (UMD 3.1) using Bowtie 2 and counted. Differential expression analysis was conducted using the limma/voom R-package and pairwise analyses were conducted to assess differential expression between risk categories and across time points, with a false discovery rate set at 0.05. There was no effect of risk category on miRNA expression on the day of calving or 4 wk post-calving. However, expression of mir-19b, mir-148a, and mir-21 in the Low Risk cows tended (adj $P = 0.1$) to be greater at 1 wk post-calving. When assessed for the effect of time relative to the day of calving, regardless of risk, expression of miR-150 and −486 increased ($P \leq 0.05$) at 1 wk post-calving and eight miRNA genes were differentially expressed at 4 wk post-calving ($P \leq 0.05$): miR-150 and −30c were greater, and miR-19b, −19a, −30d, −101–1, and −106b were lower. The results indicate that the divergent metabolic phenotype did not significantly alter miRNA in neutrophils during early lactation. However, the altered miRNA profile in neutrophils over time indicates an important role for miRNA in the regulation of immune cell function during the peripartum period.

Key Words: microRNA, dairy cows, transition

0182 Short chain nitrocompounds treatment of poultry excreta; in vitro survivability of *Salmonella*, *E. coli* and nitrogen metabolism. C. Arzola-Alvarez*[1], J. Corrales[1], O. Ruiz-Barrera[1], R. C. Anderson[2], M. E. Hume[2], Y. Castillo-Castillo[3], A. Corral-Luna[1], J. L. Guevara-Valdez[1], J. Salinas[4], and C. Rodriguez-Muela[1], *[1]Universidad Autonoma de Chihuahua, Mexico, [2]USDA-ARS, College Station, TX, [3]Universidad Autonoma de Ciudad Juarez, Chihuahua, Mexico, [4]Universidad Autonoma de Tamaulipas, Reynosa, Mexico.*

Poultry litter is a byproduct produced in large amounts by intensive poultry production systems. While it contains appreciable amounts of nitrogen as uric acid, which makes it an excellent crude protein feed supplement for ruminants, it is usually a carrier of enteropathogenic bacteria of importance to human health. The objectives of this study were to determine the bactericidal effectiveness of several nitrocompounds, assess their potential to produce a feedstuff that is environmentally safe and their potential as a sustainable technology to preserve the nitrogen contained in poultry litter. Twenty grams of 6-mo-old poultry litter (82.5 ± 1.1% dry matter) were distributed in 709-mL plastic containers, mixed with 7.5 mL of deionized water containing no added nitrocompound or 40 mM 2-nitroethanol, 2-nitro-1-propanol (2nitropropanol), nitroethane, 3-nitropropionic acid or ethylnitroacetate. Each container was then inoculated with approximately 2×10^4 CFU of a *Salmonella enterica* serovar Typhimurium (NVSL 951776), naturally resistant to 25 μg/mL novobiocin and made resistant to 20 μg/mL nalidixic acid, and incubated aerobically at 37°C for 24 h. Samples collected at 0, 6, and 24 h were enumerated for *Salmonella.* Generic *E. coli* were enumerated on 3M *E. coli*/coliform petrifilm in samples collected at 0 and 24 h. Samples were also analyzed spectrophotometrically for determination of uric acid. Data were analyzed for main effects of treatment, time of incubation and their potential interaction on temperature and pH as well as on microbiological concentrations by a repeated measures analysis of variance. Main effects of treatment ($P < 0.0001$; SEM = 0.159), time ($P < 0.0001$; SEM = 0.170) and their interaction ($P = 0.0001$; SEM = 0.417) were observed on *Salmonella* concentrations. *Salmonella* concentrations in samples treated with 44 mM 2nitroethanol, 2nitropropanol or ethylnitroacetate were decreased by 0.7 to 1.7 $\log_{10}$CFU/mL within the first 6 h of incubation. Main effects of treatment (P

= 0.0100; SEM = 0.108), time (P = 0.0020; SEM = 0.065) and their interaction (P = 0.0145; SEM = 0.160) were observed on generic *E. coli* concentrations. Main effects of treatment were observed on rates of ammonia accumulation and the residual uric acid concentrations. Urea concentrations were below the limit of detection which under conditions of use was 1 µmol/ml. Results indicate that these environmentally sustainable and safe nitrocompounds negatively impacted potential enteropathogens contained in poultry litter. Additionally, litter treated with these compounds maintained appreciable levels of nutritionally available nitrogen in the form of uric acid, enhancing the value of used poultry litter as a ruminant feed supplement.

