of the animals during the inhalation of CO_2. It can be concluded that, in a nonstressful a.m. treatment, the supplementation with elemental Mg or Trp did not affect the meat quality parameters, the genetic of the animals being the most influential factor.

Key Words: Magnesium, Tryptophan, Pork quality

Swine Species

T58 Additional heat behind farrowing sows to reduce the number of stillborn piglets. Y. Gao, H. Y. Zhang, B. Szkotnicki, and R. R. Hacker*, *University of Guelph, Guelph, ON, Canada*.

Additional heat behind a sow at farrowing might help rendering a more relaxed labor in the sow and reduce the number of stillborn pigs at parturition. To examine the hypothesized heat effect, two trials were conducted. Parturient sows were moved to farrowing crates by day 109 of gestation, and fed a 14 (in trial I) or a 16% (in trial II) CP corn-soybean meal diet (3.2-5.5 kg) twice daily. The farrowing room was maintained at 21°C or above and the creep zone was maintained above 35°C with a 175W IR heat lamp. In total, 294 sows (86 Yorkshire in trial I and 208 Hay Bay F1 York/Landrace in trial II) were randomly assigned into either a Heat (an additional 100W IR heat lamp hovered behind the sow at farrowing) or a Non-Heat group. To distinguish stillborn pigs from other pigs that were born alive and died after birth, a lung flotation test was performed on all dead piglets after completion of farrowing. In addition, 20 sows (10 from each group) from trial I were observed for the farrowing behavior. The length of time sows spent sitting, standing and lying down, average delivery interval and position change frequency were recorded. Results showed that additional heat behind a sow at farrowing did not significantly reduce the number of stillborn pigs. On average, there was one stillborn piglet per litter for either the Heat or the Non-Heat group. However, Heat did appear to make live born piglets move readily to the sows udder. Furthermore, there was no difference between the Heat and the No-Heat group for the time farrowing sows spent lying down (85.8 vs. 85.8 min), sitting (9.5 vs. 10.1 min) or standing (4.6 vs. 4.2 min). Additional heat did not significantly decrease the average piglet delivery interval (16.9 min for the Heat group and 17.2 min for the Non-Heat group), however, it significantly decreased ($P < 0.05$) position change frequency (24 vs. 30 times). Less sow movement reduces the risk of the neonatal pigs being crushed by the sow.

Key Words: Additional Heat, Stillborn, Sow Farrowing Behavior

T59 Addition of heat at birth and supplementation of energy and IgG products on improving survivability in neonatal pigs. Y. Gao, H. Y. Zhang, B. Szkotnicki, and R. R. Hacker*, *University of Guelph, Guelph, ON, Canada*.

The objective of this study was to reduce piglet mortality during the first 7d of life by providing additional heat at birth and orally administering cream (C), (10% Half-and-Half cream, Parmalat Canada), and IgG, as Porcine plasma IgG concentrate (P), (American Protein Corp. Inc., IA, USA), to provide supplemental heat, energy and immunoglobulin to neonatal pigs. The farrowing room was maintained at 21°C or above and the creep zone was maintained above 35°C with a 175W IR heat lamp. In two trials, 294 litters (86 litters from Yorkshire sows in trial I and 208 litters from Hay Bay F1 York/Landrace sows in trial II) were randomly assigned into either a Heat (an additional 100W IR heat lamp hovered behind a sow at farrowing, maintaining the farrowing zone temperature above 30°C) or a Non-Heat group. Within 12h after farrowing, the four smallest (minimum birth body weight of 0.6 kg) newborn piglets were selected from each litter and randomly assigned to one of the following four treatments: Control, C (6ml), CP-1 (7ml, 120mg/ml IgG) and CP-2 (8ml, 210mg/ml IgG) in trial I; Control, C (6ml), CP-3 (6ml, 60mg/ml IgG) and CP-4 (6ml, 120mg/ml IgG) in trial II. Results showed that the addition of heat at birth did not show any significant effect on piglet mortality at birth. There was not a significant effect on 3d or 7d piglet mortality associated with supplementation of energy or IgG to newborn pigs in this study. In addition, there was no difference for piglet weight gain from birth to 7d of life. Further investigations need to be conducted on the transfer and absorption of the supplemented IgG in neonatal pigs.

Key Words: Energy and Immunoglobulin Supplementation, Additional Heat, Piglet Mortality

T60 Effects of stocking rate and feeder space on pig performance in a wean-to-finish system. J. M. DeDecker*[1], M. Ellis[1], B. F. Wolter[2], and B. A. Peterson[1], [1]*University of Illinois, Urbana,* [2]*The Maschhoffs, Inc., Carlyle, IL*.

The objective of this study was to determine the effects of feeder space and stocking rate during the first 8 wk post-weaning on pig performance from weaning (4.8 ± 0.03 kg BW; 15 ± 1d of age) to 23 wk post-weaning. Twenty pens of crossbred pigs (n = 960) were used in a randomized complete block design with a 2 2 factorial arrangement of treatments: 1) stocking rate (Single [32 pigs/pen] vs Double [64 pigs/pen]) and 2) feeder space (two spaces [81.3 cm/pen] vs three spaces [121.9 cm/pen]. The stocking rate treatment was imposed for 8 wk post-weaning, thereafter pigs on all treatments had the same group size of 32 pigs/pen. Floor spaces/pig during the treatment period were 0.66 m^2 and 0.33 m^2 for the single- and double-stocked treatments, respectively. There were no ($P > 0.05$) stocking rate by feeder space interactions. During the 8 wk double-stocking period, daily weight gain was higher ($P < 0.001$; 494 and 467 ± 4g/d) for the single-stocked pigs resulting in heavier ($P < 0.001$; 32.3 and 30.8 ± 0.23kg) BW at the end of wk 8. Providing three feeding spaces compared to two tended ($P = 0.08$) to improve daily weight gains (485 and 476 ± 4g/d) resulting in a trend ($P = 0.09$) for heavier BW (31.9 and 31.2 ± 0.23kg) at the end of wk 8. From the end of the double-stocking period to slaughter, there was a trend ($P = 0.06$) for average daily gain (839 and 862 ± 7g/d) to be higher for the double- compared to the single-stocked pigs resulting in similar ($P = 0.77$) BW at slaughter. There was no effect of feeder space during this period and BW were similar ($P = 0.71$) at slaughter. In summary, these results suggest double-stocking pigs for 8 wk post weaning reduces growth performance and BW at 8 wk, but that difference is not maintained to slaughter. Adding an additional feeder space did not significantly improve performance of double-stocked pigs in this study.

Key Words: Pigs, Stocking Rate, Feeder Space

Animal Health

T61 Dose-dependent cytokine expression in lipopolysaccharide activated bovine alveolar macrophages. J. A. Mills*, J. E. Campanicki, and R. M. Dyer, *Dept. of Animal and Food Sciences, University of Delware, Newark*.

