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POSTER PRESENTATIONS

Animal Health II

T1 Development of kit for bovine myeloperoxidase using enzyme-linked immunosorbent assay. J. Shi*, Y. Yang, Q. Li, and Y. Lv, *Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, China.*

Myeloperoxidase (MPO) is a heme glucoprotein found in the primary granules of mammalian neutrophils. At site of infection, MPO is released extracellularly or into phagocytic vacuoles. It had shown that MPO is abundant in milk taken from mammary glands of cows with mastitis and that the amount of MPO in milk is well correlated with the somatic cell count in mastitis milk. To evaluate the potential of using MPO in the diagnosis of mastitis in cows, this study developed a specific enzyme immunoassay for MPO in milk. Bovine MPO was isolated and purified from bovine whole blood by Sephadex G-200 chromatography and ConA-Sepharose 4B affinity chromatography. Antiserum against bovine MPO were produced using the purified MPO with ConA as a coated "antibody," and mouse anti-bovine antiserum against MPO as a second detection antibody, and chicken HRP-labeled polyclonal antibody as a anti-antibody, a special sandwich ELISA for MPO was established. ELISA kit was developed. Evaluating kit by methodology showed good specificity, reproducibility (variant coefficient: 1.09 to 7.2% in batch and 1.47 to 6.7% between batches), and the detection limit was 1.1 μ g/ml. The experiment certified that this kit could maintain over one year and the detection time of the kit was about 3.5h.

Key words: bovine myeloperoxidase, purification, ELISA kit

T2 Development of kit for bovine haptoglobin using enzyme-linked immunosorbent assay. Y. Yang*, J. Shi, Q. Li, and Y. Lv, *Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, China.*

Haptoglobin (Hp) is one of the important acute phase proteins of cows with mastitis, the concentration of Hp in milk and blood is closed to the mastitis condition. So the determination of Hp in milk could be an effective diagnostic tool for mastitis. Development of sandwich-ELISA: The haptoglobin in whey was captured with bovine-hemoglobin coated ELISA plates, and the sandwich ELISA using the anti-Hp-antibody and AP-conjugated-antibody was established, the reaction conditions of ELISA were optimized. The concentration of hemoglobin for coating 96-well microtiter plate was 10%, and incubated overnight at 4°C. The plates were subsequently blocked 1 h with 2% BSA-PBST, and then incubated for 1 h with diluted whey samples (1:10), after washing the plates, the diluted anti-Hp-antibody (1:1000) was added and incubated for 1 h at 37°C. The diluted AP-conjugated-antibody (1:500) was added and incubated for 1 h at 37°C, and then substrate of PNPP solution was added for incubation 15 min at 37°C to develop color. The ELISA test gave a cut-off value of 0.996 for detection of 100 Hp-negative whey samples. The threshold for ELISA was OD405nm \geq 0.996 for positives, OD405nm $<$ 0.901 for negatives, and the other was suspectives. Correlation between analytical results of the ELISA test and the commercial kits test was similar ($R^2 = 0.996$) based on detection of 50 whey samples. The Hp detection limit of ELISA

was 0.08 μ g/mL. The ELISA test had no cross-reaction with other acute phase proteins of cows with mastitis. Packing and application of the ELISA test kit for Hp: Based on the sandwich-ELISA, the coating condition of the anti-Hp-antibody, AP-conjugated-antibody, the control positive/negative whey samples, the storage conditions of the plates and the kit were explored, and the ELISA test kit were packed. The variant coefficients of ELISA in a batch and between batches were from 3.27% to 4.98% and from 5.46% to 8.31%, respectively. The ELISA test kit provides an available technique for detection Hp of whey samples and a good foundation for the further development and commercialization of the kit.

Key words: haptoglobin, mastitis, sandwich ELISA

T3 Transcriptional factors SP1 and SP3 influence differentially the regulating sequence of the bovine osteopontin gene depending on promoter haplotype. N. Bissonnette* and C. Thibault, *Agriculture and Agri-Food Canada, Dairy Cattle and Swine Research and Development Center, Sherbrooke, Quebec, Canada.*

