

Animal Health: Swine health and transition cows

237 Protected sodium butyrate may reduce *Salmonella* spp. excretion in contaminated fattening pig farms. M. Puyalto*¹, R.C. Mainar-Jaime², S. Andres-Barranco³, E. CREUS⁴, and J. J. Mallo¹, ¹Norel S.A., Spain, ²Department of Animal Pathology, University of Zaragoza, Zaragoza, Spain, ³AgriFood Research and Technology Centre of Aragon, Spain, ⁴Agrofestiic S.L., Spain.

The objective of this study was to assess the level of *Salmonella* shedding and exposure in a fattening unit when protected sodium butyrate was added to the diet of the pigs. The study was carried out in a commercial *Salmonella*-infected fattening unit (8 pens, 110 pigs). Feed with 70% sodium butyrate protected with vegetable fat (3 kg/t) was administered to animals from 4 randomly selected pens throughout the whole fattening period (4 mo) (TG). Pigs from the remaining 4 pens were fed with regular diet without additive (CG). Individual serum and fecal samples were collected at 30, 60, and 90 d of fattening period and at slaughter. Bacteriology on fecal samples was performed following the ISO 6579:2002 protocol. Serum samples were analyzed by means of an indirect ELISA and 3 cutoff values were used (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. A difference was considered significant when the one-tail *P*-value was \leq 0.05. In addition, a repeated measures analysis was used to estimate differences in mean OD%, after taking into account sampling times and the interaction treatment \times time. The levels of shedding were significantly lower for TG when compared with CG for all samplings but the second one (60 d). No significant differences between groups were observed when cut-off values of OD% \geq 10 or %OD \geq 20 were used. However, when OD% \geq 40 was considered, significant differences in seroprevalence were observed for the sampling just before slaughter (CG 89.6% vs. TG 48%). Overall, a lower mean OD% value was observed for samplings at 60 and 90 d, and at slaughter in the TG (71, 66, and 46%, respectively) compared with the CG (88, 87, and 83%, respectively). The results indicate that the use of protected sodium butyrate at 3 kg/t may reduce the shedding of *Salmonella* spp. under this farm conditions, and therefore the risk of contact of the animals with this pathogen, as suggested by serological results.

Table 1 (Abstr. 237). Number of positive *Salmonella* animals/no. of total animals (% positive *Salmonella*)

Treatment	30 d	60 d	90 d	Slaughter
CG	19/29 (65.5%)	5/21 (23.8%)	7/21 (33.3%)	36/48 (75%)
TG	12/28 (42.8%)	2/21 (9.5%)	1/22 (4.5%)	29/50 (58%)
<i>P</i> (one-tail)	0.04	0.2	0.01	0.05

Key Words: *Salmonella* spp., sodium butyrate protected

238 Rapid cooling after acute hyperthermia alters intestinal morphology and may negatively affect pig health. J. S. Johnson*, A Sapkota, and D.C. Lay Jr., *USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN.*

Heat stress (HS) reduces livestock welfare and productivity and can negatively affect pig health. The study objective was to determine the effects of 2 HS recovery methods (rapid vs. gradual cooling) on pig welfare after acute hyperthermia. In 4 repetitions, 36 barrows (88.7 \pm 1.6 kg BW) were exposed to thermoneutral conditions (TN; n = 3/rep;

