Monday, July 25, 2005

POSTER PRESENTATIONS

Animal Health I

M1 Influence of the mycotoxin fumonisin B_1 on intestinal physiology and immune function in piglets. M. Lessard*[1], J.-P. Lallés[2], G. Boudry[2], B. Séve[2], and I. P. Oswald[3], [1]*Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre*, *Lennoxville, Qc, Canada,*, [2]*INRA Systémes d'Élevage, Nutrition Animale et Humaine*, *St-Gilles, France*, [3]*INRA Pharmacologie-Toxicologie*, *Toulouse, France.*

In this study, a fungal extract enriched in fumonisin B_1 (FB_1) was orally administered to piglets to determine the influence of this mycotoxin on intestinal morphology and function and on systemic immunity. At weaning, 18 pairs of male piglets within litters were conducted in three replicates and allocated to the control (CTR) and FB_1 groups. Ten days after weaning (d 0), pigs of the FB_1 group received 1.5 mg/kg BW of the FB_1 extract (2.3 mg/ml) diluted in glucose solution for 9 days. CTR group received only glucose solution. On d -5 and d +2, all piglets were injected with 2 mg of emulsified ovalbumin (OVA) and blood samples were taken on d -5, +2 and +9 to evaluate antibody to OVA and mitogen-induced lymphocyte responses. On d +9, piglets were slaughtered and organs weighed. Samples from the small intestine were taken for morphometry and enzyme activities and to examine physiology in Ussing chambers. Mesenteric lymph nodes were also taken to determine bacterial translocation. Feed intake was slightly restricted during the oral treatment in both groups. Growth of pigs and feed consumption were similar in both groups but liver weight was higher ($P \leq 0.01$) in FB_1 group. FB_1 did not affect small intestinal villous and crypt morphometry but it tended ($P \leq 0.10$) to increase jejunal Na^+-dependent glucose absorption and vasoactive intestinal peptide (VIP) -induced secretion in vitro. Jejunal aminopeptidase N activity was lower with FB_1 ($P \leq 0.01$). FB_1 treatment did not influence secondary antibody response to OVA, specific lymphocyte proliferation response and bacterial translocation. In conclusion, FB_1 consumption alters functions of the small intestine without effect on its architecture or systemic immunity in this trial. Increased VIP-induced jejunal secretion suggests that FB_1 may aggravate secretory diarrhea caused by gut bacterial toxins.

Key Words: Fumonisin B_1, Intestine, Pigs

M2 Effects of age and nutrition on proliferation and activation of mitogen stimulated T cell subsets from neonatal calves. M. Foote*[1], B. Nonnecke[2], M. Fowler[3], B. Miller[3], D. Beitz[1], and W. Waters[2], [1]*Iowa State University*, *Ames*, [2]*USDA, ARS, National Animal Disease Center*, *Ames, IA*, [3]*Land O'Lakes, Inc.*, *St. Paul, MN*, [4]*Land O'Lakes, Inc.*, *Webster City, IA*.

Effects of nutrition and age on mitogen-induced (i.e. in vitro) proliferation and activation of lymphocyte subsets from milk replacer-fed calves were investigated. Calves were fed standard (0.45 kg/d of a 20% crude protein, 20% fat milk replacer, n=4) or intensified (1.14 kg/d of a 28% crude protein, 20% fat milk replacer, n=4) diets from 1 to 8 wk of age. Average daily weight-gain of intensified-diet (0.66 kg/d) calves was greater (P<.05) than standard-diet (0.27 kg/d) calves. When compared to responses of pokeweed mitogen-stimulated CD4 cells from juvenile steers (5-6 m of age), CD4 cells from 1-wk old calves displayed decreased (P<.05) proliferation, delayed CD25 expression and no increase (P>.10) in CD44 expression or decrease (P>.10) in CD62L expression in response to mitogenic stimulation. The mitogen-induced decrease (P<.05) in CD62L expression by steer CD8 and γδ T cells was not seen in stimulated CD8 and γδ T cell populations from 1-wk old calves. Similarly, the mitogen-induced increase (P<.05) in CD44 expression by adult γδ T cells was not observed (P>.10) in stimulated γδ T cell populations from 1-wk old calves. At wk 8 of age, however, mitogen-induced responses (i.e. proliferation and expression of activation antigens) by T cell subsets from standard-fed calves were comparable to responses of T cell subsets from juvenile steers. Feeding an intensified diet was associated with decreased (P<.05) proliferation of stimulated CD4, CD8, and γδ T cells; CD25 expression on stimulated CD4 and CD8 cells; and CD44 expression on stimulated CD8 cells. These results indicate that the functional capacity of the calf's T cell population matures rapidly during the first weeks of life and suggest that nutrition influences maturation of T cell function during the neonatal period.