Osteopontin is a pro-inflammatory molecule which has been involved in numerous physiological aspects, from wound healing to metastasis. In cattle, osteopontin was associated to the paratuberculosis disease. In a previous study, we have identified DNA polymorphisms (SNP) in the osteopontin gene (*SPP1*) associated with the mammary health status of lactating cows. To better understand the factors that govern the expression of this gene, the activity of its regulating sequence (i.e., promoter) was study in vitro. The most prevalent haplotypes (H1-H3) of the *SPP1* promoter were cloned. Two SNP are located in the 5' untranslated region and one in the first intron. The haplotype promoter sequences were analyzed in silico for identification of transcription regulating sites using the TRANFAC software. The two transcription factors SP1 and SP3 bindings might be affected by the presence of these SNP. The luciferase reporter constructs of the haplotype containing the 1736-bp regulating sequence were compared in cotransfection assays with, without, or in presence of both SP1 and SP3 using the BOMAC (bovine macrophage), MAC-T (bovine mammary epithelial) and the MCF7 (human mammary epithelial) cell lines. The basal activity of H2 was lower than H1 and H3 ($P = < 0.0001$) in BOMAC and MCF7 cells. In MAC-T, the H2 difference for H1 remained significant ($P = 0.031$) but with a tendency for the allele H3 ($P = 0.066$). In presence of the transfection factor SP1, the expression increased globally by ~2-fold in all cell types. The allele H2 was more responsive to SP1 in both BOMAC and MCF7 (H1, $P = 0.009$ and $P = 0.004$); H3, $P = < 0.001$ and $P = 0.021$, respectively), which was also observed in MAC-T ($P = < 0.0001$). In contrast, the transcription factor SP3 had a negative impact on the promoter activity. The co-expression of SP1/SP3 recovered partially the promoter activity. In this study, we demonstrated that the transcription factors SP1 and SP3 impact gene activity through the regulating sequence and that the presence of SNP within the *SPP1* promoter may interfere with osteopontin expression.

Key words: osteopontin, transcription factor SP1/SP3, haplotype

T4 Evaluation of interleukin 5 as a biomarker for parasite resistance in goats pasture exposed to *Haemonchus contortus*. M. M. Corley* and A. A. Saeed, *Virginia State University, Petersburg.*

In the meat goat industry, an animal that exhibits disease resistance characteristics can increase the price of goat from \$250 to \$800 per head. *Haemonchus contortus*, the blood sucking gastrointestinal nematode (GIN), costs the global livestock industry billions of dollars per year in lost production and anthelmintic drug costs. The annual expenditure on anthelmintics in the US is over \$3 billion. Interleukin 5 is one of the cytokines secreted by Th2 immune cells implicated in the process of gut expulsion of GINs from humans, mice and sheep. However, studies on the mechanism of immunity to *Haemonchus contortus* infection and the ability to identify parasite resistance through IL-5 and other cytokine responses need to be assessed in goats. This study evaluated gene expression of IL-5 in selected pasture exposed parasite resistant Spanish and Myotonic goats. Whole blood, and intestinal tissues were harvested from goats exhibiting susceptibility and resistance to *Haemonchus contortus* based on (FAMACHA (FAM) eye color charts, packed cell volume (PCV), fecal egg counts (FEC) and quantitative real time PCR (qPCR) detection. Primers were designed from conserved regions of IL-5 bovine and ovine nucleotide sequence alignments. Total RNA was extracted from homogenized goat intestinal tissues and qPCR was performed to determine IL-5 expression. Results showed that parasite resistant goats expressed more ($P < 0.05$) IL-5 than susceptible goats. Gene expression of IL-5 was higher ($P < 0.05$) in infected Spanish vs. Myotonic goats. Infected does expressed higher ($P < 0.05$) IL-5 than bucks. Pearson correlation coefficients showed that there was a positive correlation between FEC, FAM, PCV ($P < 0.05$) and IL-5 expression. These data indicate that IL-5 expression can be used as a marker to screen for susceptibility or resistance to *Haemonchus contortus* infection in pasture exposed goats, allowing IL-5 based anthelmintic drug development and goat producers the ability to select parasite resistant animals.

Key words: parasite resistance, IL-5, *Haemonchus contortus*

T5 Influence of latency to collect blood samples from beef calves on ex vivo innate immune responses. L. E. Hulbert*^{1,2}, C. J. Cobb¹, M. D. Sellers¹, D. L. Hanson¹, M. L. Galyean¹, and M. A. Ballou¹, ¹*Department of Animal and Food Sciences, Texas Tech University, Lubbock,* ²*Department of Animal Sciences, University of California-Davis, Davis.*

Objective was to evaluate the influence of latency to collect a blood sample from beef calves on the ex vivo innate immune responses. Innate immune responses evaluated included total leukocyte and differential counts, neutrophil L-selectin expression, whole blood (WB) killing of *E. coli* 0111:H8, tumor necrosis factor- α (TNF- α) secretion from lipopolysaccharide (LPS)-stimulated WB, and neutrophil phagocytosis and oxidative burst responses to *E. coli* 0111:H8. Within each of 12 pens, whole blood was collected via jugular puncture from the first and last calf (n = 9 to 10 calves/pen; BW = 202 \pm 18.7 kg) to enter the squeeze chute. The time from when research personnel entered the pen to move the cattle until the blood sample was collected averaged (\pm STD) 320 and 746 \pm 44.2 s ($P < 0.001$) for the first and last calf in each pen, respectively. There were no treatment differences for secretion of TNF- α from LPS-stimulated WB or neutrophil phagocytosis. Total leukocyte counts tended ($P = 0.075$) to be less for calves sampled last in the pen vs. those sampled first. Although there were no differences between calf neutrophil:lymphocyte ratios ($P = 0.867$), neutrophil L-selectin expression tended ($P = 0.063$) to be less among calves

that were sampled last in the pen. Whole blood from the calves sampled last also tended ($P = 0.060$) to kill less *E. coli* 0111:H8. Similarly, the calves sampled last had decreased ($P = 0.043$) neutrophil oxidative burst response to the *E. coli* 0111:H8. These data indicate that it is important to control for the latency until sample collection associated with handling and movement when designing ex vivo immunological studies with beef calves.