Pro- and anti-inflammatory cytokine gene expression in bovine alveolar macrophages could be reflected by level of lipopolysaccharide (LPS) challenge. Lavage procured alveolar macrophages (n=4) were exposed to 0, 1, 10, 100 and 1000 ng/ml of LPS (*E. coli*; O111:B4) for 6 hours. Levels of mRNA expression for IL-1α, IL-1β, TNFα, IL-10, IL-12, IL-18, TGFβ and iNOS were determined by real time QPCR. Expression of IL-1α, IL-1β and iNOS and peaked at 2.5, 11.3 and 13.0 fold higher ($P<0.05$) than levels in unexposed cells, respectively at 100 ng/ml of LPS. TNFα expression changed within LPS stimulated alveolar macrophages, but differences between LPS treatments were only significant between 1 ng/ml and 100 ng/ml ($P<0.05$), and tended to differ between 1 ng/ml and 1000 ng/ml ($P<0.10$). In contrast, expression of IL-10, IL-12, IL-18 and TGFβ decreased ($P<0.05$) at all levels of LPS exposure with the greatest decrease appearing at 100 and 1000 ng/ml of LPS. These findings suggest the balance of inflammatory and anti-inflammatory cytokine expression is dependent upon levels of LPS

exposure and is strongly biased toward a pro-inflammatory response at higher doses of LPS exposure.

Key Words: Cytokines, Lipopolysaccharide, Alveolar Macrophages

T62 Cytokine and growth factor expression is regionally distributed in Holstein claws. J. A. Mills*, R. J. Grant, and R. M. Dyer, *Dept. of Animal and Food Sciences, University of Delaware, Newark.*

Horn tissue of healthy and diseased bovine claw is generated by keratinocyte replication and differentiation in the epidermal layer of the sensitive lamina. Since dermal-epidermal structures express cytokines and growth factors that regulate keratinocyte activity in other species we proposed bovine sensitive lamina expressed cytokine and growth factors. Accordingly, mRNA expression of IL-1α, IL-1β, IL-10, IL-12, IL-18, TNFα, TGFβ, KGF, and GM-CSF was determined in sensitive lamina of normal coronary band, wall, sole and heel bulb using real time PCR. Expression of IL-1α, IL-1β, IL-10, IL-12, IL-18, TNFα, TGFβ, KGF and GM-CSF was detected in the sensitive lamina and differed ($P<0.05$) between and within regions. Cytokine and growth factor expression was lowest overall in the heel bulb ($P<0.05$). Expression of IL-1α and GM-CSF were similar across regions and levels of IL-10 were extremely low or not detected across all regions. Levels of IL-1β expression were greatest ($P<0.05$) in the coronary band and wall, intermediate in the sole and lowest in the heel bulb. Expression of TNFα and KGF was highest ($P<0.05$) in the coronary band, intermediate in the wall and lowest in the bulb and sole. Levels of IL-12 and TGFβ expression were greatest ($P<0.05$) in the coronary band and wall and lowest in the bulb. IL-18 expression was greatest ($P<0.05$) in the coronary band, intermediate in the sole and wall and lowest in the bulb. Thus the results indicated growth factors and many pro- and anti-inflammatory cytokines are expressed in normal bovine sensitive lamina. Their expression differed depending upon anatomic location of the sensitive lamina.

Key Words: Cytokines, Growth Factors, Sensitive Lamina

T63 Extracellular pH alters the innate immune response by enhancing phagocytosis and decreasing reactive oxygen species production. D. C. Donovan*[1], A. J. Reber[1], R. Parks[1], L. O. Ely[2], and D. J. Hurley[1], [1]*College of Veterinary Medicine, University of Georgia, Athens,* [2]*Department of Animal and Dairy Science, University of Georgia, Athens.*

Intensive feeding management of todays feedlot and dairy cattle often results in systemic acidosis and an increase in respiratory disease. Previous research in our laboratory demonstrated that a slight change in venous pH lead to altered adaptive immune responses in steers fed acidogenic diets. In the present study, in vitro experiments were conducted on the innate immune response by evaluating the effects of media pH on phagocytosis and radical production of neutrophils and monocytes. Sixty milliliters of blood was obtained by jugular venipuncture from late lactation multiparous Holstein cows housed at the Athens Dairy Research Center of UGA (331 +/- 81 DIM) with an average body condition score of (3.2 +/ 0.3) for use in ROS assays (n = 12) and (337 +/- 82 DIM) with an average body condition score of (3.22 +/ 0.36) for phagocytic activity of neutrophils and macrophages (n = 12). Phosphate Buffer Saline was supplemented with 0.5% Bovine Serum Albumin and 5 mM Glucose and adjusted to pH 6.0, 6.4, 6.8, 7.2, 7.6, & 8.0. Data were analyzed by mixed procedures of SAS 8.2 (2002) with pH as the main effects. After stimulation with phorbol myristate acetate (PMA) for 1 h incubation at 37 C, the production of reactive oxygen species (ROS) was evaluated using a response ratio (PMA stimulated cells/unstimulated cells). Statistical analysis indicates that pH ($P < 0.01$) greatly effects the production of ROS, and acidosis decreased ($P = 0.031$) the production of ROS relative to alkalotic conditions. Additionally, phagocytosis of bodipy labeled E. coli and S. aureus tended ($P = 0.12$) to be increased under acidotic pH compared to alkalotic pH. Preliminary finding suggest nitric oxide production, after stimulation with lipopolysaccharide, also appears to be decreased at acidic conditions. These results suggest that acidotic pH alters the innate immunity.

Key Words: Acidosis, Immunity, Phagocytosis

T64 Elevation of tumor necrosis factor-α and α_1-acid glycoprotein in lambs with consolidation of lung tissue. J. A. Daniel*[1], T. H. Elsasser[2], and W. Epperson[1], [1]*South Dakota State University, Brookings,* [2]*USDA-ARS Growth Biology Lab.*

Previous research in cattle and sheep has indicated that lung lesions result in decreased animal growth, but mechanisms by which lung lesions result in decreased growth have not been identified. The objective of this research was to identify possible changes in circulating mediators of inflammation associated with lung lesions. As part of a study to determine timing of the onset of lung lesion development, lambs were slaughtered at three time points after weaning (day 1 n=21, day 50 n=20, and day 71 n=21). Serum was harvested and stored at -80°C until analysis. Percentage consolidation of each lobe of the lung was estimated for each lamb. Lambs were classified as having normal (<5 % consolidation of any lobe), moderate lesions (5-50% consolidation of any lobe) or severe lesions (>50% consolidation of any lobe). Circulating concentrations of tumor necrosis factor-α (TNF) were determined by RIA and circulating concentrations of α_1-acid glycoprotein (AGP) were determined by radial immunodiffusion. For statistical analysis, sheep with AGP levels below the minimum detectable concentration (50 μg/ml) were assigned an AGP concentration of 50 μg/ml; effect of lesion scores and level of severity on circulating concentrations of TNF and AGP were tested using one-way ANOVA. Lambs with lesions, moderate or severe, had elevated circulating concentrations of TNF and AGP relative to normal lambs (75.9 $\pm$ 2.3 vs. 68.7 $\pm$ 2.2 pg TNF /ml and 183.6 $\pm$ 44.6 vs. 76.0 $\pm$ 21.3 μg AGP /ml respectively, $P < 0.05$). Circulating concentrations of TNF and AGP did not differ between lambs with moderate or severe lesions (76.4 $\pm$ 3.14 vs. 75.3 $\pm$ 3.52 pg TNF /ml and 139.3 $\pm$ 74.2 vs 232.3 $\pm$ 45.3 μg AGP /ml respectively, $P > 0.05$). These data indicate that circulating concentrations of TNF and AGP are elevated in lambs with lung lesions. Elevations in circulating concentrations of TNF and AGP in lambs with lung lesions are consistent with an inflammatory state that compromises health and growth.

