625 Analysis of immune-relevant genes expressed in spleen of *Capra hircus* kids fed with Cr-Met supplement. M. J. Najafpanah* and M. Sadeghi, *University of Tehran, Tehran, Iran.*

Various metals are responsible for many immunological activities of the body as micronutrients. Chromium has a significant role in altering the immune response by immunostimulatory or immunosuppressive processes. Our objective is to survey the effect of Chromium (Cr^{3+}) on expression of B2M, MHCII-DRA, MHCII-DRB and RAP2A genes in spleen that it houses immune cells such as T and B cells. Here, we investigated the effect of Chromium on genes expression in goat that treated with Chromium as chromium-methionine (Cr-Met) supplement. Twenty-four, male kids of Mahabadi goat were used and randomly allocated to 1 of the 4 dietary treatments according to live weight. They were individually penned for 90-d feeding period. The treatments were included 3 levels of 0.5, 1 and 1.5 mg/day of chromium plus standard control diet and the fourth treatment was standard control diet without Cr-Met supplement. After feeding, the kids were slaughtered and samples taken from the spleen. Total RNA was extracted and first-strand cDNA synthesis was performed. Real-time PCR was performed on iQ5 Bio-Rad system using HSP-90 as a reference gene. The results showed that expression of each 4 genes affected by chromium have increased significantly compared with the control treatment ($P < 0.05$). The highest and the lowest level of expression was related to the treatment containing 1.5 and 0.5 mg/day of chromium, respectively. Consequently, it may be concluded that supplemental chromium has some beneficial effects on the health status and it may lead to the increased susceptibility of animals to resist certain diseases such as bacterial infection or parasitic illness.

Key Words: chromium, Mahabadi goat, MHC gene

626 Prenatal transportation alters the acute phase response (APR) of bull calves exposed to a lipopolysaccharide (LPS) challenge. N. C. Burdick Sanchez*[1], J. A. Carroll[1], D. M. Price[2,4], B. P. Littlejohn[2,4], M. C. Roberts[2,4], R. C. Vann[3], T. H. Welsh Jr.[4], H. D. Hughes[5], J. T. Richeson[5], and R. D. Randel[2], [1]*Livestock Issues Research Unit, USDA-ARS, Lubbock, TX,* [2]*Texas A&M AgriLife Research, Texas A&M University System, Overton,* [3]*MAFES-Brown Loam, Mississippi State University, Raymond,* [4]*Texas A&M AgriLife Research, Texas A&M University System, College Station* [5]*Department of Agricultural Sciences, West Texas A&M University, Canyon.*

This study was designed to determine if prenatal transportation influences the APR to a postnatal LPS challenge. Pregnant Brahman cows (n = 96) matched by age and parity were separated into transported (TRANS; n = 48; transported for 2 h on gestational d 60, 80, 100, 120 and 140) and non-transported control groups (CONT; n = 48). From these cows, bull calves (n = 16 per TRT) were identified at weaning (176 ± 2 d of age) to subsequently receive a LPS challenge. We previously reported an effect of TRANS on temperament (TEMP); therefore bulls were also grouped based on TEMP score [Calm (C); Intermediate (I), or Temperamental (T)]. On d −3 bulls were fitted with rectal temperature (RT) probes and were transported from Overton to Lubbock, TX on d −2. On d −1 bulls were fitted with jugular cannulas and placed in individual stanchions. On d 0 blood samples and sickness behavior scores (SBS) were collected at 0.5-h intervals from −2 to 8 h and again at 24 h relative to LPS challenge (0.5 μg/kg BW). Serum was analyzed for cortisol, interferon-γ (IFNγ), tumor necrosis factor-α (TNFα), and interleukin-6 (IL6) concentration. All variables increased following LPS ($P < 0.01$). Both pre- and post-LPS TRANS bulls had greater RT than CONT bulls ($P < 0.01$), and T bulls had greater RT than C and I bulls ($P < 0.01$). The SBS was greatest in the C TRANS bulls post-LPS and post-LPS cortisol was lowest in the T bulls ($P < 0.01$). Pre- and post-LPS TNFα was greater in TRANS than CONT bulls ($P = 0.03$ and < 0.01). Post-LPS TNFα was greatest in the T TRANS bulls ($P < 0.01$). Pre-LPS IL6 was greater in TRANS than CONT bulls ($P = 0.02$), yet was greater in CONT than TRANS bulls post-LPS ($P = 0.04$). Pre-LPS, IFNγ was greater in CONT than TRANS bulls ($P < 0.01$), yet was greater in TRANS than CONT bulls ($P < 0.01$) post-LPS. Post-LPS IFNγ was greatest in the I TRANS bulls ($P < 0.01$). Prenatal transportation influenced the physiological and APR before and after LPS, and altered the response within temperament groups. These data demonstrate that prenatal transportation can alter the acute phase response to LPS, and may affect subsequent health and performance of these calves.