Key words: immunity, cattle, stress

T6 Characterization of bovine leukocyte differentiation molecules in Egyptian cattle using flow cytometry. G. S. Abdellazeq*¹, M. M. El-Naggar¹, and W. C. Davis², ¹*Alexandria University, Edfina, Behara province, Egypt,* ²*Washington State University, Pullman.*

Evaluation of the efficacy and cross-protectivity of any applied vaccine in Egypt requires preliminary studies to investigate the characteristics of the immune system in a chosen farm animal species including the proportions of subpopulations of leukocytes. In this report, we investigate the comparative changes of leukocytes, T cell subsets and MHC molecules. Twenty ml blood samples were collected from healthy Egyptian cattle from young (age ~6–8 mo, no 4) and adult cattle (age ~3–5 year, no 4). Blood was processed, stained with a panel of monoclonal antibodies (mAbs) and analyzed by flow cytometry (FC). FC analysis revealed that the whole leukocyte population in young animals was composed of granulocytes representing about 30%, monocytes representing about 11% and lymphocytes representing about 59% while in adult animals, the granulocytes represent about 22%, monocytes represent about 7% and lymphocytes represent about 71%. CD4+ T cells represent about 41% of T cells in young animals and the proportion of this cell subset represents about 44% of T cells in adult animals. The CD8+ T cell subset was found to represent about 22% of T cells in young animals while representing about 41% of T cells in adult animals. We conclude that the age-related changes of leukocytes and T cell subsets in Egyptian cattle are basically the same as those found in pure Holstein cattle. These results will serve as a reference value and need to be taken in consideration when attempting to analyze the immune response in Egyptian cattle to *M. a. paratuberculosis* and other pathogens and their derived vaccines.

Key words: leukocyte differentiation molecules, flow cytometry, Egyptian cattle

T7 Comparative evaluation of gene expression in bovine and caprine neutrophils. M. Worku*, N. Mikiashvili, and H. Ishamel, *North Carolina A&T State University, Greensboro.*

Recognition and destruction of bacteria by polymorphonuclear neutrophil leukocytes (PMN) is a major defense against infections by pathogens. Impaired innate immune defense to gram-negative *Escherichia coli* infections is associated with delayed influx of PMN into the mammary gland. An evaluation of gene expression in response to exposure to *Escherichia coli* O111-B4 lipopolysaccharide (LPS) was conducted in isolated bovine and caprine PMN. Blood samples were collected from 3 Holstein Friesian cows and 3 Spanish \times Boer goats. Isolated blood PMN, were incubated with LPS (0, 10 or 100 ng/ml, at 37C for 15 or 30 min in the presence of 95% humidity and 5% CO₂). Eight cytokines were measured in cell culture supernatants using Signosis Inflammation ELISA Strips for quantitative profiling and measuring TNF- α , IFN γ , G-CSF, GM-CSF, IL-1 α , IL-8, IP-10, and Rantes. Quantitative reverse transcriptase-PCR was employed to test the synthesis of specific mRNAs. Both bovine and caprine neutrophils

expressed TLR4 (452 bp) and CD14 (600bp). Exposure to LPS significantly increased CD14, TLR4 ($P < 0.001$) and TNF α ($P < 0.05$), transcripts in cow PMN. In goat PMN exposure to 100ng LPS for 30 min increased transcripts for CD14 and TNF- α ($P < 0.05$). Changes were not observed with TLR4 transcript levels. Treatment affected secretion of TNF- α , IFN γ , G-CSF, GM-CSF, IL-1 α , IL-8, IP-10 and Rantes. Exposure to 100ng of *E. coli* LPS caused a strong induction of TNF- α , IFN γ , G-CSF, GM-CSF cytokine synthesis, in cows. The concentrations of TNF- α and IL-8 increased 2 fold. Thus in both bovine and caprine neutrophils exposure to LPS results in transcription and translation involving TLR4 CD14, and TNF α . This response is affected by dose and exposure time to LPS and varied by species. The role played by neutrophils in immunity might be affected by species variations in gene expression.