Key Words: Milk-fed calf, Neonatal immunology, T lymphocyte

M3 Gastrointestinal leukocyte and peripheral blood mononuclear cell populations within piglets nursing sows supplemented with phosphorylated mannan oligosaccharides during gestation and lactation. C. L. Bradley*[1], D. C. Brown[1], M. E. Davis[1], C. V. Maxwell[1], E. A. Halbrook[1], Z. B. Johnson[1], R. Dvorak[2], and B. Lawrence[3], [1]*University of Arkansas*, *Fayetteville*, [2]*Alltech, Inc.*, *Nicholasville, KY*, [3]*Hubbard Feeds, Inc.*, *Mankato, MN*.

Three weeks prior to farrowing, 36 gestating sows were allotted to two dietary treatments to determine the effects of mannan oligosaccharide (MOS) supplementation on peripheral blood mononuclear cell (PBMC) and enteric leukocyte populations within their offspring. Diets consisted of a control or the control with 0.3% MOS fed to sows for 3 wk of gestation and throughout lactation. Dietary treatments were assigned to individual sows in a completely randomized design with sows stratified for equal parity and genotype representation. At an average of 16 d of age, 12 pigs (6 pigs per treatment) were randomly selected, and blood samples were obtained for the isolation of PBMC for flow cytometric analysis. Twenty-four hours later, the same 12 pigs were euthanized and jejunal intraepithelial (IEL) and lamina propria (LP) leukocytes were isolated for flow cytometric analysis. Myeloid cell lineage was affected in pigs nursing MOS-supplemented sows, which was evident by a reduction ($P < 0.04$) in monocytes (SWC1+CD14+ and CD14+SWC9-), intermediate monocytic cells (SWC1+SWC9+ and SWC1+MHCII+), activated macrophages (CD14+SWC1-, SWC9+MHCII+, SWC9+CD14+, and MHCII+CD14+) and antigen presenting cells (MHCII+CD14-) within the PBMC population. In addition, cellular immunity in nursing pigs was altered by MOS supplementation to the sow, with a reduction ($P < 0.02$) of CD3+ (T cells), CD4+ (T helper cells), CD21+ (B cells), CD25+ (activated T and/or B cells), CD4-CD8-CD25+ (activated immature T cells), TCR1+ (T cells with the gamma/delta T cell receptor), and CD4-CD8-TCR1+ (immature T cells with the gamma/delta T cell receptor) leukocytes within the PBMC population. However, there were no differences ($P > 0.10$) in populations of LP leukocytes or IEL between the two treatment groups. This study suggests that MOS supplementation to the sow may alter innate and cellular immunity in developing piglets, creating a quiescent immune response in the nursing offspring.

Key Words: Leukocytes, Mannan oligosaccharide, Swine

M4 Gene identification in bovine neutrophils. M. Worku*, T. Harris, and P. Matterson, *North Carolina A&T State University*, *Greensboro*.