Key Words: cattle, immune, transportation

627 Circulating immune cell subpopulations in pestivirus persistently infected calves and non-infected calves varying in immune status. S. M. Falkenberg*, J. Ridpath, and F. V. Bauermann, *USDA-ARS-National Animal Disease Center, Ruminant Immunology Group, Ames, IA.*

The circulating immune cell subpopulations in cattle representing varying stages of immune status categorized as; colostrum deprived (CD), receiving colostrum (COL), colostrum plus vaccination (VAC) and persistently infected with a pestivirus (PI) were compared. The PI calves were infected with a HoBi-like virus, which is a member of the Pestivirus genus and similar to bovine viral diarrhea virus. All calves in the PI group tested positive for viral protein using a commercial ELISA test, all other calves tested negative. Calves in the CD and COL group averaged 60 d of age (DOA), VAC averaged 150 DOA and PI 24 DOA. Blood samples were collected and analyzed by flow cytometry within 2 h after collection. The leukocyte (LEUK) and granulocyte populations were identified using forward and side scatter plots. Primary antibodies were used for identification of the cell markers CD4, CD8, B cell, Gamma-delta (GDTCR) and CD14. Comparisons between immune status groups for total circulating cell populations (cell/mL) revealed differences ($P < 0.0001$) for total LEUK, CD8, B cell, GDTCR and CD14 with the PI group having the lowest total numbers of cells in circulation. While no differences were observed for the absolute number of circulating CD4 among the groups, the proportion of CD4 within total LEUK populations was significantly different ($P < 0.0001$). Significant differences ($P < 0.002$) were also observed in the CD8, B cell, GDTCR and CD14 populations. The PI group had a greater proportion of CD4 cells and the VAC group had the greatest proportion of CD8, while the COL group had the greatest B cell, GDTCR and CD14. No differences ($P > 0.05$) in the absolute number or proportion of circulating cells identified as granulocytes. These results suggest immune cell populations vary between immune status groups with the greatest differences observed for the PI calves. Defining differences associated with different immune statuses could provide insight into immune function.

Key Words: cattle, immune, pestivirus

628 Ontogenetic changes of ochratoxin A on growth performance, serum biochemistry and nephrotoxic damages in cherry valley male ducks. W. Wang[1], L. Zhong[1], H. Ye[1], H. Zhang[2], and L. Yang*[1], [1]*College of Animal Science, South China Agricultural University, Guangzhou, China,* [2]*China National Key Laboratory of Animal Nutrition, Beijing Animal and Veterinary Science Institute, Chinese Agricultural Academy, Beijing, China.*