19.5 \pm 0.1°C) for 6h, or HS (36.4 \pm 0.1°C) for 3h, followed by a 3h recovery period of rapid cooling (HSRC; n = 3/rep; immediate TN exposure and water dousing) or gradual cooling (HSGC; n = 3/rep; gradual decrease in HS room temperature to TN). To evaluate heat dissipation, a thermal circulation index was calculated using gastrointestinal tract (T_{GI}), ambient (T_A), and skin (T_{SKIN}) temperatures collected every 15 min [TCI; $(T_{SKIN} - T_A)/(T_{GI} - T_{SKIN})$]. In repetitions 1 and 2, blood was collected at 60, 180, 210 and 240 min for lipopolysaccharide (LPS) analysis, and then pigs were euthanized at 360 min and duodenum, ileum and colon tissue was collected to determine intestinal morphology. HS treatment reduced ($P < 0.02$) TCI in HSRC (-0.69) and HSGC pigs (-0.64) compared with TN controls (1.31); however, during recovery HSRC reduced TCI ($P < 0.01$; 53.5%) compared with HSGC and TN treatments. In the duodenum and ileum, HSRC and HSGC treatment reduced villus height ($P < 0.01$; 29.4 and 21.1%, respectively) and increased crypt depth ($P < 0.01$; 56.9 and 32.2%, respectively) and villus width ($P < 0.01$; 30.5% and 32.5%, respectively) compared with TN controls. In addition, HSRC reduced duodenal and ileal villus height ($P < 0.01$; 27.6 and 38.2%, respectively) and increased crypt depth ($P < 0.01$; 43.8 and 35.2%, respectively) compared with HSGC pigs. While no colon crypt depth differences were observed for HSRC and HSGC pigs (156.8 μ m), crypt depth was reduced ($P < 0.01$; 37.1%) in HSRC and HSGC pigs compared with TN controls. During HS, circulating LPS was similar for all treatments ($P < 0.57$; 56.2 EU/mL); however, during recovery HSRC pigs had increased circulating LPS ($P < 0.05$; 68.5 and 52.4%, respectively) compared with TN and HSGC pigs. In summary, rapid cooling after acute hyperthermia reduces heat dissipation capacity, damages intestinal tissue, and increases circulating LPS compared with gradual cooling.

Key Words: heat stress, pig, recovery

239 Mycotoxin-contaminated diets affect immunity parameters of piglets. Simone Schaumberger*, Sabine Masching, and Ursula Hofstetter, *BiomIn Holding GmbH, Herzogenburg, Austria.*

Important aspects of more than one multi-mycotoxin in feed, are the synergistic and additive effects which may result in negative effects on immunity and liver health of animals. The aim of the feeding trial was to investigate the effects of a multi-mycotoxin contaminated diet on immune parameters and liver health in weanling piglets. Twenty-four female weanling piglets were randomly assigned to 2 groups with 12 piglets each. Piglets were assigned to a vaccination program including hog cholera, pseudorabies and foot and mouth disease. Naturally mycotoxin contaminated corn was used to prepare the feed. Groups were as following: negative control group (A) and a mycotoxin contaminated (ZEN 1183 ppb, DON 1740 ppb, FUM 988 ppb) feed group (B). Parameters evaluated included antibody titers for pseudorabies referred to as sample to negatives ratio (S/N 0.6 was defined as positive), plasma CD4+/CD8+, IL-2, TNF- α , IgA, IgG, IgM, total plasma protein (TP) and liver enzymes (ALT, AST). The ratio of S/N in group B was significantly higher compared with group A at d 42 ($P < 0.05$). There were no significant differences of plasma CD4+/CD8+ among the 2 groups. The plasma IL-2 levels of group B were significantly lower than group A at d 14, 28 and 42 ($P < 0.05$). The plasma TNF- α level of group B was significantly lower than group A at d 28 and 42 ($P < 0.05$). The IgA, IgG levels of group B were significantly lower than group A at d 28 and 42 ($P < 0.05$). There was no significant difference of IgM among the 2 groups. Plasma TP levels of group B were significantly

lower than group A at d 14, 28 and 42 ($P < 0.05$). Liver enzymes AST and ALT of group B were significantly higher compared with group A at d 14, 28 and 42 ($P < 0.05$). To conclude, the combination of mycotoxins can damage the liver and impair immune response in weanling piglets. The decreased antibody titer against pseudorabies and the increase of S/N ratio in the group fed with mycotoxin-contaminated diet, suggests animal's decreased protection against pseudorabies when mycotoxins are present. This points to the need for greater awareness of a multiple mycotoxin contamination in feed and the protection of animals against said toxins.

Key Words: mycotoxin, piglet, immunity

240 Butyrate enhances disease resistance of piglets through up-regulated gene expression of endogenous host defense peptides. Haitao Xiong*, Bingxiu Guo, and Yizhen Wang, *College of Animal Sciences of Zhejiang University, Hangzhou, Zhejiang, China.*