Polymorphonuclear leukocytes (PMN) are key cells in the inflammatory response to bacterial products such as endotoxin. Endotoxins (LPS) are components of the outer cell wall of gram negative bacteria. Few studies have addressed the effect of LPS on gene expression in bovine blood PMN. The objective of this study was to investigate the expression of 5′-lipoxygenase (5′-LOX), cylcooxygenase 1 and 2 (COX-1 and COX-2), ubiquitin, tumor necrosis factor alpha (TNF-A), lactoferrin (Lf), and c-kit in PMN before and after exposure to LPS. Bovine PMN were isolated by differential centrifugation and hypotonic lysis of red blood cells. Cells were exposed to E. coli LPS (10ng/ 1x 10^6cells, 30 minutes, @ 37°C) and unexposed cells were used as control. RNA from control and endotoxin exposed PMN was isolated using TRI-REAGENT® (Sigma). RNA was reverse transcribed to cDNA and gene specific primers were used to amplify COX-1, COX-2, TNF-A, Lf, ubiquitin, 5′-LOX, and c-kit using RT-PCR. Bands observed on a 2% agarose gel were documented. The genes for 5′-LOX, COX-2, ubiquitin, and c-kit were expressed following LPS exposure. The genes for COX-2 and c-kit were expressed in the control samples. There was no expression for genes encoding COX-1, TNF-A, and Lf in bovine PMN. The expression of the gene encoding ubiquitin following LPS exposure may reflect its role in the regulation of the immune responses. Further studies are needed to elucidate the implications for inhibition of TNF-A-mRNA expression. The interplay between these genes and the levels of gene expression may contribute to the resolution of inflammation in response to LPS in bovine PMN.

Key Words: Bovine, PMN, Genes

M5 Use of PCR to amplify RNA in bovine neutrophils. M. Worku* and P. L. Matterson, *North Carolina A&T State University*, *Greensboro*.

Mature neutrophils (PMN) are post-mitotic cells that synthesize lower levels of protein and RNA than do dividing cells. Further, they contain endogenous RNases complicating RNA isolation. Neutrophils are critically important for determining the outcome of acute infections and serve as indicators of inflammation. Bacteria and their products such as endotoxin (LPS) evoke several functional responses in PMN that contribute to innate immunity. With the availability of the draft bovine genome sequence and the development of specific microarrays it is possible to conduct genome level expression studies to decipher key mechanisms of innate immunity. Gene expression studies using microarray analysis require sufficient quantities of good quality RNA. Studies were conducted to isolate sufficient quantities of good quality RNA from bovine blood PMN which was isolated from two cows using TRI-REAGENT®(Sigma). RNA quantity was measured using a spectrophotometer (OD 260), evaluations and concentrations calculated by multiplying OD x dilution factor (200) x 40 (extinction factor). Quality was assessed from the OD 260/280 ratio and using electrophoretic documentation. RNA was amplified by PCR using the cDNA Synthesis System (Roche) and purified using the High Pure RNA Tissue Kit (Roche). A concentration of 0.108 µg/µl RNA was isolated from LPS treated and 0.100 µg/µl from untreated samples. The purity was 1.07 and 1.1 respectively. PCR amplification yielded 0.348µg/µl of RNA from treated and 0.212 µg/µl from untreated samples to conduct microarray experiments. Endotoxin treated amplified samples showed an increased amount of RNA over untreated. Additionally, PCR amplification resulted in a 61% overall increase in the amount of RNA. Amplification of RNA with PCR using poly(A) primers may be a feasible approach to overcome the low concentration of RNA in bovine PMN in order to facilitate studies on gene expression at the transcriptional level.

Key Words: PCR, Bovine, Microarray

M6 In vitro effects of leptin on bovine immune cells. H. Florez-Diaz* and E. B. Kegley, *University of Arkansas*, *Fayetteville*.

Leptin is a 16 kDa protein that regulates feed intake and energy expenditure and has been proposed to act as a link between nutritional status and immune function. However, the role of leptin as a regulator of immune function in cattle has not been studied. Therefore, the objective of this study was to investigate the effect of leptin on in vitro lymphocyte proliferation and macrophage phagocytosis in beef cattle. Blood samples were collected from steers (n = 4) for peripheral blood mononuclear cells isolation. Recombinant ovine leptin was added to cell cultures at concentrations of 0, 1, 5, 10, 50, 100, 500, and 1000 ng/mL for lymphocyte proliferation without (unstimulated) or with mitogens phytohemagglutinin (PHA), pokeweed mitogen (PWM), concanavalin A (ConA), and lipopolysaccharide (LPS), and at concentrations of 0, 1, 10, 100, and 1000 ng/mL for macrophage phagocytosis. To discard endotoxin contamination of leptin, unstimulated and LPS stimulated cell cultures, in the absence or presence of leptin, were cultured for lymphocyte proliferation with or without the addition of polymyxin B sulfate. Linear, quadratic, and cubic effects of leptin were tested by GLM and ORTHOREG procedures of SAS. No evidence of endotoxin contamination was detected ($P \geq 0.06$). Leptin increased (linear effect, $P < 0.0001$) lymphocyte proliferation in PHA stimulated cells and maximum lymphocyte proliferation was obtained at leptin concentrations of 1000 ng/mL. Leptin increased stimulation index in cells cultured with PHA (cubic effect, $P < 0.01$). In ConA stimulated cells leptin increased (cubic effect, $P < 0.05$) stimulation index and maximum proliferation was obtained at leptin concentrations of 500 and 1000 ng/mL. Leptin did not affect ($P \geq 0.06$) lymphocyte proliferation in unstimulated, or PWM and LPS stimulated cells. Macrophage phagocytosis was affected by leptin (cubic effect, ($P < 0.05$). Maximum phagocytosis was obtained at leptin concentrations of 10 ng/mL. These results provide some evidence of the possible role of leptin in the immune response of cattle.