The objective of this study was to investigate the ontogenetic changes of ochratoxin A (OTA) on growth performance, serum biochemistry and nephrotoxic damages in cherry valley male ducks. Eight hundred one-day-old ducks were assigned to 5 treatment groups with 8 replicates of 20 birds each. The experiment was conducted across 2 periods. Period 1 was d 1 to 21; group I (control group) was fed with commercial diet I free of OTA. Groups II to IV were fed diets containing 2.11, 4.22, 6.33, 8.44 μg/g OTA, respectively. In period 2, all 5 groups were fed with commercial diet II from 22 to 45 d. Our results showed that OTA-contaminated feed reduced ADG and mean intakes significantly, but F/G was not different across treatments in Period 1. In Period 2, ADG of ducks fed 6.33 μg/g diet OTA were decreased. There were no significances of ADFI and F/G in 5 groups. The relative kidney damage in groups D and E from 9 to 35 d was remarkable higher than that in control groups ($P < 0.05$). As to the relative liver weights, group E had the higher damage index compared with the control group after 21 d ($P < 0.05$). Mean blood urea nitrogen, and albumin levels in toxic groups were higher than in control group, especially at second period ($P < 0.05$). No significant difference was observed of TP between toxic groups and control group, but Alb in toxic groups was increased after 21 d. Nephritic glutamic pyruvic transaminase in toxic groups were decreased from 9 to 21 d, and nephritic glutamicoxaloacetic transaminase levels were decreased from 9 to 45 d respectivelyacross periods. Obvious pathomorphological damage in renal tissues of the toxic groups were seen at 3, 9, 15, 21, 35, 45 d. Hyperaemia and granular degeneration in renal tubular epithelial cells were increasingly severe with the higher OTA concentration. These results showed that OTA-contamined feed reduces growth performance and induces nephrotoxic damage in cherry valley male ducks. The damage was not improved even after OTA was eliminated from the feed.

Key Words: ochratoxin A, growth performance, nephrotoxic damage

629 Anamnestic antibody response to BVDV 1b challenge in Angus-Nelore steers. E. D. Downey*[1], X. Fang[1], C. A. Runyan[1], J. E. Sawyer[4], T. B. Hairgrove[3], J. F. Ridpath[2], and A. D. Herring[1], [1]*Texas A&M University, College Station,* [2]*National Animal Disease Center, USDA-ARS, Ames, IA,* [3]*Texas AgriLife Extension, College Station,* [4]*Texas AgriLife Research, College Station.*

Bos taurus-Bos indicus F_2 and F_3 steers were examined over 3 years (2010–2012) for differences in anamnestic antibody responses to a BVDV 1b challenge following a standard vaccine protocol. Angus-Nelore F_2 (n = 122) and F_3 (n = 156) yearling steers were randomly allocated to 1 of 3 vaccine treatments: non-vaccinated (n = 94), a 2-shot killed vaccine (n = 91), or a single-immunization modified-live vaccine (MLV; n = 94). Steers were vaccinated against 5 viral pathogens, BRSV, IBR, PI_3, BVDV 1a and 2, approximately 21d pre-challenge. Serum samples were collected at challenge (d0) through d42 at 14-d intervals. The anamnestic response was measured as the $\log_2$ titer of the area under the curve (AUC) for 42 dpi, calculated using the trapezoid rule for each of the 4 viral responses (IBR, BVDV 1a, 1b, and 2). The measured anamnestic response appeared lowest for IBR and greatest for BVDV 1b, with the largest range in antibody response to BVDV 1b. Factors influencing AUC for the 4 viral pathogens were investigated using mixed model analyses with fixed effects of vaccine treatment, year, composition (F2 or F3), and sire nested within composition along with covariates of d0 titer ($\log_2$), weaning temperament score and d0 weight; pen nested within year was a random effect. The 4 antibody responses were affected ($P < 0.05$) by vaccine treatment and d0 titer. Animals vaccinated with the killed vaccine had significantly higher BDVD 1b AUC responses to the challenge than MLV or non-vaccinated animals (values of 405.6, 256.2, and 183.3, respectfully). Similar trends were seen for the other viruses. A 1-point $\log_2$ increase in titer at time of challenge increased the anamnestic response (AUC) by 15.7, 17.9, 17.4, and 26.5 for BVDV 1b, 1a, 2 and IBR, respectively. Sire nested within composition affected ($P < 0.05$) for BVDV 1a and b and approaching significance for BVDV2 ($P = 0.08$), however no trends across sires were apparent. These results indicate that both genetic and environmental factors affect the anamnestic antibody response mounted to a BVDV challenge and that the d0 titer from the vaccine treatment pre-challenge has significant influence on the response.