Key words: neutrophil, TLR4, ruminant

T8 Detection and expression of the gene encoding low density lipoprotein receptor-related proteins 6 (LRP6) in goat peripheral blood. M. Worku*, H. Mukhtar, and N. Mikiashvilli, *North Carolina Agricultural and Technical State University, Greensboro.*

Previous studies in our lab have reported the identification and expression of Wingless (Wnt) 1 and Frizzled in the goat. Wnt ligands conduct their functions in canonical Wnt signaling by binding to 2 receptors, the single transmembrane low density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) and 7 transmembrane Frizzled receptors. Polymorphisms of the LRP6 gene are associated with bone mineral density. The objectives of this study were to detect the LRP-6 gene and evaluate its expression in goat peripheral blood. Blood from goats ($n = 9$) was collected on FTA elute cards for DNA extraction and in PAX-gene Blood tubes for RNA extraction. RNA samples were reverse-transcribed and the cDNA was obtained. Specific LRP6 primers were used for PCR amplification. The amplified product was run on a 1% agarose gel. GAPDH was used as loading control and primers in the absence of DNA were used as negative controls. Gels were stained with ethidium bromide and visualized. The amplicon was sequenced commercially and the BLAST tool was used to compare the sequence with the NCBI database. An ~400 bp fragment of LRP6 was present in genomic DNA and expressed at the RNA level the amplified sequences had 100% similarity to human LRP6. The results of this study show that LRP-6 is conserved between goats and humans. Human mutations in LRP6 cause early onset coronary disease, osteoporosis, late onset Alzheimer's and cancers. In goats osteoporosis has been reported to occur due to mineral deficiency and gastrointestinal parasitism. Further, the goat has been developed as a large osteoporotic animal model that resembles human osteoporotic changes. Thus, this gene could aid in studies in skeletal development and diseases of goats and humans.

Key words: goat, osteoporosis, Wnt LRP6

T9 Comparison of commercially available enzyme-linked immunosorbent assay with serum neutralization for measuring bovine viral diarrhea virus specific antibodies. M. Gonda*¹, X. Fang¹, G. Perry¹, and C. Maltecca², ¹South Dakota State University, Brookings, ²North Carolina State University, Raleigh.

Our laboratory is focused primarily on mapping loci that affect bovine viral diarrhea virus (BVDV) vaccine response in beef cattle. Several methods for measuring humoral BVDV vaccine response are available: 1) a BVDV antibody enzyme linked immunosorbent assay (ELISA) which measures total BVDV antibodies, and 2) serum neu-

tralization (SN) which measures only protective antibodies specific for BVDV-1 or BVDV-2. The SN tests are more biologically relevant, but the ELISA is cheaper and faster for measuring BVDV antibodies, and could be effectively used if the ELISA and SN tests were highly correlated. Our objective was to test if BVDV total antibody ELISA sample-to-positive (S/P) ratios were correlated with BVDV-1 and BVDV-2 SN antibody titers. A total of 406 blood samples were collected from 193 Angus and Angus cross calves raised in 2 South Dakota beef herds that vaccinate cows for BVDV-1 and BVDV-2. Serum or plasma were collected from blood and then BVDV-specific antibody concentration was measured on each sample by 1) IDEXX (Liebefeld-Bern, Switzerland) antibody ELISA, 2) BVDV-1 SN, and 3) BVDV-2 SN. Higher BVDV ELISA S/P ratios were positively correlated with higher BVDV-1 SN ($\rho = 0.809$) and BVDV-2 SN ($\rho = 0.638$) titers ($P < 0.0001$), although the relationship was weaker when SN titers were $< 1:64$. Higher BVDV-1 SN titers were also correlated with higher BVDV-2 SN titers ($\rho = 0.708$; $P < 0.0001$). We conclude that IDEXX BVDV total antibody ELISA S/P ratios can be used as a surrogate for BVDV-1 and BVDV-2 SN antibody titers when mapping loci affecting BVDV vaccine response.

Key words: ELISA, serum neutralization, bovine viral diarrhea virus

T10 Effects of *Camellia L.* plant extract and mannan-oligosaccharide on growth performance, gut health, blood parameters, cecal microflora and immunity of broiler chicks. K. Hatami and M. Zaghari*, *Department of Animal Science, College of Agriculture and Natural Resource, University of Tehran, Karaj, Karaj, Alborz, Iran.*

An experiment was conducted with 128, 1-d-old, male broilers to investigate the effects of sacchariterpenin (composed of triterpenoid saponin and saccharide from the plant *Camellia L.*; CL) and Techno-Mos (rich in mannan-oligosaccharides and β -1,3-glucanes) on growth performance, gut health, blood parameters, cecal microflora and immune response of broiler chicks. Experiment was done as a completely randomized design with 4 treatments (control, 0.3 and 0.5 g/kg CL and 0.5 g/kg mannan-oligosaccharide), 8 replicates and 4 chicks in each battery cage. Two basal diets were formulated for starter (1 to 21) and grower (22 to 42) periods and levels of CL and MOS were added to basal diet. Body weight gain, feed intake and feed conversion ratio were measured at 21, 35 and 42 d of age. Plasma triglyceride, cholesterol, LDL, VLDL and HDL concentrations were measured at 42 d of age. Differential counting of monocytes, lymphocytes, eosinophils and neutrophils percent were done at 42 d of age. pH of gastrointestinal sections were measured at 42 d of age. Effects of diet on villi length and crypt depth were measured at 42 d of age. Immune response to PHA-P was measured at 35 and to SRBC at 28 and 42 d of age. Supplementation of diets with 0.5 g/kg CL decreased body weight gain, feed intake and feed conversion ratio significantly throughout the experiment ($P < 0.01$). Proventriculus, gizzard and ileum pH differed among the treatment but duodenum and jejunum pH were not affected ($P < 0.05$). Supplementation of diets by CL and MOS affected cholesterol level of plasma but had no effect on plasma levels of triglyceride, LDL, HDL ($P < 0.03$). Cecal microbial population did not differ among treatments. Supplementation of diets with CL and MOS significantly increased villi length and crypt depth ($P < 0.0001$). CL had no effect on immune response against PHA-P at 36 d of age, however an increase was observed with addition of CL and MOS, also CL and MOS decreased antibody titer against SRBC at 28 d of age ($P < 0.01$).