Key Words: Lambs, Tumor Necrosis Factor-Alpha, Alpha$_1$-Acid Glycoprotein

T65 Failure to down regulate tumor necrosis factor-α (TNF-α) responses to repeated endotoxin (LPS) challenge in subpopulations of cattle constitutes a pathophysiologically relevant marker of risk for increased morbidity to disease. T.H. Elsasser* and S. Kahl, *USDA, Agricultural Research Service, Beltsville, MD.*

The tolerance phenomenon is a progressive physiological downregulation of the cytokine cascade responses to repeated challenge with inflammatory stimuli like LPS. Tolerance is considered necessary to attenuate the development of multiorgan failure that occurs from the formation of oxy-nitrogen free radicals and initiation of the complement C9 membrane attack complex (MAC) pathway. Using repeated, graded doses of *E. coli* 055:B5 LPS, we have identified and characterized subpopulations of cattle who fail to develop tolerance in their presentation of significantly greater changes in the immune response event-initiating proinflammatory cytokine TNF-α and corresponding indicators of increased morbidity and disease response. Crossbred beef heifers (n = 32, avg BW 323 kg) were injected twice in five days with LPS (0.8 μg/kg $BW^{0.75}$) with representative blood samples obtained for measurement of plasma TNF-α by RIA. The TNF-α response index (T_{IND}, area response to the first challenge divided by the area response to the second challenge) for each heifer was ranked (Z-score) and outliers identified and grouped (tolerance failure heifers, TFH, n = 6) and retested with a contemporary group of tolerant heifers (TH, n = 6) at 9.46 μg LPS/kg $BW^{0.75}$. T_{IND} responses to LPS were highly repeatable across dose and correlated ($P<0.02$). Replenishment of lost BW following LPS was delayed ($P<0.02$) in TFH vs TH. Plasma acute phase proteins (APP: serum amyloid-A and haptoglobin) concentration changes were greater and temporally prolonged ($P<0.01$) after the repeated LPS challenge in TFH vs TH. Immunohistochemical quantification indicated an increased number of cells and greater intensity of staining positive for MAC at 24 h post LPS in TFH vs TF ($P<0.05$). The data indicate that TNF-α and APP responses to repeated LPS challenge testing can identify animals at risk of greater morbidity response to disease vector challenge.

Key Words: Endotoxin, Immune tolerance, Regulation

T66 Exogenous testosterone (T) modulates tumor necrosis factor-α (TNF-α) and acute phase proteins (APP) responses to repeated endotoxin (LPS) challenge in steers. S. Kahl* and T. H. Elsasser, *USDA, Agricultural Research Service, Beltsville, MD.*

Clinical responses to some disease agents differ between sexes and this dimorphism has been attributed to the immunomodulating effects of estrogens and androgens. Our objective was to determine in steers the effect of T treatment on circulating concentrations of immune response mediators after two consecutive LPS challenges (LPS1 and LPS2, 5 d apart; 0.25 μg/kg BW, i.v., *E. coli* 055:B5). Crossbred steers (n=16; 328 $\pm$ 6 kg), fed a forage-concentrate diet (15% CP) to appetite, were assigned to control (C) or T treatment (n=8). Testosterone cypionate (100 mg/m^2 body surface, i.m.) was injected 12 and 2 d before LPS1. Mean plasma concentrations of T before LPS were 0.02 $\pm$ 0.01 and 3.9 $\pm$ 0.17 ng/ml in C and T, respectively. For each challenge, jugular blood samples were obtained at 0, 1, 2, 4, 7, and 24 h relative to LPS injection. The response to LPS challenge was calculated as area under the time$\times$concentration curve (AUC) for the parameter measured. After LPS1, TNF-α AUC was greater in T than C (3.17 vs 1.91 ng/ml$\times$h, P<0.05). Serum Amyloid-A (SAA) and plasma haptoglobin (HG) concentrations increased (P<0.01) after LPS1 and LPS2. In all steers SAA AUC was greater after LPS1 than LPS2 (P<0.01) but the response was augmented over C with T treatment (2.70 vs 2.05 mg/ml$\times$h, P<0.05). HG response to LPS1 within 24 h was not affected by T. However, 5 d after LPS1 mean plasma HG concentration remained higher in T than C (0.95 vs 0.27 mg/ml, P<0.01). HG response to LPS2 was greater in T than C (17.7 vs 9.7 mg/ml$\times$h, P<0.01). Results indicate that the presence of circulating T increases the magnitude of the TNF-α response to LPS challenge as well as the subsequent increases in APP. Effects of T on increases in TNF-α and APP may underlie a differential presentation of disease symptoms. The data also suggest a role for T in the development of tolerance to repeated immune challenge through its effect on the increased magnitude and duration of HG response.

Key Words: Acute Phase Proteins, Endotoxin, Testosterone

T67 Concomitant dual wavelength fluorescence evaluation of inducible nitric oxide synthase (iNOS) and cytokine responses to endotoxin (LPS) stimulation of bovine peripheral blood mononuclear cells (PBMC). C. Li*, D. Carbough, S. Kahl, and T. Elsasser, *Animal and Natural Resources Institute, Beltsville, MD.*

Cytokines exert autocrine and paracrine effects on the tissue response to immune challenge. Characterization of induced cytokine proteins and intracellular response mediators like nitric oxide (NO) can be used to characterize specific aspects of host responsiveness or resistance to disease challenge. LPS induces rapid formation of proinflammatory cytokines like tumor necrosis factor-alpha (TNF) and many TNF actions are mediated by NO generated from arginine through protein kinase b/AKT- mediated TNF upregulation of the iNOS gene promoter. Once formed, the 17 kD form of TNF is rapidly released from cells making it difficult to evaluate the cellular level of cytokine produced as well correlate its production to that of iNOS. In the present work, we optimized an immune challenge protocol, to rapidly evaluate TNF and iNOS responses in individual beef cattle. L PS (E. coli,055:B5) was added to whole blood (jugular, heparinized) to a final concentration of 2.0 μg/ml. Brefeldin-A, an antibiotic that blocks vesicular protein transport out of cells (Golgi-Block (tm), BD Scientific) was added at 1.0 μl/ml blood. After discrete time periods, subsamples of the whole blood were permiabilized to permit passage of antibodies into cells; blood was incubated with anti-bovine TNF or anti-mouse iNOS. Red blood cells were eliminated by hypotonic lysis. PBMC TNF and iNOS responses were quantified by specific immunofluorescent staining using a dual wavelength fluorescence flow cytometer (Beckman-Coulter Cytomics Fc 500). Our data indicate that upon activation, the intracellular levels of TNF accumulate in the cells treated with brefeldin-A and iNOS increases significantly with a differential time course to that of TNF. The levels of TNF and i-NOS are correlated in PBMCs (P<0.01). By rapidly evaluating the functional interactions between iNOS and cytokines using whole blood in vitro challenge, we can develop strategies to more closely monitor animal health status.