Key Words: antibody response, BVDV challenge, cattle

630 Increasing the dietary ratio of n-3 to n-6 fatty acids increases the n-3 concentration of peripheral blood mononuclear cells in Holstein calves. L. C. Nagengast*, A. L. Lock, S. N. Woodruff, C. M. Ylioja, N. A. Martinec, C. V. Vanderson, C. L. Preseault, N. L. Trottier, M. J. VandeHaar, and E. L. Karcher, *Michigan State University, East Lansing.*

Our objective was to test if increasing the ratio of n-3 to n-6 fatty acids (FA) in diets for weaned calves alters growth, the n-3 FA content of peripheral blood mononuclear cells (PBMC), or inflammatory response. Twenty-seven Holstein calves (16–20 wks-old) were housed individually and fed 1 of 3 diets (9 calves/treatment). Diets were total mixed rations of alfalfa silage, corn grain, and soybean meal supplemented with (1) 4% Ca-salts of soybean FA (N6) containing 50% 18:2 n-6; (2) 4% Ca-salts of flaxseed FA (N3) containing 35% 18:3 n-3; or (3) a 50:50 mix of N6 and N3 (MIX). Blood was sampled on d 1 and 28 to determine FA composition of PBMCs. Blood from d 28 was incubated with endotoxin (LPS) for 2 h, and gene expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and osteopontin determined. On d 28, calves were challenged with Pasteurella vaccine and rectal temperatures measured for 48 h. Treatment did not alter body weight or average daily gain. As a percent of total FA, PBMC of N6 calves had 30% total n-6, 11% 18:2 n-6, 15% 20:4 n-6, 6% total n-3, 0.6% 18:3 n-3, 0.4% 20:5 n-3, and 1.0% 22:6 n-3. Compared with N6, N3 resulted in 22% less total n-6 FA, 15% less 18:2, 17% less 20:4, 39% more total n-3 FA, 93% more 18:3, 213% more 20:5, and 11% more 22:6 (all $P < 0.01$, with MIX intermediate). Compared with N6, MIX (but not N3) decreased expression of osteopontin mRNA in PBMC ($P < 0.05$). Compared with N6, N3 tended to decrease expression of IL-1β mRNA in PBMC ($P = 0.08$). Treatment did not alter expression of IL-8 or TNF-α ($P > 0.12$) and had no effect on expression of genes after LPS-stimulation ($P > 0.13$). Treatment did not alter change in rectal temperature following vaccination. We conclude that increasing the dietary ratio of n-3 to n-6 FA for 28-d resulted in corresponding changes in the FA profile of PBMC in postweaned Holstein calves. However, these changes in FA profile of PBMC were not associated with changes in growth or health of calves during the study.

Key Words: dairy calf, immune response, n-3 fatty acid

631 Epigenetic mechanisms control over cytokine gene expression of biased immune response dairy cattle. M. Paibomesai* and B. Mallard, *University of Guelph, Guelph, ON, Canada.*

The adaptive immune response is composed of 2 branches: (1) the antibody-mediated immune response (AMIR; high IFN-γ production), which responds primarily to extracellular pathogens; and (2) the cell-mediated immune response (CMIR; high IL-4 production), which responds primarily to intracellular pathogens. These immune processes are both genetically and epigentically controlled. Epigenetics is defined as modification to DNA which control gene expression without changes to the DNA sequence (ex. DNA methylation). Epigenetics provides the link between environment and genetics, defining the potential that a gene has to be expressed. T- helper cells (CD4+) are the mediators of AMIR and CMIR, producing cytokines that help direct other cell types to an appropriate response to an invading pathogen. Biased immune responder cattle were used to investigate mechanisms of immune response variation and the role of epigenetics in controlling T-helper cell cytokine production in cattle. Biased immune responder cattle respond strongly with either a high AMIR or CMIR to test antigens (H-AMIR/L-CMIR n = 10 and H-CMIR/L-AMIR n = 11). Isolated T-helper cells (CD4+) from H-CMIR/L-AMIR and H-AMIR/L-CMIR cows were stimulated with a T-cell mitogen (ConA) and cell culture supernatant was harvested at the 24 h time point to quantify IFNγ and IL-4 by ELISA. Simultaneously, DNA was extracted from unstimulated and stimulated cells and DNA methylation status of the promoter regions for both IFNγ and IL-4 was determined by bisulfite pyrosequencing. Previously it was shown that H-CMIR/L-AMIR produced significantly more cytokine than the H-AMIR/L-CMIR cows for both IL-4 and IFN-γ when sampled 21 d into lactation. Increased IFN-γ secretion was associated with decreased methylation status both in the promoter region and at specific CpG sites proximal to the transcription start site, but requires more individuals to be confirmed. Increased DNA methylation at the IL-4 promoter did not associate with IL-4 secretion. These preliminary results suggest that immune response variation in cattle is partially controlled by DNA methylation at gene promoter regions.