Dietary substances can manipulate the expression of endogenous host defense peptides (HDPs), which may provide a promising strategy for disease control and prevention, especially for antibiotic-resistant infections. We hypothesized that butyrate can induce HDP expression, which likely contributes to the elimination of *Escherichia coli* O157:H7 in the intestine and reduces the severity of inflammation caused by *E. coli* O157:H7 challenge. Piglets treated with or without butyrate (2 g/kg of diet) 2 d before *E. coli* O157:H7 challenge was employed to investigate the relationship among porcine HDP expression, status of inflammation and *E. coli* O157:H7 load in feces. The effects of butyrate on HDP gene expression, antibacterial activity of 3D4/2 macrophages and IPEC-1 cells, and phagocytic capacity of 3D4/2 and polymorphonuclear (PMN) leukocytes in vitro were also examined. Data were represented as mean \pm SEM. One-way ANOVA or Student's *t*-test was used to compare differences between treatment groups, $P < 0.05$ was considered statistically significant. Results showed that butyrate treatment (1) alleviated clinical symptoms of *E. coli* O157:H7-induced hemolytic uremic syndrome and severity of intestine inflammation, (2) significantly reduced *E. coli* O157:H7 load in feces 24 h after the last inoculation ($P < 0.01$), (3) significantly upregulated multiple HDPs, such as pBD2, pBD3, PMAP23, PR-39, and PG1-5 ($P < 0.05$), but not all HDPs, in vitro and in vivo, and (4) enhanced the antibacterial activity of macrophage 3D4/2 and IPEC-1 and the phagocytic capacity of 3D4/2 and PMN cells in vitro. Our findings indicate that butyrate enhances disease resistance, promotes clearance of *E. coli* O157:H7, and alleviates clinical symptoms of hemolytic uremic syndrome and inflammation partially by affecting endogenous HDP expression.

Key Words: butyrate, *E. coli* O157:H7, host defense

241 The effect of antiseptic compounds on umbilical cord healing and infection rates in neonatal piglets from a commercial facility. Amanda L. Robinson*, Jessica D. Colpoys, Glenn D. Robinson, Elizabeth A. Hines, Leo L. Timms, Erika M. Edwards, Kenneth J. Stalder, Anna K. Johnson, and Howard D. Tyler, *Iowa State University, Ames, IA.*

Umbilical cord antiseptics are often not used in swine production systems. The objective of this study was to determine if treating the umbilical cord with antiseptics reduces infection and enhances healing within the first 48 h after birth in newborn piglets. A total of 421 mixed sex commercial piglets from a breed-to-wean sow farm were enrolled. Piglets were alternately assigned by birth order within a litter

to 4 treatment groups; (1) iodine (2%), (2) trisodium citrate (10%), (3) a dry dip created using an antibacterial peptide (nisin) mixed with talc, and (4) no treatment. All treatments were applied within 1 h of birth. 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). Prior to treatment, diameter of the umbilical cords (as an indicator of cord drying and healing) was 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. These measurements were repeated at 24 ± 1 h of age and at 48 h of age. In addition, umbilical stump redness and swelling (indicators of infection) were evaluated visually at 24 and 48 h. All data were analyzed using mixed model methods. Models included the fixed effects of umbilical diameter at birth, sex (female or male), stall conditions and treatment. No treatment differences were noted between dips on change in diameter of the umbilical cord during the first 24 h (6.60 ± 0.057 mm at birth vs. 3.25 ± 0.072 mm at 24 h). There was no difference in umbilical cord stump surface temperature, redness or swelling at 24 h or 48 h. Stall conditions at birth did not affect the change in umbilical diameter, surface temperature of the umbilical stump, or visual indications of infection. In conclusion, there was no benefit observed when applying an antiseptic treatment on piglet umbilical cords to improve healing or reduce the incidence of infections during the first 48 h of life.

Key Words: piglet, umbilical cord, antiseptic

242 Porcine hepcidin protects piglet intestinal epithelial cells by aggregating *Escherichia coli* K88. Huahua Du*, Dan Liu, and Zhenshun Gan, *Zhejiang University, Hangzhou, China.*