Key Words: Endotoxin, iNOS, Cytokines

T68 The effects of anti-inflammatory agents on expression of cyclooxygenase-2 by bovine neutrophils. N. Cunningham*, P. Matterson, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro.*

Neutrophils (PMN) play a very important role in the inflammatory response of mammals. Cyclooxygenase-2 (COX-2) is one of two isoforms of the enzyme that catalyzes the production of prostaglandins. Bovine PMN release the COX-2 enzyme when stimulated with Escherichia coli lipopolysaccharide (LPS). Nonspecific steroidal anti-inflammatory drugs (NSAIDS) have been very effective at inhibiting COX-2 gene expression in man. The objectives of the present study were to evaluate the effect of commonly used NSAIDS on COX-2 gene expression in resting and stimulated bovine PMN. Blood collected from the jugular vein of four lactating Holstein cows was used to isolate PMN by differential centrifugation and hypotonic lysis of red blood cells. Viability was determined by Trypan blue dye exclusion, purity by differential cell counts and cell concentrations by using a hemacytometer. Subsequently, untreated or LPS stimulated PMN(5x10^6 cells/ml) were incubated for 30 minutes in the presence of either naproxen sodium, flunixin meglumine, acetaminophen, Ibuprofen, sodium salicylate, nordihydroguaiaretic acid, Indomethocin, Dexamethasone, or NS-398. Each NSAID was diluted to a final concentration of 1.0 úM/ treatment. Cells were harvested, lysed and fractionated using SDS-PAGE. Cyclooxygenase-2 was positively identified on LPS treated samples using a specific antibody for western blot analysis. A band of 72 Kd was detected by enhanced chemiluminescence. All NSAIDS tested inhibited the expression of COX-2 on LPS stimulated bovine PMN. The tested reagents may be useful in targeting COX-2 gene expression for modulation of inflammatory diseases such as Mastitis.

Key Words: Neutrophil, Cyclooxygenase-2, NSAID

T69 Extracts of shitake mushrooms modulate receptors for immunoglobulins on bovine neutrophils. K. Gyenai*, M. Worku, O. Ishekhumen, and P. Matterson, *North Carolina Agricultural and Technical State University, Greensboro.*

Phagocytic destruction of opsonized bacteria by bovine neutrophils (PMN) is mediated by receptors (FcR) binding IgM and IgG $_2$. Receptor-specific induction of genes ultimately determines cell fate. Binding of ligand to FcR results in signal transduction through the action of mediators. Expression of FcR by PMN is a marker of apoptosis. The aqueous extract of the Shitake mushroom (Lentinus edodes) decreases production of inflammatory mediators and apoptosis in human PMN. Two different extracts were evaluated for their effect on the modulation of immunoglobulin binding to and FcR expression on bovine PMN. Blood PMN from a Holstein cow were isolated by differential centrifugation and hypotonic lysis of red blood cells. Extracts of 20 grams of Shitake were prepared in phosphate buffered saline (PBS) and incubated at room temperature (RT) or at 90 ^{o}C for six hours. Isolated, viable PMN were treated with 100úl of extracts in the presence of E.coli lipopolysaccharide (LPS) or PBS. Cells were then incubated with FITC labeled bovine IgG $_2$or IgM for flow cytometric analysis of 10,000 cells in duplicate. The mean is presented. The percentage of untreated PMN binding IgM was 76%. Treatment with LPS or RT extract slightly decreased IgM binding 69% and 68% respectively. Treatment with the 90 ^{o} C extract decreased binding (44%). Expression of FcR for IgM in untreated PMN was 112. Treatment with LPS increased receptor expression to 180 and to 107 with the RT extract. The 90 ^{o} C extract decreased expression of FcR for IgM to 93. The percentage of untreated PMN binding IgG_2 was 44%. Treatment with LPS or mushroom extract prepared at 90 ^{o}C had no effect on IgG_2 binding (47% and 45% respectively). The RT extract increased binding (54%). Expression of FcR for IgG_2 in untreated PMN was 88. Treatment with LPS and the 90^o C extract did not change receptor expression (72). The RT extract up-regulated expression of FcR for IgG_2 to 165. Extracts of Shitake mushroom differentially modulated binding and expression of receptors for IgM and IgG_2 on bovine PMN. Methods of extraction may determine the effect of mushroom derived compounds for therapeutic use in animal health. Further studies are warranted.

Key Words: Neutrophil, Fc Receptor, Mushroom

T70 Expression of 5'-lipoxygenase on bovine blood neutrophils. T. Harris*[1], M. Worku[1], P. Matterson[1], and D. Fargo[2], [1]*North Carolina Agricultural and Technical State University,* [2]*University of North Carolina at Chapel Hill, Chapel Hill.*

Exposure to bacterial products such as endotoxin (LPS) results in activation of neutrophils (PMN) and associated inflammation. Studies have shown that following exposure to LPS components of somatic cells express genes for 5'-lipoxygenase (5'-LOX) a key enzyme in the synthesis of leukotrienes. Bovine PMN contribute to the Somatic cell count in milk in response to LPS exposure. The objectives of this study were to determine if bovine PMN express the gene for 5'LOX and to asses the effect of exposure to LPS. Blood collected from the jugular vein of two lactating Holstein cows was used to isolate PMN by differential centrifugation and hypotonic lysis of red blood cells. Viability was determined by Trypan blue dye exclusion, purity by differential cell counts and cell concentrations by using a hemacytometer. Cells were exposed to E. coli LPS (10ng LPS for 30 minutes at 37o C or unexposed. RNA from unexposed and LPS exposed PMN was isolated using TRI-Reagent (Sigma). Specific forward and reverse primers for 5'-LOX were used. RNA was reverse transcribed to cDNA and amplified, observed on a 2% agarose gel and documented. The PCR product was sequenced commercially and Genbank and the Basic Local Alignment System (BLAST) were used to determine homology. The PCR product appeared as a band of 332 base pairs. Two sequences of length 320 and 349 were isolated and consequently showed homology with sequences form Bos Taurus partial mRNA, Mus musculus, and Homo sapiens. The PCR results and sequence information confirm that bovine PMN express 5'LOX. The results indicate that the gene is expressed in LPS exposed and unexposed cells. Quantitative studies will be conducted to asses the levels of gene expression following LPS exposure. Bovine PMN may contribute to the production of leukotrienes in inflammatory diseases such as mastitis through 5'-LOX gene expression. The gene for 5'-LOX is an ideal target for the design of therapeutics based on 5'-LOX inhibition to block leukotriene synthesis.

Key Words: 5'lipoxygenase, Neutrophil, Sequence

T71 Ergovaline transport across human gastrointestinal cells (Caco-2). N. W. Shappell* and D. J. Smith, *USDA-ARS Biosciences Research Laboratory, Fargo, ND.*

The gastrointestinal (GI) cell model Caco-2 (derived from human colon carcinoma) was used to assess ergovaline transport. Cells were grown in transwell inserts until monolayers were established (∼day 20 in culture). A pre-equilibrated mixture of ergovaline/ergovalinine (60:40; 10 and 40 μM concentrations) was added to the apical wells (equivalent to mucosal side) in media containing phenol red. Basal media (equivalent to serosal side) contained no phenol red or ergovaline/inine. Diffusion of compounds through filters with no cells present was also measured. Apical and basal media were extracted, and isomer concentrations were determined by HPLC with fluorescence detection. Monolayer integrity was maintained throughout the 12h experimental period, as assessed by the absence of phenol red in basal media. Kinetics of isomers were identical. In the absence of cells, basal accumulation of isomers was essentially linear for three h at 10 and 40 μM concentrations, after which basal accumulation plateaued (regression curves best described by ln equation; R^2 from 0.85 to 0.96). A second order polynomial equation best described the regression analyses of basal isomer accumulation in the presence cells (R^2 from 0.94 to 1.00). The linear phase of accumulation extended to 240 min with cells. Little change in basal accumulation was observed from 6 to 12 h. After six h in the presence of cells, ∼25% and 40% of dose had accumulated in the basal compartment for 10 and 40 μM ergovaline, respectively. These experiments show that both ergovaline and its naturally occurring isomer, ergovalinine, readily cross GI mucosal cells intact and with similar kinetics. Because both isomers were transported, either isomer, or a combination of both, could be involved in the pathogenesis of fescue toxicosis at sites distal to the G.I.