Key Words: epigenetics, immune response

632 The effect of feeding endophyte-infected fescue on the metabolic response to a provocative immune challenge in beef heifers. A. W. Altman*[1], N. C. Burdick Sanchez[2], J. A. Carroll[2], T. B. Schmidt[3], E. S. Vanzant[1], and K. R. McLeod[1], [1]*Department of Animal and Food Sciences, University of Kentucky, Lexington,* [2]*Livestock Issues Research Unit, USDA-ARS, Lubbock, TX,* [3]*Department of Animal Science, University of Nebraska-Lincoln, Lincoln.*

To determine the effect of endophyte-infected fescue on the metabolic response of beef heifers to a lipopolysaccharide (LPS) challenge, Angus heifers (n = 22; 292 ± 9.0 kg BW) were paired by body weight and randomly placed on either an endophyte-infected (E+) or endophyte-free (E−) diet for 10 d. Heifers were fed at 1.8 × NEm. Diets contained 20% fescue seed, 30% cottonseed hulls, 36% cracked corn, 10% supplement, and 4% molasses, and were balanced to meet protein and mineral requirements. On d −1, heifers were fitted with indwelling jugular cannulas. On the day of challenge, blood samples were collected and serum isolated from heifers at 0.5-h intervals from −2 to 8 h, and again at 24 h relative to LPS administration (0.5 μg/kg BW at time 0 h). Serum was analyzed for glucose, insulin, nonesterified fatty acids (NEFA), blood urea nitrogen (BUN), creatinine, and β-hydroxybutyrate (β-HB). Data were analyzed separately within pre- and post-challenge periods with the Mixed Procedure of SAS, using repeated measures in a completely randomized design. Within period, no treatment by time interactions were detected ($P > 0.10$). Insulin was decreased both pre-and post-LPS in E+ heifers ($P < 0.10$; 0.50 vs 0.46 ng/mL for pre- and 1.17 vs. 1.02 ng/mL post-LPS). Glucose was unaffected ($P = 0.87$) by endophyte status pre-LPS, and was decreased ($P < 0.01$) for E+heifers post-LPS (78.1 vs 71.2 mg/dL). Concentrations of NEFA were decreased ($P = 0.06$) in E+ heifers pre-LPS (0.11 vs 0.08 m*M*) but were unaffected ($P = 0.85$) by endophyte status post-challenge. Both BUN and creatinine differed ($P < 0.01$) between E+ and E− during both periods, BUN was decreased in E+ heifers (1.10 vs 1.04 mg/dL pre-LPS and 1.17 vs 1.04 mg/dL post-LPS) whereas creatinine was increased in E+ heifers (1.31 vs 1.41 mg/dL pre-LPS and 1.16 vs 1.29 mg/dL post-LPS). Concentrations of β-HB were not different ($P = 0.47$) pre-LPS, and were decreased ($P < 0.01$) in E+ heifers post-LPS (1.06 vs 0.97 n*M*). These results indicate that exposure to fescue endophyte alters the metabolic response of heifers during a provocative immune challenge.

Key Words: endophyte, metabolism, LPS

633 Gut microbiome profile of early weaned piglets in response to crowding stress, *Escherichia coli* K88$^+$ challenge, and anti-*E. coli* K88 probiotics. P. M. Munyaka*[1], R. J. Hartmann[1], J. C. Rodriguez-Lecompte[2], J.-E. Ghia[1], D. O. Krause[1], and E. Khafipour[1], [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*University of Prince Edward Island, Charlotte, PEI, Canada.*