Key Words: Cattle, Leptin, Immune Response

M7 Tumor necrosis factor-α (TNF-α), nitric oxide (NO), and xanthine oxidase (XO) responses to endotoxin (LPS) challenge in heifers: effect of estrous cycle phase. S. Kahl* and T. H. Elsasser, *USDA, Agricultural Research Service*, *Beltsville, MD*.

The severity of host response in some diseases differs between sexes and this dimorphism has been attributed to the immunomodulating effects of steroid hormones. In females, puberty, pregnancy, menopause, and age have been shown to affect the immune response to a disease stress through the prevailing sex steroid milieu. Our objective was to determine in heifers whether the phase of estrous cycle affected the plasma concentration changes of immune response mediators after LPS challenge (2.5 µg/kg BW, i.v., *E. coli* 055:B5). Sixteen beef heifers (426 ± 9 kg) were synchronized to a similar stage of the estrous cycle with the two-injection protocol of dinoprost tromethamine (Lutalyse, Pfizer). Heifers were challenged with LPS 3 d (E, estrus; n = 8) or 10 d (D, diestrus) after the last i.m. injection of Lutalyse. Blood samples were collected at 0, 1, 2, 3, 4, 7, and 24 h after LPS injection. Plasma progesterone (P4) concentrations before LPS challenge (0 h) were 0.3 ± 0.1 and 4.2 ± 0.6 ng/mL in E and D, respectively. In all heifers, plasma TNF-α peaked 2 h after LPS ($P < 0.01$) and returned to basal level by 7 h. With TNF-α concentrations higher ($P < 0.01$) in E than D at the 1, 2, and 3 h samplings, the integrated TNF-α response (area under the time × concentration curve, AUC) was greater in E than in D (27.1 vs. 16.8 ng/mL × h, $P < 0.05$). Plasma concentrations of nitrate+nitrite (NO_x), an estimate of NO production, and XO activity, a mediator of superoxide production, were measured. NO_x increased ($P < 0.01$) in all heifers at 7 and 24 h after LPS; plasma NO_x AUC after LPS was greater in E than D (146 vs. 65 µM × h, $P < 0.01$). Plasma XO responses were also greater in E than D (235 vs. 150 mU/mL × h, $P < 0.05$). Results indicate that the estrous cycle phase is a major source of variability in the magnitude of immune response to bacterial toxins like LPS. The discrimination in responses between cycle phases may reside in the prevailing P4 concentrations at the time of challenge encounter.

Key Words: Endotoxin, Estrous cycle, Tumor necrosis factor-α

M8 Microarray analysis of LPS-induced mastitis in a mouse model. J. Zheng*, A. Watson, and D. Kerr, *University of Vermont*, *Burlington*.