Key Words: Ergovaline, Fescue Toxicosis, Caco-2

T72 Assessment of Tasco^{TM} and YCWP on ergovaline toxicity in Caco-2 cells. N. W. Shappell* and L. O. Billey, *USDA-ARS Biosciences Research Laboratory, Fargo, ND.*

The seaweed extract Tasco^{TM} and yeast cell wall preparation (YCWP) were tested to evaluate efficacy in attenuation of ergovaline toxicity to Caco-2 cells. Initially cells were treated with 0.1% to 0.001% Tasco, to identify potential Tasco^{TM} toxicity. By ∼ 24h at 37 °C in the presence of media (with or without cells) globules formed. After consultation with manufacturers, it was concluded that calcium and magnesium concentrations present in the medium were causing coalescence of the alginates present in Tasco^{TM} resulting in globules. Tasco^{TM} was toxic at high concentrations (72h, 0.001% to 0.1% yielded ∼ 66% to 1% of control values, respectively, as assessed by metabolic activity (using the alamarBlue^{TM} assay) and total protein. Ergovalines toxicity (100 μM) was tested +/- Tasco^{TM} (0.0001% and 0.00005%, 72 h) on undifferentiated cells. At these concentrations, Tasco^{TM} was non-toxic and it did not alter the toxicity of ergovaline (∼40% reduction of metabolic activity and/or total protein relative to control cells with ergovaline treatment). As YCWP is not water soluble, DMSO extraction was used (0.1g/ml). The cellular effect of YCWP in DMSO (ranging from 0.1 0.5%) was evaluated on cells. DMSO alone was found to reduce metabolic activity and total protein at concentrations greater than 0.2%. Therefore YCWP was tested in 0.05% and 0.1% DMSO. At the 0.05% DMSO/YCWP there was a slight amelioration of the toxicity caused by ergovaline (10-20%). At the 0.1% concentration, no amelioration was seen and ergovaline toxicity may have been increased slightly (<10%). Further investigation of Tasco^{TM} and YCWP at different concentrations may be warranted. An unexpected finding was the reduction of ergovaline toxicity when cells were treated in the presence of DMSO.

Key Words: Ergovaline, Yeast Cell Wall Prep, Tasco

T73 Effects of an oral rehydration solution with added bovine serum proteins on small intestinal absorptive capacity. S. I. Kehoe*[1], J. D. Quigley, III[2], and H. D. Tyler[1], [1]*Iowa State University, Ames,* [2]*American Protein Corporation, Ames, IA.*

Calves commonly become infected with viruses and bacteria that damage the intestinal lining. Although calves cannot absorb IgG after 48 h of age, oral IgG may bind to intestinal lining. This IgG binding may decrease the levels of pathogens in the gut. To enhance recovery of small intestinal function following a coronavirus challenge, bovine serum proteins, containing IgG, TGF-β and other growth factors, were added to an oral rehydration solution (ORS) for 16 Holstein and Jersey calves. Calves were housed individually and offered water ad libitum and milk replacer at 10% of BW. Treatments consisted of a control ORS (CON) and ORS with added bovine serum proteins (GFR). After a 2 d adjustment, calves were orally challenged with bovine coronavirus. Xylose (0.5 g/kg) was administered orally once daily for 6 d and jugular blood was sampled at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 h post-dosing. Hematocrits, fecal dry matter, rectal temperatures, attitude scores and dehydration scores were recorded once daily. Concentrations of serum xylose were numerically higher on d 6 (27.1± 4.9 mg/dl for CON and 24.9 ± 5.3 mg/dl for GFR) than d 3 (13.7 ± 4.6 mg/dl for CON and 17.0 ± 4.7 mg/dl for GFR) but treatments did not differ significantly. Hematocrits and other scores were not significantly different between treatments. In this model, bovine serum proteins did not enhance intestinal recovery from a coronavirus challenge when added to ORS.

Key Words: Calves, Oral Rehydration Solution, Bovine Serum Proteins

T74 Effects of small intestinal absorption in calves treated with an oral rehydration solution supplemented with fat-soluble vitamins. S. I. Kehoe*[1], H. D. Tyler[1], M. L. O'Brien[2], K. J. Touchette[2], and J. A. Coalson[2], [1]*Iowa State University, Ames,* [2]*Merrick's, Inc., Middleton, WI.*

Young calves commonly become infected with viruses and bacteria that damage the intestinal lining. Deficiencies in fat-soluble vitamins also occur in young calves and may impair the rate of differentiation of intestinal epithelial tissues, especially following enteric infections. To enhance recovery of small intestinal function following a coronavirus challenge, fat-soluble vitamins were added to an oral rehydration solution (ORS) for 16 Holstein and Jersey calves. Calves were housed individually and

offered water ad libitum and milk replacer at 10% of BW daily. Treatment one consisted of a control ORS (CON). Treatment two consisted of added antioxidants, to provide 70,000 IU of vitamin A and 300 IU of vitamin E per day (VIT). After a 2 d adjustment, calves were orally challenged with $5x10^6$ plaque-forming units of a moderately virulent bovine coronavirus isolate. Xylose (0.5 g/kg of BW) was administered orally once daily for 6 d and jugular blood was sampled at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 h post-dosing. Hematocrits, fecal dry matter, rectal temperatures, attitude scores and dehydration scores were recorded once daily. Serum xylose concentrations were higher on d 6 (27.1 ± 4.9 mg/dl for CON and 20.2 ± 3.7 mg/dl for VIT) than on d 3 (13.7 ± 4.6 mg/dl for CON and 14.9 ± 4.2 mg/dl for VIT) but were not significantly different between treatments. Hematocrits and other clinical scores were not significantly different between treatments. In this model, antioxidant vitamins did not appear to enhance intestinal recovery from a coronavirus challenge when added to ORS.

Key Words: Calves, Oral Rehydration Solution, Fat-Soluble Vitamins

T75 Effectiveness of ground endophye-infected tall fescue seed in production of fescue toxicosis in cattle. L. E. Wax*, D. E. Spiers, G. E. Rottinghaus, and T. J. Evans, *University of Missouri, Columbia*.

Cattle fed endophyte-infected tall fescue (E+) in the form of whole seed during heat stress experience increased hyperthermia, and reduction in both feed intake and weight gain. This study determined if intake of diet containing ground infected fescue seed would increase fescue toxicosis as a result of increased absorption of toxin. Steers (n=18; 300kg avg. BW) were housed in the Brody Climatology Laboratory and randomly assigned to daily diet treatments of either E+ (20-40μg ergovaline/kg BW) or control (endophyte-free, E-) seed. Animals were exposed to a 14 day heat challenge (HC) reaching 36°C during the day and 26°C at night and fed twice daily at 0800 and 1600 with water available *ad libitum*. Core temperature was measured continuously using telemetric, temperature transmitters (CowTemp, Model BV-010) with respiration rate, skin and rectal temperatures recorded four times daily. Ground E+ decreased feed intake below control by day 7 at thermoneutral (TN) ($p<.0001$). Maximum reduction in feed intake at TN was 25% from pretreatment with an additional 46% decrease during HC. By the end of HC, E+ feed intake increased to E- levels, suggesting there is a recovery from the effect. In contrast to previous studies using whole seed, there is no evidence of change in core temperature from control levels. A preliminary study was done using fistulated steers to determine if this level (20μg ergovaline/kg BW) of seed is capable of producing an increase in core temperature above the E- level. Steers were fed E- seed for 3 days switched to E+ seed for 5 days followed by E- seed for 3 days, during HC. Peak rectal temperature rose 1.3°C from E- to E+ treatment periods. Therefore core temperature response and feed intake are independent of each other with feed intake being more sensitive. Ground fescue seed elicits a robust temperature response, once the large effect on feed intake is overcome.