Post-weaning diarrhea (PWD) in pigs is usually caused by enterotoxigenic *Escherichia coli* K88 (ETEC).The current study was conducted to determine the effect of stress and anti- *E. coli* K88$^+$ probiotics on the gut microbiome of early weaned piglets challenged with *Escherichia coli* K88$^+$. To model stress, 2 floor spaces were used; Standard floor allowance (SFA) or half standard floor allowance (HSFA). Ninety 6 male piglets weaned at 21 ± 1 d and fed a basal mash diet were allocated in to 6 experimental treatments: 1) SFA, 2) HSFA, 3) SFA + *E. coli* K88$^+$ (ESFA), 4) HSFA + *E. coli* K88 (ESHFA), 5) ESFA + probiotic *E. coli* UM2 and *E. coli* UM7 (PSFA), 6) EHSFA + probiotics *E. coli* UM2 and *E. coli* UM7 (PHSFA). DNA was extracted from ileal digesta samples and the V1-V3 regions of bacterial 16S rRNA genes amplified and sequenced using Roche 454 pyrosequencing. Sequences were analyzed using mothur software package comparing to the Silva 16S rRNA bacterial database. Data was evaluated using partial least squared determinant analysis and SAS 9.2 to identify significant taxa associated with each treatment. *Paraprevotella* spp., *Dorea* spp., and *Roseburia* spp. were significantly associated with non-stress treatments. Contrary, *Bacteroidales*, *Lachnospiraceae*, *Proteobacteria*, *Achromobacter* spp., *Stenotrophomonas* spp., *Sporobacter* spp., *Delftia* spp., *Papillibacter* spp., *Finegoldia* spp., and *Sporacetigenium* spp. were associated with stress treatment. *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* contributed to approximately 97% of all phyla within the treatments. Probiotics *E. coli* UM2 and UM7 helped to maintain the gut microbiome similar to that of non-infected pigs; however, stress reduced the population of *Firmicutes* ($P = 0.006$) but increased *Proteobacteria* ($P < 0.0001$) and *Actinobacteria* ($P = 0.01$). Data suggest that stress induces notable changes in the bacterial composition of the gut. The use of anti-ETEC probiotics could effectively reduce negative effects of ETEC in a piglet challenge model; however, probiotics alone may not attenuate the effects of stress.

Key Words: microbiome, probiotic, stress

634 Breed susceptibility to pathogenic enterotoxigenic *Escherichia coli* strains in piglets from South Africa. N. S. Chaora*[1], F. C. Muchadeyi[2], E. Madoroba[2], E. F. Dzomba[1], and M. Chimonyo[1], [1]*University of KwaZulu Natal, Pietermaritzburg, South Africa,* [2]*Agricultural Research Council, Pretoria, South Africa.*

Escherichia coli is an important cause of diarrhea in piglets. It is responsible for economic losses through mortality, morbidity, decreased growth rate and cost of medication. The increase in *E. coli* strains' resistance to various antibiotics and the increase in incidences of post weaning diarrhea have necessitated the exploration of alternative methods for control. The pathogenesis of *E. coli* involves the production of fimbriae that adhere to specific receptors present on the intestinal epithelium and enterotoxins which release water and electrolytes into the gut lumen. Earlier reports show that *E. coli* causing post weaning diarrhea usually carry the F4ab/ac and F18 fimbrial strains. Molecular studies have positioned the receptor(s) for F4ab/ac located on pig chromosome 13 and for F18 on chromosome 6. To date, the causative mutations for these receptor(s) remain unknown. After fine mapping the loci for F4ab/ac receptor(s), the region of interest was mapped between markers SW207 and S0075, where candidate genes such as MUC4, MUC13, MU20 and TRFC surfaced. A recent study investigating collibacillosis in South African pigs observed absence of F4ab/ac and F18 fimbrial adhesins carried out in 263 piglets aged between 9 and 136 d of age. However, non-fimbrial adhesins such as, AIDA-1, PAA and EAST-1 were detected in 14.5, 17.9 and 20.15% of the piglets respectively. Such findings necessitate further investigations into the South African pig populations to these new and prevalent *E. coli* strains. An in vitro adhesion test will identify receptors in the intestinal tissues of the local pigs to which the fimbrial and non-fimbrial adhesins attach to during infection. Analysis of these receptors will help identify genes conferring resistance/susceptibility to *E. coli* infections in South African pigs. The presence of resistant pigs to these newly found strains could help in developing a resistant population through marker assisted selection methods.

Key Words: adhesion, *Escherichia coli*, susceptibility