Hepcidin is a liver-expressed iron-regulating hormone that also is an antimicrobial peptide. Though the iron regulatory function of porcine hepcidin (pHepc) has been extensively investigated, the studies on the relationship between pHepc and bacteria are limited. The aim of current study was to evaluate its antibacterial activity against *Escherichia coli* K88 (*E. coli* K88) and investigate the effect of pHepc on bacterial infection in vitro. The antibacterial activity against pathogen bacteria was evaluated via radial diffusion, colony forming count, transmission electron microscopy (TEM) and DNA binding assays. Invasion assay and immunofluorescence microscopy were employed to determine its effect on bacterial infection. The results showed that pHepc exerted an iron-independent bacteriostatic activity against *E. coli* K88 in a dose-dependent manner. pHepc-treated *E. coli* K88 exhibited longer cells and cytoplasm unevenly distribution. To determine whether pHepc inhibited bacterial infection, piglet intestinal epithelial cells were challenged with pHepc-treated or untreated *E. coli* K88. The ability of pHepc-treated bacteria to invade IPEC-1 epithelial cells was impaired. pHepc significantly reduced 33.5% amount of *E. coli* K88 which adhered or invaded to cells ($P < 0.05$). Another human epithelial colorectal adenocarcinoma cell Caco-2 exhibited the same inhibition pattern by pHepc treatment ($P < 0.05$). By scanning electron microscopy (SEM), pHepc-treated *E. coli* K88 was aggregated, and higher magnification revealed a net-like meshwork of fibrils emanating from the bacterial surface that entangled the bacteria. It suggested that pHepc could afford protection against infection of *E. coli* K88 by a novel aggregation strategy, which will contribute broadly to piglet innate immunity.

Key Words: porcine hepcidin, *E. coli* K88, aggregation

243 Limited sun exposure increases 25(OH)D serum concentration and affects mRNA expression of the vitamin D enzymes in liver and kidney of growing pigs at high altitude. Samanta R. Fensterseifer*¹, D. Enette Larson-Meyer², Bennett C. Ingold², Kathleen J. Austin¹, Kacey C. Myers¹, and Brenda M. Alexander¹, ¹Department of Animal Science, University of Wyoming, Laramie, WY, ²Department of Family and Consumer Sciences, University of Wyoming, Laramie, WY.

Traditional confinement production management limits exposure of pigs to sunlight. With vitamin D primarily obtained from the diet, producers risk suboptimal vitamin D status. Ten weaning pigs (67.6 ± 6.1 kg; 76d ± 11) were randomly divided into control (CON, n = 5, traditional swine confinement) or sun (SUN, n = 5, exposed to one hour of sunlight at solar noon for 2-weeks during the summer solstice and fall equinox) groups. Blood samples were collected via cranial cava venipuncture before and after the 2-week sunlight exposure to determine serum concentration of 25-hydroxyvitamin D [25(OH)D]. Following fall sun exposure, pigs were slaughtered and liver and kidney tissue collected and frozen. Expression (mRNA) of vitamin D binding protein (VDBP), vitamin D receptor (VDR) and the enzymes CYP2R1 and CYP27B1 were determined. Serum 25(OH)D concentration did not differ ($P = 0.1$) before summer sunlight exposure (39.5 ± 2.6 ng/mL). Sunlight exposure increased ($P = 0.001$) serum concentration of 25(OH)D by 54.5% in SUN pigs during summer exposure, with 25(OH)D 87.5% greater ($P = 0.001$) compared with CON pigs (66.5 ± 4.8 vs 35.5 ± 2 ng/mL, respectively). Serum 25(OH)D concentration returned to CON values ($P = 0.3$) by the fall exposure period (45.1 ± 2.1 ng/mL). Following fall sun exposure, serum 25(OH)D concentration was again increased ($P < 0.001$) in SUN compared with CON pigs (80.8 ± 3.3 vs. 39.9 ± 3 ng/mL). CYP27B1 mRNA expression was decreased in kidney ($P = 0.03$) but increased in the liver ($P = 0.05$) of SUN pigs when compared with CON pigs. The relative mRNA expression of VDBP, VDR and CYP2R1 did not differ ($P > 0.05$) among pigs. In conclusion, 2-week sunlight exposure at high altitude during the summer solstice and fall equinox increases serum concentration of 25(OH)D and influences expression of CYP27B1 in kidney and liver of sun exposed pigs. Limited sun exposure enhances vitamin D status in confinement-raised pigs and may improve health and production outcomes dependent on vitamin D.

Key Words: vitamin D, sun exposure, pig

244 Relationship between left displacement of the abomasum and daily milk yield in high-producing Chilean dairy cows. Pedro G. Melendez*¹, Catalina Romero², Maria P. Marin², Mario Duchens³, Patrick Pithua¹, and Pablo J. Pinedo^{4,5}, ¹University of Missouri-Columbia, Columbia, MO, ²University Santo Tomas, Viña del Mar, Chile, ³University of Chile, Santiago, Chile, ⁴Texas A&M AgriLife Research, Amarillo, TX, ⁵Texas A&M University System, College Station, TX.