Key Words: Cattle, Endophyte, Heat Challenge

T76 Effect of Eprinex® on subsequent 90-day production in Virginia Holstein herds. K. L. Rossini*[1], M. L. McGilliard[1], and R. H. Nutt[2], [1]*Virginia Tech, Blacksburg,* [2]*Valley Feed Co., Staunton, VA*.

One trial involving four Holstein herds and 270 total dairy cows was conducted to determine if cows treated once with Eprinex® (Merial) produced more milk in subsequent months than did untreated cows. Cows were generally confined and averaged 33 kg/d of milk. Odd-numbered cows in each herd were treated with Eprinex® (1 ml/10 kg body weight) whereas even-numbered cows were left untreated. A single treatment was administered on or near one DHI test day in March, April or May. Test-day data were down-loaded for that initial test day and the next 3 monthly tests. Change in production from month zero was analyzed with a model containing effects of treatment, herd (random), month (repeated), parity (first and older), and initial days in milk (<90, 90-270, >270 d). Variables analyzed were test-day milk, percentages of fat and protein, and SCC score. A total of 211 cows remained for all four test days. Change in milk per cow from mo 0 to 1 was 2.7 kg/d (0.5 SE) greater for treated cows. The advantage was maintained at 2.7 kg/d (0.8 SE) to mo 2, and 3.1 kg/d (0.8 SE) to mo 3 (cumulative from mo 0). For cows in milk less than 90 d, treated cows had an advantage of 5.4 kg/d (1.1 SE) to mo 1, 6.6 kg/d (1.7 SE, NS) at mo 2, and 6.5 kg/d (1.7 SE) at mo 3. For cows in early lactation, 7 of 13 untreated cows increased in milk from mo 0 to 1, whereas 10 of 11 treated cows increased. Initial days in milk were 72 and 69 d for those untreated and treated groups. The advantage for cows more than 90 d in milk at treatment was not significant at less than 2 kg/d. Fat changed 0.33% more by the mo 2 for cows dosed in mid-lactation. Change in percentage protein and somatic cell score did not differ between treated and untreated cows. At a cost of $4.00 per cow for product and labor, 0.35 kg of feed dry matter consumed for each kilogram of additional milk, $0.15/kg of dry matter, and $0.28/kg milk, the net value for 90 d of 3.1 kg/d would be $0.66/d per cow ($0.86 milk - $0.04 product and labor - $0.16 feed).

Key Words: Eprinex, Milk Yield, Eprinomectin

T77 Frequencies of calving-related diseases of Holstein dairy cows injected with low doses of bovine somatotropin during the transition period. M. Liboni*, M. J. Hayen, M. S. Gulay, T. I. Belloso, and H. H. Head, *Department of Animal Sciences, University of Florida, Gainesville*.

Objective was to evaluate effects of injecting bST (0.4 mL, 10.2 mg/d, POSILAC®) during the prepartum and/or early postpartum periods on incidence rate of calving-related disorders of dairy cows during the first 60 d postpartum. Multiparous Holstein cows (n=150) were assigned randomly to a 2x2 arrangement of treatments to give four groups (I=no bST, n=26; II=bST postpartum, n=25; III=bST prepartum, n=27; IV=bST prepartum and postpartum, n=25) plus a group of cohorts (V=cohort, n=47). Biweekly injections of bST were in left or right ischiorectal fossa beginning 3 wk before expected calving through 70 DIM. Disease frequencies were collected from farm records, and incidence rates (number of diseased cows divided by the total number of cows) were reported for each group. Across all TRT groups (n=150) the observed incidence rates were: retained fetal membranes (RFM, 15%), metritis (MET, 36%), clinical mastitis (MAT, 22%), digestive problems (indigestion [DIG], 11%), ketosis (KET, 4%), milk fever (MF, 1.3%), displaced abomasum (DA, 3.3%) and lameness (LAME, 4.6%). Incidence rates of RFM, MET, MAT and DIG for TRT I, II, III, IV and V were 19.2, 16, 7.4, 0.0 and 25.5 %; 42.3, 32.0, 37.0, 32.0 and 42.5%; 30.7, 28.0, 14.8, 20.0 and 21.2%; and 19.2, 16, 11.1, 0.0 and 8.5 %, respectively. The proportions of sick cows in a group (number of cows that had one or more cases of disease divided by the total number of cows) were 76.9, 64.0, 59.2, 48.0 and 65.9 %, respectively. Significant Chi-Square values were detected between TRT I vs. TRT IV cows for RFM and DIG ($P<0.028$). In addition, there tended to be greater number of healthy cows on TRT IV than on TRT I ($P<0.0649$). No differences were detected between TRT I and cohorts. Results indicated that bST injected during the transition period did not increase the incidence of calving-related disorders. Furthermore, cows on TRT IV were less likely to have RFM, DIG and tended to be healthier than non-injected cows.

Key Words: bST, Transition Period, Diseases

T78 The effect of Johne's disease on culling and milk production in nine Ontario dairy herds. S. H. Hendrick*[1], T. F. Duffield[1], D. F. Kelton[1], K. E. Leslie[1], K. D. Lissemore[1], and M. Archambault[2], [1]*Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Animal Health Laboratory, University of Guelph, Guelph, ON, Canada*.

The objective of this study was to evaluate the influence of Johnes disease (JD) on culling and milk production on Ontario dairy herds. During the summer of 2002, 9 Holstein dairy herds with a previous history of JD were enrolled in this study. Blood and fecal samples were collected from all milking and dry cows (868 cows). Serum samples were submitted to the AHL, for a commercial ELISA. Fecal samples were sent to AntelBio Systems for traditional fecal culture. Milk samples were collected from all milking cows at the herds next DHI test day (690 cows). The milk samples were sent to AntelBio for an in-house milk ELISA. Test results were not returned to the producers until January 2003. Milk production and culling data were retrieved from the DHI database. 305-day milk production was compared to JD status as predicted by the three diagnostic tests. A separate model was made for each test with the effects of mastitis, DIM, parity and herd controlled in each of the models. Culling data was collected between the farm visit date and Dec. 31, 2002. Proportional hazards models were used to evaluate the days to culling stratified by JD status with separate model

for each test, and controlling for herd, DIM, parity, pregnancy status and linear score. Fecal culture positive cows produced 548 kg less milk than fecal culture negative cows. Similarly, milk ELISA positive cows had a decrease of 457 kg versus milk ELISA negative cows. There was no statistical difference in 305-day milk production in seropositive cows. Survival analysis showed that fecal culture positive cows were 3.16 times more likely to be culled than non-shedding cows. Milk ELISA positive cows were 2.27 times more likely to be culled than milk ELISA negative cows. There was a tendency for serum ELISA positive cows to be culled 1.72 times more than seronegative cows. For the 9 herds in this study, JD significantly limited milk production and cow longevity.