Key words: *Camellia* extracts, performance, mannan-oligosaccharide

T11 Gastrointestinal nematode infection in Nelore and cross-bred cattle. M. C. S. Oliveira*¹, M. C. D. Beraldo², E. Nakandakari³, L. Boschini¹, M. M. Alencar¹, R. Giglioti⁴, A. C. S. Chagas¹, B. Rubert⁵, S. C. Bogni², and A. M. G. Ibelli⁵, ¹Embrapa Pecuaria Sudeste, São Carlos, SP, Brazil, ²UNICEP, São Carlos, SP, Brazil, ³Uniará, Araquara, SP, Brazil, ⁴UNESP, Jaboticabal, SP, Brazil, ⁵UFSCAR, São Carlos, SP, Brasil.

Cattle nematodes in Brazil are mainly controlled through application of anthelmintics. However, this causes concern about the presence of drug residues in meat and dairy products, prompting studies of alternative control methods. Among these, the use of animals that are genetically resistant is a very promising complementary strategy. Resistance to gastrointestinal nematodes was compared in males and females Nelore (NI, n = 28) and a 3 breed cross, 1/2 Angus 1/4 Canchim (5/8 Charolais + 3/8 *Bos indicus*) + 1/4 Nelore (TC, n = 17) that were born from October to December 2008. The animals were kept without treatment, in rotational paddocks of Tanzania grass. Monthly collections were conducted totaling 810 observations (August 2009 to January 2011). The feces samples were collected from each animal for fecal cultures and determination of the number of eggs per gram of feces (EPG). Blood samples were collected monthly for packed cell volume determination (PCV) that was an indicator of animal health. The count data of EPG were submitted to log₁₀ (n+1) transformation. The data were analyzed using the MIXED procedure of SAS (2002–2003), according to a model considering repeated measures on the animal, structured with a compound symmetry variance matrix, and also included the effects of genetic group, sex, month/year of collection and 2-way interactions involving these 3 factors. The means of PCV were significantly higher ($P < 0.01$) for NI animals (40.6%) compared with TC (38.6%). No significant effects of genetic groups or interaction between the genetic groups and month/year of collection on the EPG were found, but there was a significant influence of the month/year of collection ($P < 0.01$). The following nematode genera were found in the coprocultures: *Haemonchus*, *Cooperia*, *Esophagostomum* and *Trichostrongylus*, the latter in smallest proportion. There was no significant difference between the genetic groups for averages of all parasites identified, except *Cooperia*, which were present in higher numbers in the animals of the NI group ($P < 0.05$). These data confirm previous findings that showed greater susceptibility of purebred Nelore animals to *Cooperia*.

Key words: nematodes, resistance, cattle

T12 Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. R. F. Cooke*¹, B. I. Cappellozza¹, F. N. T. Cooke¹, D. W. Bohnert¹, and J. D. Arthington², ¹Oregon State University–Eastern Oregon Agricultural Research Center, Burns, ²University of Florida–Range Cattle Research and Education Center, Opa.

Our laboratory determines plasma concentrations of haptoglobin using a low-cost colorimetric procedure that measures haptoglobin-hemoglobin complexing by estimating differences in peroxidase activity (CPPA). Results are expressed as arbitrary units based on absorption readings, given that the CPPA method does not contain a standard curve. Conversely, commercially available ELISA methods generate results based on standards with known haptoglobin concentrations. Therefore, the objective of this study was to determine if the CPPA method generates results compatible with a commercial ELISA kit. Nine Angus steers were vaccinated against *Mannheimia haemolytica* (One Shot, 2 mL s.c) to stimulate an acute-phase response and blood samples were collected before vaccination (d 0), and on d 1, 3, 5, 7,