Left displacement of abomasum (LDA) is a metabolic disease affecting dairy cows within the first 30 DIM. Dairy farms in central Chile, consisting of Holstein cows maintained under confinement, use advanced technology including computerized record keeping systems. These farms have well-established postpartum health monitoring programs and LDA is regularly diagnosed. However, due to a scarcity of immediate veterinary assistance, many cows with LDA are treated conservatively by administering oral fluids, anti-inflammatory drugs and/or antibiotics. The hypothesis was that cows with LDA treated conservatively would produce less milk than cows with LDA treated surgically. The objective was to compare daily milk production among cows with LDA that received a conservative treatment, had surgery (right omentopexy or

toggle suture) or were not affected (negative controls). Data between 2010 and 2012 from 4 high-producing dairies in Central Chile were analyzed. Cases (n = 146) were categorized as treated surgically (DAs, n = 72), treated with toggle suture (DA_t, n = 16), or treated conservatively (DA_c, n = 58). For each case, 2 healthy contemporary cows (controls) were selected at random and matched by farm, parity and DIM (±15 d; n = 289). A mixed model for repeated measures was developed for the analysis of daily milk yield up to 90 DIM with the corresponding covariance structure based upon the best goodness of fit criteria. The mean and median DIM for LDA diagnosis was 13 and 9 d, respectively. Average daily milk up to 90 DIM was 43.0 kg/d for controls, 30.4 kg/d for DAs, 30 kg/d for DA_t, and 14.2 kg/d for DA_c. Controls produced more milk than DAs, DA_t, and DA_c group ($P \leq 0.01$). DAs and DA_t produced a similar amount of milk ($P > 0.05$), but more milk than the DA_c group ($P \leq 0.01$). It is concluded that cows with LDA receiving conservative treatment produced substantially less milk than control cows (30 kg/d) and cows with LDA treated with surgery or toggle suture (16 kg/d). Furthermore, control cows produced more milk than cows with LDA treated with surgery or toggle suture (13 kg/d). In light of these results, it is recommended that all cows developing LDA should be treated surgically or with toggle suture.

Key Words: displaced abomasum, treatment

245 Comparison of hematological parameters in dairy cows during periparturient period. Samir Kumar Dash*¹, Hemant Dasharath Kadam¹, Asmita Anant Kulkarni¹, Mohua Das Gupta¹, Narayan Laxman Phadke¹, and Arun P. Phatak², ¹BAIF Development Research Foundation, Central Research Station, Uruli Kanchan, Pune, Maharashtra, India, ²Waterford, CA.

The periparturient period ranging from 3 wk before calving to 3 wk after calving presents enormous physiological challenges for the dairy cows. It is a period that is affected by metabolic stressors, major changes in endocrine status and altered immune function which together results in an increased risk of diseases including hypocalcaemia, ketosis, hepatic lipidosis, laminitis, mastitis, retained placenta and metritis. The present investigation was undertaken in Central Cattle Breeding Farm, Central Research Station, BAIF, India to study the hematological changes in puerperal period in 30 cows of HF pure, JE pure and their crosses under tropical conditions to compare the Hgb, TLC and CBC/diff. count 10 d before and 10 d after parturition to assess the extent of metabolic changes, infection and stress. The results were analyzed using ANOVA. The study revealed a nonsignificant difference and decrease in the Hgb count from 11.41 ± 0.30 to 10.59 ± 0.30 which may be due to the increased blood supply to fetus and milk adipose tissues with hemorrhagic loss during calving. The TLC count displays a significant ($P < 0.05$) increase from 8.57 ± 0.39 to 10.00 ± 0.58 in postpartum period confirming the reports of previous studies which states that there is a decrease in immunity level due to stress and rise in infection. Neutrophil count increased from 47.03 ± 1.77 to 51.27 ± 2.14 nonsignificantly as the infection in postpartum period may rise. Eosinophil count, Lymphocyte and monocyte level did not reveal any significant changes. It can be concluded that due to lower hemoglobin, hemorrhagic stress and lower immunity due to rise in infection, the animal's health and production are always under threat; hence careful attention is required during the periparturient period.