Key Words: *E. coli*, poultry excreta, *Salmonella*, uric acid

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0183 Effect of protected sodium butyrate on *Salmonella* spp. excretion in a pig fattening unit.

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This study was conducted to evaluate if the addition of protected sodium butyrate (SB) to a pig diet affected the level of *Salmonella* shedding in feces. The study was performed in a commercial *Salmonella*-infected fattening unit (8 pens, 110 pigs). Feed with 70% SB protected with vegetable fat (3 kg/t) was administrated to animals from 4 randomly selected pens during the fattening period (4 mo) (BUT). Pigs from the remaining 4 pens were fed the same diet without additive (CON). Individual serum and fecal samples were collected at 30, 60, and 90 d of fattening and at slaughter, where mesenteric lymph nodes (MLN) were also collected. Bacteriology on fecal and MLN samples were performed following the ISO 6579:2002 protocol. Serum samples were analyzed by means of an indirect ELISA using 3 cut-off values (OD% $\geq$ 10, $\geq$ 20 and $\geq$ 40). Chi-squared analyses were performed to compare microbiological and serological results between groups at different time periods, and a repeated measures analysis was used to estimate differences in mean OD% after taking into account sampling times and the interaction treatment × time. Although a lower proportion of positive animals in BUT was observed for samplings at 60d (4 vs. 0%), 90d (8 vs. 4%), and at slaughter (9.3 vs. 6.2%), no significant differences were detected, which was likely associated to the overall low prevalence of infection/shedding in both groups. In addition, the proportion of dead/withdrawn pigs in CON was significantly higher than in BUT (13.7 vs. 1.9%; P = 0.03). A higher (P < 0.05) seroprevalence was observed in CON compared with BUT for the sampling just before slaughter and for all cut-off values used (82.2 vs. 64.7%; 53.3 *vs*. 33.3% and 31.1 vs. 13.7%, at OD% $\geq$ 10, $\geq$ 20 and $\geq$ 40, respectively). Also, an overall significant positive relationship was observed between serology before slaughter (cut-off OD% $\geq$ 40) and shedding at slaughter (P < 0.01). The withdrawn of sick pigs in CON may have contributed to its low prevalence of infection/shedding, despite of which a higher seroprevalence was detected in this group at slaughter which, in general, appeared to be positively related to shedding. Thus, overall results suggested that the addition of protected SB at 3 kg/t may reduce the shedding of *Salmonella* spp. under farm conditions. The lowest number of pigs removed from pens in BUT also indicated an overall positive effect on health status of pigs in this group.

Key Words: *Salmonella*, protected butyrate, fattening pigs

0184 Study of genetic basis of immune response in gilts vaccinated with a modified live PRRS virus in a swine farm from southern Sonora Mexico.

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Porcine respiratory and reproductive syndrome (PRRS) is a disease of high negative impact on Mexican porcine production; one of the main reasons is the highly-variable response to vaccination. The objective of this study was to validate the favorable relationship among genotypes and immune response after PRRS vaccination for SNP previously associated with serum antibody response (SAR) and rectal temperature (RT). This study included 6-mo-old 3/4-Landrace × 1/4-Yorkshire replacement gilts (n = 100). After a 7-d acclimation period, all gilts were vaccinated with a modified live PRRS virus (d 0). The antibody response was measured from blood serum samples collected the d 7, 21, and 35 after vaccination using a commercial antibody ELISA kit (IDEXX Laboratories, Inc.). Rectal temperature data were collected the d 7, 14, 21, 28, and 35 using a digital GLA M750 thermometer (GLA Agricultural Electronics). A blood sample was also collected from each gilt approximately 40 d after vaccination and spotted onto FTA cards. All cards were processed for genomic analyses using a low-density chip to obtain genotypes from 8826 SNP (Infinium BeadChip, Illumina, San Diego, CA). In a previous analysis of these data, multi-locus mixed models performed in SNP Variation Suite 7 identified nineteen SNP associated with immune response (P < 0.001). The associative relationship between these SNP and the phenotypes SAR and RT was validated using a mixed effects model; this model included SNP genotype and age of

dam as fixed effects, and sire as a random. Allele substitution effect was also calculated using a regression model that included genotype term as a covariate. Mean S/P values for SAR were 2.26 ± 0.08 and mean values for RT were 39.0 ± 0.05°C. From the SNP previously identified, ASGA0019937, ALGA0025501 and H3GA0020133 were associated with SAR after PRRS vaccination ($P < 0.01$), and the favorable alleles increased S/P levels in 0.21 ± 0.06, 0.19 ± 0.07 and 0.46 ± 0.10, respectively. However, only ALGA0017541 was associated to RT ($P < 0.01$), and the favorable allele reduced 0.21 ± 0.01°C the rectal temperature. In conclusion, four specific genetic markers that underlie genetic variation in response of gilts to PRRS vaccination were validated using a SNP genotype to phenotype association analysis. We propose such SNP as candidates for use in a marker assisted selection program that further evaluates the immune response to PRRS vaccination in gilts of southern Sonora production systems.