and 10. Plasma samples were frozen in triplicates at -80°C . One set of the triplicates was analyzed for haptoglobin concentrations using the CPPA procedure. A day effect was detected ($P < 0.01$) given that haptoglobin peaked on d 1, 3, and 7 relative to vaccination. A second set of the triplicates was analyzed using a commercial ELISA kit. A similar day effect ($P < 0.01$) was detected. When Pearson coefficients were calculated among results obtained from CPPA and ELISA methods, a strong correlation was detected ($r = 0.98$; $P < 0.01$). Based on the ELISA results, the plasma sample with the greatest haptoglobin concentration was serially diluted with PBS (1:1 through 1:32 dilution) and used as known reference to generate a standard curve for samples from the third set of triplicates analyzed with the CPPA method. A linear standard curve was generated ($r^2 = 0.99$) and a day effect ($P < 0.01$) was again detected. However, the values generated by the CPPA procedure with standard curve differed ($P < 0.01$) when compared with those generated by ELISA. In conclusion, assessing concentrations of haptoglobin in bovine plasma using the CPPA method yields results highly correlated to ELISA. Therefore, the CPPA method can be adopted to evaluate plasma haptoglobin concentrations in cattle when absolute values are not required.

Key words: bovine, haptoglobin, assay

T13 Feed and water restriction elicits an acute-phase protein response in beef cattle. B. I. Cappellozza*, R. F. Cooke, C. Trevisanuto, V. D. Tabacow, F. N. T. Cooke, and D. W. Bohnert, *Oregon State University–Eastern Oregon Agricultural Research Center, Burns.*

The acute-phase protein response is an important component of the innate immune system, but can be highly detrimental to cattle productivity. A comprehensive understanding of the causes and mechanisms that stimulate the bovine acute-phase protein response is required for development of management strategies to modulate this immune reaction. Therefore, the objective of this study was to determine if feed and water restriction stimulates an acute-phase protein response in overtly healthy beef steers. Nine Angus \times Hereford steers were ranked by initial BW (average 244 ± 8 kg) and assigned to 1 of 2 treatments: 1) CONT - ad libitum access to feed and water during the study (d 0 to d 10), and 2) RESTR - feed and water restriction for 24 h (d 0 to d 1 of the study) and subsequent ad libitum access to feed and water (d 1 to d 10). Blood samples were collected from all steers on d 0 (before restriction period), 1 (at the end of the restriction period), 3, 5, 7 and 10. Samples were harvested for plasma, and immediately stored at -80°C until analyzed for concentrations of haptoglobin and ceruloplasmin. Hay DMI was evaluated daily by measuring refusals. Results were analyzed with the MIXED procedure of SAS. Plasma haptoglobin concentrations tended to be greater ($P = 0.06$) for RESTR steers compared with CONT on d 3 of the study (6.35 vs. 4.62 absorbance at $450 \text{ nm} \times 100$, respectively). Plasma ceruloplasmin concentrations tended to be greater ($P = 0.15$) for RESTR steers compared with CONT on d 3 (18.0 vs. 14.4 mg/dL, respectively) and d 7 of the study (19.3 vs. 15.8 mg/dL, respectively). A treatment \times day interaction was detected ($P = 0.01$) for hay DMI because RESTR steers had greater ($P < 0.01$) hay DMI on d 1 (2.98 vs. 1.97% of BW, respectively) and tended to have greater ($P = 0.11$) hay DMI on d 2 (2.74 vs. 2.32% of BW, respectively) compared with CONT steers. Results from this study indicate that feed and water restriction elicits an acute-phase protein response in overtly healthy beef cattle, which may detriment subsequent health and productivity parameters.

Key words: acute-phase proteins, feed and water restriction, beef cattle

T14 Natural infestation by external parasites in beef cattle in southern Brazil. M. C. S. Oliveira*¹, E. Nakandakari², M. C. D. Beraldo³, M. M. Alencar¹, A. C. S. Chagas¹, L. Boschini¹, R. Giglioti⁴, and A. M. G. Ibelli⁵, ¹Embrapa Pecuaria Sudeste, São Carlos, SP, Brazil, ²UNIARA, Araraquara, SP, Brazil, ³UNICEP, São Carlos, SP, Brazil, ⁴UNESP, Jaboticabal, SP, Brazil, ⁵UFSCAR, São Carlos, SP, Brazil.

The existence of genetic differences between groups regarding the degree of infestation by external parasites suggests the possibility of using crosses between breeds to increase the benefits of complementarity and heterosis for adaptive traits. In this experiment natural infestations were compared in males and females Nelore (NI, n = 28) and a 3 breed cross, 1/2 Angus 1/4 Canchim (5/8 Charolais + 3/8 *Bos indicus*) + 1/4 Nelore (TC, n = 17) that were born from October to December 2008., regarding the resistance to the tick *Rhipicephalus microplus*, to the horn fly (*Haematobia irritans*) and the botfly (*Dermatobia hominis* larvae). The animals were kept without chemical treatment, in rotational paddocks of Tanzania grass. Monthly collections were conducted totaling 810 observations (August 2009 to January 2011), being counted all engorged female ticks with more than 4.5 mm diameter located on the left and the botfly around the animal's body. Horn flies were counted with the aid of photographs of the lumbar region. Blood samples were also collected monthly for packed cell volume determination (PCV) that was an indicator of animal health. The count data of external parasites were submitted to log₁₀(n + 1) transformation and analyzed using the MIXED procedure of SAS (2002–2003) according to a model considering repeated measures on the animal, structure with a compound symmetry variance matrix, and included the effect of genetic group, sex, month/year of collection and interactions involving these factors. The means of PCV were significantly higher for NI animals (40.6%) compared with TC (38.6%), and there was significant influence of collection month/year ($P < 0.01$). Animals of NI group had lower external parasites infestations than TC animals ($P < 0.01$). Means and standard errors for NI and TC animals were, respectively, 0.07 ± 0.01 and 0.36 ± 0.02 for *R. microplus*, 0.86 ± 0.05 and 1.25 ± 0.06 for *H. irritans* and 0.06 ± 0.03 and 0.47 ± 0.04 for *D. hominis* larvae. These results indicate that the control of external parasites should be done differently for each genetic group.