Contd.

Table 1 (Abstr. 245). Blood parameters in Holstein cows 3 wk pre- and post-calving

Parameter	Hgb (g %)	TLC ($\times 10^3$)	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Pre	11.41 ± 0.30	8.57 ± 0.39	47.03 ± 1.77	6.07 ± 0.43	44.03 ± 1.86	2.73 ± 0.17
Post	10.59 ± 0.30	10.00 ± 0.58	51.27 ± 2.14	5.57 ± 0.42	40.70 ± 2.18	2.53 ± 0.20
“F”	0.0572	0.0454	0.1299	0.5808	0.2494	0.5318

Key Words: tropical, puerperal, immunity

246 Characterizing the effect of feed restriction on biomarkers of leaky gut. Sara K. Stoakes^{*1}, Erin A. Nolan¹, David J. Valko¹, Mohannad Abuajamieh¹, Jake Seibert¹, Maria V. Sanz Fernandez¹, Patrick J. Gorden¹, Howard B. Green², Katie M. Schoenberg², William E. Trout², and Lance H. Baumgard¹, ¹Iowa State University, Ames, IA, ²Elanco Animal Health, Indianapolis, IN.

Study objectives were to determine the magnitude of feed restriction (FR), which causes “leaky gut” and characterize the temporal consequences on production and blood variables. Twenty-three lactating Holstein cows (157 \pm 46 DIM; 713 \pm 54 kg BW; parity 2–4) were enrolled in 2 experimental periods. Period 1 lasted 5 d (ad libitum feed intake) and served as baseline for period 2 (P2), which lasted 5 d during which cows received one of 5 dietary treatments: 100% of ad libitum feed intake (AL; n = 3), 80% of ad libitum feed intake (AL80; n = 5), 60% of ad libitum feed intake (AL60; n = 5), 40% of ad libitum feed intake (AL40; n = 5), or 20% of ad libitum feed intake (AL20; n = 5). As the magnitude of FR increased, milk yield, MUN, and milk lactose content decreased linearly ($P < 0.01$) whereas milk fat content linearly increased ($P = 0.02$). Heart rate declined linearly with increasing FR ($P < 0.02$) while respiration rate declined similarly for all FR treatments ($P = 0.02$). Body weight loss increased linearly with increased FR ($P < 0.01$). Both plasma insulin and BUN decreased, whereas NEFA increased linearly with greater FR ($P < 0.01$). Lipopolysaccharide binding protein, haptoglobin, and serum amyloid A tended to increase linearly with increasing FR ($P = 0.09$, $P = 0.07$, $P = 0.10$, respectively). Circulating lymphocytes increased with increasing FR ($P = 0.04$). AL40 and AL-fed cows were killed to determine the effect of FR on intestinal histology. Jejunum villus width, jejunum goblet cell area, ileum height and ileum crypt depth were or tended to be reduced ($P = 0.03$, 36%; $P = 0.02$, 52%; $P = 0.06$, 22%; $P = 0.03$, 28%, respectively) in AL40 cows compared with AL controls. Liver weight tended to be decreased (15%; $P = 0.07$) in AL40 compared with AL cows. Liver fat and moisture percentages did not differ between treatments. In summary, FR tended to increase circulating acute phase proteins, which we speculate is due to an increase in leaky gut as demonstrated by the deterioration in intestinal architecture. Consequently, FR appears to be a simple and viable model to study intestinal integrity and barrier function.

Key Words: lipopolysaccharide, intestinal integrity, feed restriction

247 Neutrophil function is dysregulated over the transition period. Mallory A. Crookenden^{*1}, Caroline G. Walker¹, Axel Heiser^{6,5}, Juan J. Looor², Kasey M. Moyes³, Jane K. Kay¹, Susanne Meier¹, Alan Murray⁵, Venkata S. R. Dukkipati⁵, Murray Mitchell⁴, and John R. Roche¹, ¹DairyNZ, Hamilton, New Zealand, ²University of Illinois, Champaign-Urbana, IL, ³University of Maryland, College Park, MD, ⁴University of Queensland, Brisbane, Queensland, Australia, ⁵Massey University, Palmerston North, New Zealand, ⁶AgResearch, Palmerston North, New Zealand.