Key Words: Paratuberculosis, Culling, Milk Production

T79 ***Escherichia coli*** **and** ***Staphylococcus aureus*** **elicit differential innate immune responses following intramammary infection.** D. Bannerman*[1], M. Paape[1], J.-W. Lee[2], X. Zhao[2], J. Hope[3], and P. Rainard[4], [1]*Bovine Functional Genomics Laboratory, USDA-Agricultural Research Service, Beltsville, MD,* [2]*Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, QC, Canada,* [3]*Institute for Animal Health, Berkshire, UK,* [4]*Institut National de la Recherche Agronomique, Nouzilly, France.*

Staphylococcus aureus and *Escherichia coli* are among the most prevalent species of Gram-positive and Gram-negative bacteria, respectively, that induce clinical mastitis. The innate immune system comprises the immediate host defense mechanisms to protect against infection and contributes to the initial detection of and pro-inflammatory response to infectious pathogens. The objective of the current study was to characterize the differential innate immune response to experimental intramammary infection with *E. coli* and *S. aureus*. The cytokine response and changes in the levels of soluble CD14 (sCD14) and lipopolysaccharide (LPS)-binding protein (LBP), two proteins that contribute to host recognition of bacterial cell wall products, were studied. Intramammary infection with either *E. coli* or *S. aureus* elicited systemic changes that were statistically significant including decreased milk output, a febrile response, and induction of the acute phase synthesis of LBP. Infection with either bacteria resulted in significantly increased milk levels of IL-1β, IFN-γ, IL-12, sCD14, and LBP. Significantly higher levels of the complement cleavage product C5a, and the anti-inflammatory cytokine IL-10, were detected at several time points following *E. coli* infection, whereas, *S. aureus* infection elicited a slight but significant increase in these mediators at a single time point. Significant increases in IL-8 and TNF-α were only observed in quarters infected with *E. coli*. Together, these data demonstrate the variability of the host innate immune response to *E. coli* and *S. aureus* and suggest that the limited cytokine response to *S. aureus* may contribute to the well-know ability of this bacterium to establish chronic intramammary infection.

Key Words: Mastitis, Innate Immunity, Inflammation

T80 Ultrasonographic characteristics of the uterus of Holstein cows with late endometritis. J. K. Haskell*[1], D. S. Hammon[2], and G. R. Holyoak[3], [1]*Department of Forest, Range and Wildlife, Utah State University, Logan,* [2]*Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan,* [3]*Department of Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater.*

Endometritis in dairy cows is common during the prebreeding period and has a profound negative impact on reproductive efficiency. Further characterization of endometritis and easily utilized, non invasive, and accurate methods to diagnose endometritis are needed. The objective of this study was to determine the potential for using ultrasonography as a method for diagnosing subclinical endometritis in early lactational dairy cows. To assess subclinical endometritis, primiparous and multiparous Holstein dairy cows were examined 40 to 60 days postpartum. The reproductive tract of each cow was examined using transrectal ultrasonography, and measurements of uterine horn diameter, and endometrium height were obtained by electronic caliper. Frozen ultrasound images of uterine horn transverse sections were recorded for later evaluation to determine the presence of endometritis, based on echoic particles and fluid within the uterine horn lumen. Uterine cytology was performed as a definitive diagnosis of endometritis for each cow. Specificity and sensitivity of ultrasound were established based upon the results of two investigators and compared to uterine cytology. Specificity was 97% and 64%; and sensitivity was 53% and 57% for evaluator 1 and 2, respectively. Measures of uterine endometrium height difference between horns, and maximum endometrium height were significantly associated with the presence of endometritis. This finding supports the use of ultrasound to accurately detect endometrial changes, which are associated with endometritis.

Key Words: Subclinical Endometritis, Uterine Cytology, Ultrasonography

T81 Antimicrobial Susceptibility Of Coagulase-Negative Staphylococci Isolated From Hands Of Urban Children, Farm Workers, and Dairy Cow Teat Skin. M. M. Pol*, C. C. M. Hulland, and P. P. L. Ruegg, *University of Wisconsin, Madison.*

There is increasing concern that antimicrobial use in animals can create a reservoir of resistant bacteria that could be transferred to humans. The aim of this study was to compare results of antimicrobial susceptibility tests of coagulase-negative staphylococci (CNS) isolated from teat skin of cows, and hands of children and farm workers. Swabs (Quick Swab, 3M) were obtained from hands of farm workers (n = 39), urban children (n = 36), and from teat skin of dairy cows (n = 32). Swabs were cultured on blood agar plates and incubated at 37 C. CNS were speciated using a commercial microbial identification system. MIC values were obtained using a commercial microdilution system (Sensititre, Westlake, OH). Isolates classified as intermediate were reclassified as resistant for statistical analysis. MIC values were obtained for CNS isolated from children (n = 57), farm workers (n = 64), and teat skin (n = 42). There were no significant differences in MICs of CNS isolated from farm workers among farms. MICs of CNS obtained from teat skin varied among farms for erythromycin and sulfadimethoxine (P #8804 0.05). For most antimicrobials, there were no significative differences in MIC based on source of the isolate. MICs for erythromycin and sulfadimethoxine were significantly higher for farm workers as compared to urban children (P #8804 0.05). MICs for tetracycline were significantly higher for CNS obtained from farm workers as compared to CNS obtained from children and teat skin ($P < 0.001$). Source of isolate was significantly associated with resistance for ampicillin ($P = 0.04$), penicillin ($P = 0.02$), erythromycin ($P = 0.03$), pirlimycin ($P < 0.001$), sulfadimethoxine ($P = 0.04$), and tetracycline ($P < 0.001$). The probability of resistance was 0.3, 0.4, 0.1 and 0.2 for children as compared to farm workers for ampicillin, erythromycin, pirlimycin and tetracycline respectively. The probability of resistance was 0.3, 0.2 and 0.04 for children as compared to teat skin for erythromycin, penicillin, and pirlimycin, respectively.

Key Words: Antimicrobial Susceptibility, Coagulase-Negative Staphylococci

T82 Heel erosion in dairy cattle. L. G. Baird*[1], L. C. Pinheiro Machado Filo[2], M. A. G. von Keyserlingk[1], D. M. Weary[1], and K. A. Beauchemin[3], [1]*Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada,* [2]*Universidade Federal de Santa Catarina, Brazil,* [3]*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.*

Heel erosion, one of the most common hoof disorders in dairy cattle, begins as shallow irregular grooves in the heel horn and can progress to deep oblique grooves resulting in major loss of heel structure. Although some research is available for European farms, to date no published studies have addressed this disorder for North American dairy herds. The objective of this study was to monitor the progression of heel erosion in a cohort of Holstein cows, examining the effects of parity and stage of lactation. The hind hooves of 20 heifers and 38 multiparous cows were scored at least once pre-calving, in early lactation (0-100 DIM) and in mid-lactation (100 200 DIM). Specifically, we noted any grooving on the four hind claws (medial and lateral, left and right), and if these grooves were irregular or oblique. Any claw with oblique grooves or major loss of heel structure was classified as having severe erosion. During pre-calving observations, the average number of claws per cow with severe heel erosion was 0.59±0.12, but by mid-lactation this score had increased to 2.7±0.20 ($P < 0.001$). Erosion was also related to parity, with the older cows experiencing higher scores ($P < 0.001$), especially in early and mid-lactation ($P < 0.02$ for period by parity interaction). The

results of this study indicate that heel erosion is a common affliction, especially for mid-lactation multiparous cows.