Key words: cattle, parasites, resistance

T15 Cinnamaldehyde enhances in vitro parameters of immunity and reduces severity of in vivo infection against avian coccidiosis. S.-H. Lee¹, H. Lillehoj*¹, S. Jang¹, K. Lee¹, and D. Bravo², ¹Animal and Natural Resources Institute, ARS USDA, Beltsville, MD, ²Pancosma S.A., Le Grand Saconnex, Geneva, Switzerland.

We examined the effects of cinnamaldehyde (CINN) on in vitro parameters of immunity (splenocyte proliferation, macrophage activation, and killing of tumor cells and parasites), and in vivo protection against avian coccidiosis (body weight gain, oocysts production, antibody responses, and intestinal cytokine levels). In vitro stimulation of chicken spleen lymphocytes with CINN (25, 50, 100, and 400 ng/ml) induced greater cell proliferation compared with the media control ($P < 0.001$). CINN activated cultured macrophages to produce higher levels of nitric oxide at 1.2, 2.5, and 5.0 $\mu\text{g/ml}$ ($P < 0.001$), inhibited the growth of chicken tumor cells at 0.6, 1.2, and 2.5 $\mu\text{g/ml}$ ($P < 0.001$), and reduced the viability of *Eimeria tenella* parasites at 10 and 100 $\mu\text{g/ml}$ ($P < 0.01$ and $P < 0.001$, respectively), compared with media controls. In in vivo experiments, chickens were fed a non-supplemented diet (control) or a diet supplemented with 14.4 or 125

mg/kg of CINN from hatch for 14 d. The levels of IL-1 β , IL-6, IL-15, and IFN- γ transcripts produced by intestinal lymphocytes were 2- to 47-fold higher in CINN-fed chickens compared with controls ($P < 0.001$). To determine the effect of CINN-supplemented diets on body weight gain and excretal oocyst shedding following *E. tenella*, *E. acervulina*, *E. maxima* infection, chickens were fed a non-supplemented diet (control) or diets supplemented with 125 (*E. acervulina*-infected birds) or 14.4 mg/kg (*E. maxima*- or *E. tenella*-infected birds) of CINN from hatch for 24 d, and uninfected or orally infected with $2 \times 10,000$ sporulated oocysts of the homologous parasites at 14 d of age. CINN-fed chickens showed 16.5% and 41.6% increased body weight gains between 0 and 9 d post-infection (DPI) with *E. acervulina* or *E. maxima*, reduced *E. acervulina* oocyst shedding between 5 and 9 DPI, and increased *E. tenella*-stimulated EtMIC2 antibody responses at 9 DPI compared with controls. This study provides the first evidence that CINN enhances in vitro and in vivo parameters of immunity against experimental avian coccidiosis.

Key words: cinnamaldehyde, chicken, immunity

T16 Comparison of different levels of vitamin premix on chicken meat quality in floor and battery cage broiler raising. M. A. Shahrasb, H. Moravej, and M. Shivazad*, *Department of Animal Science, Faculty of Agriculture and Natural Resources, Tehran University.*

This experiment was conducted to comparison of different levels of vitamin premix (VP) on broiler finisher diets at 29 to 42 d of age on meat quality in floor and battery cage rearing systems. A total of 616 male broiler chicks (Ross 308) were allocated to 7 treatment groups (different levels and access time to VP), with 4 replicates per treatment group and all data were analyzed in a randomized complete block design. The oxidative stability, evaluated by thiobarbituric acid reactive substances (TBARS) on the thigh meat samples that storage 180 d in -20°C . The results of TBARS values in trial floor system showed there are not significant differences between TBARS values of thigh meat samples for birds slaughtered at 35 d of age, however, at 42 d of age TBARS values of treatments without VP were significantly higher than other treatments ($P < 0.05$). The results of TBARS values in trial battery cage system showed the highest TBARS values of treatments belong to the treatment without VP at 35 d of age ($P < 0.05$), although TBARS values of treatments without VP and 33% VP were significantly higher than other treatments of thigh meat samples for birds slaughtered at 42 d of age ($P < 0.05$). Finally, the results of this study demonstrated that (1) it is not possible withdrawal but it can be possible to reduce VP in finisher broiler diets without negative effects on performance and meat quality during the time of freezing in both methods of rearing; (2) it is possible to reduce the VP levels in diet of broilers rearing in floor system more than cage system.