Key Words: gilts, immune response, PRRS vaccination.

UNDERSTANDING INFLAMMATION AND INFLAMMATORY BIOMARKERS TO IMPROVE ANIMAL PERFORMANCE

0185 Overview of the inflammatory response and its nutritional costs.

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Innate immune cells respond quickly to a potential pathogen due to the presence of a common set of receptors on all phagocytic cells that recognize broad categories of pathogens. Thus, a very large number of cells can recognize invading microbes and respond to them quickly. A consequence of this is pathogen clearance, usually by phagocytosis, followed by the release of inflammatory cytokines and chemokines that amplify the local infiltration of additional inflammatory cells and activate them. If the challenge is large or if it is accompanied by damage to host tissue, cytokines are released in sufficient amounts that they have endocrine-like effects throughout the body. This cytokine storm induces metabolic changes, including increased protein degradation and insulin resistance in skeletal muscle, which diverts nutrients from muscle and other tissues so that they become available for the increased demands of leukocytes and for the production of protective proteins. Importantly the liver transitions from maintaining homeostasis and supporting the nutritional demands of growth or reproduction to the production of protective proteins such as complement, mannan binding protein, and C-reactive protein that aid in the detection and neutralization of pathogens. This transition is accompanied by hepatic hypertrophy. A study of the costs of a systemic inflammatory response in chickens to *Salmonella* that examined the amount of nutrients in 6 different leukocyte types in 5 different tissues (blood, spleen, bursa, thymus, bone marrow) and 12 protective proteins (acute phase proteins and immunoglobulins) found that the amount of essential amino acids in the protective proteins greatly exceed that in the cellular component of the immune system during both a normal and an inflammatory state. The ideal balance of amino acids for the acute phase of an inflammatory response differs greatly from that needed for growth and there is a critical need for additional cysteine and threonine. Ongoing research indicates that higher metabolic rate, decreased intake of food, a mismatch between the nutrient balance needed for the inflammatory response relative to that in body tissues and less efficient digestion that accompany a robust inflammatory response are, together, even more costly than the direct use of nutrients by inflammatory cells and the liver. Together, these costs result in decreased productivity that cannot be completely reversed by supplying additional nutrients.

Key Words: inflammation, cytokines, nutrients

0186 Ruminal microbes, microbial products, and systemic inflammation.

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The ruminal ecosystem is inhabited by complex communities of microbes that include bacteria, archaea, protozoa, fungi and viruses. The immune system of the animal has evolved to maintain tolerance to innocuous gut commensals and induce protective responses to pathogens. Besides fermentative role, ruminal microbes do have the potential to influence the overall health of the host because of their ability to induce systemic inflammation. The ruminal epithelium-vascular interphase allows absorption of fermentation products and also serves as a selective barrier to prevent translocation and systemic dissemination of bacteria, bacterial toxins, and immunogenic factors. Ruminal dysbiosis that increases ruminal acidity and osmolarity may increase permeability and even induce a breach in the integrity of the epithelial and vascular endothelial barriers, thus facilitating entry of bacteria or bacterial antigens into the portal vein. A classic example is the delivery of ruminal bacterium, *Fusobacterium necrophorum,* into the liver to cause abscesses, which is facilitated by ruminal damage induced by excessive accumulation of lactic acid or VFA. Bacteria that manage to exit or bypass the liver can cause systemic inflammation in other organs, such as lungs, heart, joints, hoof, etc. Shifts in microbial populations associated with dysbiosis result in increased concentrations of potentially toxic and inflammatory substances, which include endotoxic lipopolysaccharide (LPS), biogenic amines, ethanol, etc. A bacterial product that has received a lot of interest is LPS, a component of all Gram negative bacteria. The entry of LPS into the systemic circulation, either from the rumen or the lower gut, could trigger release of proinflammatory cytokines, reactive oxygen and nitrogen intermediates, and bioactive lipids. The inflammatory response to the presence of ruminal LPS in the blood is evidenced by increase in acute phase proteins, such as haptoglobin and LPS binding protein.