There is a high incidence of infectious and metabolic disease in the transition period. During this time, there is a period of observed immunosuppression that appears to be ‘natural’. The effect of parturition on neutrophil function is of particular interest; we hypothesized that neutrophil dysfunction occurs around the transition period, as evidenced by altered gene expression. To determine this, we extracted neutrophils from peripheral blood of 45 cows at 5 time points: 1 wk pre-calving (–1 wk), day of parturition (d 0), and post-calving at wk 1, 2, and 4. Key pathways of neutrophil function were investigated by qRT-PCR. The ‘bovine immune panel’ (BIP) included 96 targets for detection with Fluidigm 96.96 integrated fluidic circuits and the Biomark HD system. Data were analyzed using SAS 9.3; the effect of time was analyzed using a mixed model approach to repeated measures ANOVA and Tukey’s *t*-test for pairwise comparisons between weeks. Data were submitted for pathway enrichment analysis using the protein analysis through evolutionary relationships tool. The top 3 pathways enriched from gene targets on the BIP included inflammation signaling (14-fold enrichment, $P < 0.001$), apoptosis signaling (19-fold enrichment, $P < 0.001$), and interleukin/cytokine signaling (18-fold enrichment, $P < 0.001$). Biological processes enriched at –1 wk, d 0, and 1 wk post-calving included the immune system process, response to stimulus, and the immune response. The immune response process was most highly enriched (8-fold enrichment, $P < 0.05$) at 1 wk post-calving, which coincided with greatest NEFA concentration (1.08 \pm 0.52) and, therefore, greatest NEB. No biological processes were enriched at 2 wk or 4 wk post-calving. Results indicate that the gene expression profile of neutrophils is altered over the transition period. This provides insight into neutrophil activity around parturition and the increased susceptibility to disease during this time.

Key Words: neutrophil, transition period, dairy cow

248 The effects of grain-induced subacute ruminal acidosis on blood plasma proteomic characterization in dairy cows. S. C. Li^{*1}, A. M. Danscher², P. Azevedo¹, P. H. Andersen³, P. Ezzati⁴, J. A. Wilkins⁴, E. Khafipour¹, and J. C. Plaizier¹, ¹Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, ²Department of Large Animal Sciences, University of Copenhagen, Copenhagen, Denmark, ³Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, ⁴Manitoba Centre for Proteomics & Systems Biology, Winnipeg, Manitoba, Canada.

Subacute ruminal acidosis (SARA) resulting from excessive grain feeding to dairy cows is accompanied by an immune response and has been associated with impaired liver function and blood composition. We, therefore, investigated the proteome in blood plasma to discover potential biomarkers for SARA. Four lactating Danish Holstein cows were used in a study that included a 4-week control period followed by a one-week SARA challenge period. During the control period, all cows received a total mixed ration (174 g crude protein/kg DM; 192 g starch/kg DM; 6.28 MJ NE_L/kg DM) ad libitum. During the SARA period, 40% of the ration was gradually substituted with grain pellets (50:50 wheat:barley) within 3 d to induce SARA. This SARA induction diet was then fed for another 4 d. Rumen pH was monitored with indwelling pH probes to verify that cows had rumen pH lower than 5.6 for 3 h daily during the SARA induction. Blood samples were obtained from the jugular vein at 7 h post-feeding on d 4 before SARA induction and on the second of 4 SARA-induction days. The blood plasma samples were then processed for the enrichment of low molecular weight serum proteins using acetonitrile precipitation before mass spectrometry-based proteomic analysis on a nano-RPLC-MS/MS system. The analysis

identified 14 high-confidence proteins with significant quantitative differences between control and SARA periods as defined by MS intensity, of which 9 were potential differentially expressed (PDE) proteins LASP1, TMSB4, and the precursors of KNG1, APOE, SERPINA3–8, CRP, SERPINF2, SERPIND1 and CATHL4 reportedly involved in acute phase response, cytoskeletal proteins, blood coagulation, cholesterol transport, liver disease, and innate immune defense molecules. Among these PDE proteins, LASP1 and the precursors of APOE, SERPINA3–8,

SERPINF2, SERPIND1 and CATHL4 were upregulated and others were downregulated ($P < 0.05$). These results suggest that it may be possible to identify proteins that are differentially expressed during SARA. Studies are ongoing to validate the possible candidates and identify additional proteins for the understanding of ethological concepts of SARA.

Key Words: dairy cow, SARA, plasma proteome