Key words: broiler, vitamin premix, meat quality

T17 Effects of feeding OmniGen-AF to rats on gastrointestinal gene expression: Microarray analysis. B. R. Ou², Y. Q. Wang¹, and N. E. Forsberg*¹, ¹OmniGen Research, Corvallis, OR, ²Tunghai University, Taichung, Taiwan, ROC.

OmniGen-AF is widely fed in the United States dairy industry and has been reported to reduce somatic cell counts and improve herd health. Yet, the fundamental mechanism(s) by which this product brings about these benefits remains unknown. Our hypothesis was that, because the product is fed, it alters gastrointestinal (GI) gene expression. To test

this hypothesis, 16 growing male CD rats (ca. 225 g) were assigned to 2 treatments: a control treatment (fed a Teklad 8604 powdered diet) and an OmniGen-AF treatment (Teklad supplemented with 0.5% w/w OmniGen-AF [Prince Agri Products, Quincy, IL]). Rats were maintained on diets for 28 d then euthanized. A 10 cm portion of small intestine was removed from each rat and RNA preserved via Trizol. Quality of RNA was assessed in an Agilent Bioanalyzer. RNA was hybridized to Agilent arrays containing 23000 genes. Normalization of the probe set was performed using the Robust Multiarray Analysis method. Gene expression intensities were compared using a moderated *t*-test and a Bayes smoothing approach. Analysis was performed using the affyGUI Graphical Interface for the Limma microarray package. Differentially expressed genes and pathways were derived using DAVID functional analysis. Of the 23000 genes on the array, 288 were upregulated and 385 were downregulated ($P < 0.05$). DAVID pathway analysis identified 13 GI pathways which were specifically affected ($P < 0.05$) by the additive. Of these 13 pathways, 6 were related to "immune function." These included complement and coagulation, antigen processing and presentation, autoimmune thyroid disease, allograft rejection, graft versus host disease and viral myocarditis. Other regulated pathways included starch and sucrose metabolism and endocytosis. A limitation of these data are that we do not know which cell type, of the many GI cell types, was affected by feeding the product. Still, these data demonstrate that significant changes in GI metabolism apparently result from feeding the product. We propose that any nutrient or additive will similarly exert changes in GI gene expression. The specific pathways/patterns affected likely underlie efficacy.

Key words: OmniGen-AF, gene expression, intestine

T18 Inhibition of inflammatory processes in Caco-2 intestinal epithelial cells by an ethanolic extract of a polyphenol-rich grape seed meal. R. Ringseis¹, M. Siebers¹, J. Keller¹, A. Steinbeck², B. Eckel*², and K. Eder¹, ¹*Institute of Animal Nutrition and Nutrition*

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Several pathologic stimuli, including bacteria and viruses, are known to stimulate inflammatory processes in the intestinal mucosa by cytokine-mediated activation of the proinflammatory transcription factor NF- κ B. Through the subsequent release of inflammatory mediators which enter the circulation, the inflammatory process may also affect other tissues, and cause stimulation of protein catabolism in skeletal muscle and formation of acute phase proteins in the liver. Considering that such processes lead to an impairment of animal performance, the inhibition of inflammatory processes in the intestine is a reasonable approach to maintain performance characteristics of livestock animals. Therefore, the objective of the present study was to explore the anti-inflammatory potential of a polyphenol-rich grape seed meal (AntaOx E) using Caco-2 intestinal epithelial cells. Caco-2 cells were grown to confluence, and differentiated for 6 days. Subsequently, the differentiated Caco-2 cells were treated with the cytokine TNF α alone as a control or with TNF α and different dilutions of an ethanolic extract of AntaOx E for 24 h. Cell viability of Caco-2 cells treated with increasing dilutions of the ethanolic extract of AntaOx E was not impaired. Reporter gene assays using an NF- κ B-driven reporter plasmid revealed that the lowest dilutions (1.00E-03, 1.00E-04) of the ethanolic extract of AntaOx E inhibited the TNF α -induced transactivation of NF- κ B by 35% and 25% ($P < 0.05$), respectively, whereas higher dilutions (1.00E-05, 1.00E-06) had no effect. Moreover, the lowest dilution (1.00E-03) of the ethanolic extract of AntaOx E reduced the TNF α -induced mRNA levels of the NF- κ B target genes IL-1 β , IL-8, MCP-1 and CXCL10 by 20 to 35% ($P < 0.05$). It is shown that AntaOx E is capable of inhibiting the activation of the proinflammatory transcription factor NF- κ B and, thereby, the expression of several inflammation-related genes in intestinal epithelial cells. Thus, polyphenol-rich grape seed meal may be useful in the inhibition of inflammatory processes in the intestinal mucosa of livestock animals.

Key words: inflammation, intestine, phytochemicals