Key Words: Heel Erosion, Dairy Cattle, Hoof

PSA - Pathology

T83 Identification of a c-reactive protein gene in cardiomyopathic turkeys: A possible genetic marker for turkey cardiomyopathy. A. E. Hauser* and M. M. Corley, *Tuskegee University, Tuskegee, AL*.

C-reactive protein (CRP) is an inflammatory protein released by the body in response to infection and injury. Elevation of this serum protein has been linked to increased risks of heart disease through inflammation that is believed to play a key role in the hardening of arteries resulting in a heart attack or stroke. Consequently, CRP could serve as a genetic marker for an eminent cardiac event. Thus far, the poultry industry has experienced considerable loss due to turkey cardiomyopathy. Furthermore, the gross and microscopic lesions in tissue from cardiomyopathic turkeys, have been shown to be very similar to human cardiomyopathic heart tissue. Therefore investigation of those genes involved in turkey cardiomyopathy can lead to further insight of cardiovascular disease and thus benefit both the poultry industry and the human population. In this study, we attempted to identify a crp gene from turkeys that carry a genetic trait (unknown) that renders them susceptible to cardiomyopathy. The identification and expression of this gene as it relates to heart disease in these turkeys has not been investigated. The reverse transcriptase polymerase chain reaction (RT-PCR) was used to target this gene. Total RNA, extracted from frozen heart tissue (0.1g) of a cardiomyopathic turkey was used to generate turkey cDNA, and oligonucleotide primers were designed from a partial crp gene sequence derived from a chicken liver cDNA library to amplify the crp gene. The expected 504 bp RT-PCR product was successfully amplified. Identification and analysis of the gene that codes for CRP in turkeys will lend further insight into the etiology of turkey cardiomyopathy.

Key Words: Cardiomyopathic, C-reactive Protein, Turkeys

T84 Identification and analysis of an apolipoprotein- A gene in cardiomyopathic turkeys. T. A. Dugger* and M. M. Corley, *Tuskegee University, Tuskegee, AL*.

Apolipoprotein A is a major constituent of high-density lipoproteins, which aids in the regulation of high cholesterol levels in the blood and peripheral tissues. If this process is defective, cholesterol molecules can accumulate in arteries, result in arterial blockage, thereby leading to a cardiac event (heart attack). The turkey cardiomyopathic heart resembles that of humans in gross and microscopic morphology. Therefore analysis of genes implicated in turkey cardiovascular disease may be beneficial to both poultry and human health. Heart tissue from cardiomyopathic turkeys was used to identify an apoA gene and its possible involvement in cardiovascular disease. The role of this gene as it relates to turkey cardiomyopathy has not been investigated. To isolate this gene, total RNA was isolated from heart tissue samples and purified. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed using primers designed from the chicken apoA-1 gene. The RT-PCR product was visualized via agarose gel electrophoresis (1.5% w/0.5 μg/ml ethidium bromide). The expected 519 bp RT-PCR product was observed, indicating successful amplification of the apoA gene from cardiomyopathic turkey heart tissue. Identification and analysis of the turkey apoA gene will lead to further knowledge of the etiology of turkey cardiomyopathy.

Key Words: Apolipoprotein, Cardiomyopathy, Turkeys

T85 Isolation and characterization of an anti-*Salmonella* phage collection for use as antibacterial agents. E. Kozhina*, P. Herrera, and S. Ricke, *Texas A&M University, College Station*.

Interest in phage therapy has increased in the past several years due to the rise in the antibiotic resistance. There are several problems to overcome before bacteriophages can be routinely used as antibacterial agents. One problem is that most bacteriophages are highly specific infecting only a few host strains of bacteria. However some bacteriophages exhibit a wider host range. Another problem is the maintenance of the stability and viability of a phage collection. Our objective is to isolate a stable phage collection capable to lysing a number of different *Salmonella* strains. First, we isolated phages from the environmental water samples. This was followed by five consecutive step propagation procedures in a mixture of *Salmonella* strains and in four different *Salmonella* strains. A number of plaques grew on a bacterial lawn containing the 4 *Salmonella* strains. The largest and clearest plaques were chosen for further study. We investigated stabilizer (YT media supplemented with 10 mM MgSO4, 1% BSA and 5% sucrose) for storage of phage collection. We found that it result in 100% recovery of newly isolated phage collection stored for a month in 4°C (compare to 98% of recovery in SM buffer (100 mM NaCl, 8 mM MgSO4, 50 mM Tris-Cl, pH 7.5). The DNA from these phages were isolated, digested with EcoRI restriction enzyme, and the banding patterns analyzed by agarose electrophoresis. In order to select for the most stable phage isolates, the mixtures of primary phage isolates were stored for 5 months at 4°C. Phages isolated from these mixtures were tested for their ability to survive while incubated in non-sterile bovine rumen fluid at 37°C. Stabilizers, such as 1M MgSO4 and 1% gelatin, were added to determine if they could enhance the bacteriophages survival. In all cases phages were isolated from rumen fluid after 3 days of incubation. The presence of stabilizer did not influence phage stability in rumen fluid. Initial results indicate that these procedures, with minor modification, can be used routinely to generate a useful and stable anti-*Salmonella* phage collection.

Key Words: *Salmonella*, Phage, Isolation

T86 Pathology of listeriosis resulting from respiratory infection of turkey poults with Listeria monocytogenes Scott A. G. R. Huff*[1], W. E. Huff*[1], J. N. Beasley[2], M. G. Johnson[2], R. Nannapaneni[2], J. M. Balog[1], and N. C. Rath[1], [1]*USDA/ARS/PPPSRU,* [2]*University of Arkansas*.

The pathogenesis of a human epidemic strain of *Listeria monocytogenes*, Scott A, was studied by challenging day-old turkey poults with air sac inoculation of 10^0 (Control), 10^4, 10^5, 10^6, 10^7, or 10^8 cfu. Respiratory challenge with all levels resulted in listeriosis. Mortality at 2 wk post-infection ranged from 25-100% and was directly correlated with level of challenge. Gross pathology included enlarged gall bladders and pale livers, some of which were also yellowish, mottled, or cooked in appearance. Ruptured yolk sacs were common. Lungs were necrotic and hearts were swollen and surrounded by fluid. Sections of liver, heart, spleen, bursa, lung, and brain were fixed in 10% buffered formalin. Paraffin-embedded sections were cut at 5μ, stained with hematoxylin and eosin as well as Gram stain and were examined for histological lesions. Lesions were observed in liver, heart, spleen, bursae, and lung, however no significant changes were present in brain. The myocardial lesions consisted of large infiltrations of mononuclear cells deep in the myocardium and were associated with Gram positive rods. In the liver, focal infiltrations of mononuclear cells were small and scattered and were also associated with Gram positive rods. Congestion and reticuloendothelial hyperplasia were prominent in the spleen and there was necrosis of scattered cells. Lymphocytes and mononuclear cells infiltrated areas surrounding bronchi in the lung. In the bursae there was depletion of lymphocytes in bursal follicles. Listeria challenge also resulted in significantly decreased relative weight of the bursa of Fabricius and increased relative weight of the spleen. *L. monocytogenes* was isolated by direct plating of liver, gall bladder, pericardium, brain, yolk sac, lung, cecal tonsil, and both left and right knee synovium cultures on UVM Listeria selective agar. These results suggest that respiratory infection with *L. monocytogenes* can be invasive in young turkeys and may be responsible for some unexplained cases of early poult mortality as well as the initiation of chronic infection leading to product contamination.

Key Words: Listeria Monocytogenes, Respiratory Infection, Turkeys