Bovine mastitis is an inflammation of the mammary gland that is usually due to bacterial infection. In order to better understand the acute host response to mas-

titis, we have taken a microarray approach to study the genomic response to an intramammary infusion of LPS in a mouse model. On day 10 of lactation, LPS (1ug/gland) or saline (control) was infused into two glands/mouse (n=3/treatment). Mice were euthanasized 4h post-infusion and glands were recovered for analysis. Expression levels of approximately 23,000 genes were then examined by microarray (Affymetrix genechip; Moe430A). Microarray data was background corrected, normalized, and expression indexing was performed using RMA. Statistical analysis was performed using the R statistics programming environment (www.r-project.org). We found that a total of 299 genes were significantly ($p<0.005$) affected (≥2-fold change) of which 260 were induced and 39 were repressed. Northern blot analysis with phosphoimager quantification of band intensity (pixel intensity) confirmed induction ($P<0.01$) of CXCL1 (64,158 ± 16,063 vs 1,761 ± 52), serum amyloid A3 (74,629 ± 24,258 vs 397 ± 90), CD14 (245,893 ± 58,744 vs 4,354 ± 491), and IL-6 (3,459 ± 483 vs 614 ± 141). The fold changes in expression of these genes obtained by northern blot analysis were 36, 188, 56, and 6, respectively, and were of similar magnitude to those obtained by microarray analysis, 70, 93, 13, and 8, respectively. Overall, the importance of chemotaxis signals in the acute response was indicated by the induction of numerous chemokines including CXCL1, CXCL2, CXCL10, S100A8, and S100A9. Microarray analysis indicated that the fold induction of these genes was 70, 114, 21, 169, and 71 respectively. Immunohistological techniques are being used to determine if the epithelial cells are the source of these acute infection response signals.

Key Words: Innate, Chemotaxis, Chemokine

M9 Temporal response of signal transduction elements during endotoxin (LPS) challenge in cattle liver cells: effects of growth hormone treatment. C. Li*, T. Elsasser, S. Kahl, and D. Carbaugh, *Agricultural Research Service, USDA*, *Beltsville, MD*.

Exogenous GH treatment has been explored as a potential adjunct for management of catabolic processes that further challenge the host response to infection stress. Few definitive studies in cattle, if any, have addressed NO production as affected by GH treatment prior to the onset of immune challenge as well as signal transduction pathways after the challenge. Using Western blot, we examined the protein expression level of inducible nitric oxide synthase (iNOS) and the activities of potential signal transduction pathway elements in cattle liver cells in response to LPS challenge and the modification of these responses by daily treatment with recombinant GH prior to LPS challenge (3.0 µg/kg BW, *i.v*. bolus, *E. coli* 055:B5). Animals (n = 24) were divided into GH- and non-GH-treatment groups (n = 12/group, GH-treated: recombinant bovine GH, Monsanto Inc., St. Louis, MO; 0.1 mg/kg BW, i.m., daily for 12 d) in a factorial arrangement of GH treatment (+/-) and biopsy sampling time. In responses to LPS challenge, the protein level of iNOS increased significantly ($P < 0.001$) after 3 h and remained higher until 24 h. In GH treaded animals, the level of iNOS protein increased at 0, 3, and 6 h and was significantly higher than that in non-GH treated animals ($P < 0.001$). GH treatment stimulated the phosphorylation of Akt/Protein kinase B (PKB). Family of mitogen-activated protein kinases (MAPK), Erk, SAPK/JUK and p38 showed different patterns of response. While the temporal profile of phospho-Erk only responded to LPS in GH-treated animals ($P < 0.001$), phospho-SAPK/JUN responded to LPS in both GH-treated and non-GH-treated animals with significant higher level of phosphorylation ($P < 0.001$) in GH treated group. P38 showed no temporal response to LPS in both groups. These data support the notion that GH is able to activate signaling pathways similar to those used by cytokines and protect or enhance immune cell's function during stress conditions.

Key Words: GH, LPS, Signal transduction

M10 The effects of anti-inflammatory agents on gene expression of bovine neutrophils. N. Cunningham, M. Worku*, and P. Matterson, *North Carolina A&T State University*, *Greensboro*.

The objective of this study was to evaluate the effect of commonly used NSAIDS on COX-2 gene expression in resting and LPS (E. coli) stimulated bovine neutrophils (PMN). Blood PMN were isolated by differential centrifugation and red blood cells lysis. Cell viability was determined by Trypan blue dye exclusion, purity by differential cell counts and concentration by hemacytometer. Isolated PMN ($5x10^6$) were incubated in the presence or absence of $10ng/1x10^6$ cells LPS or NSAIDS (Naproxen Sodium, Flunixin Meglumine, Ibuprofen, Acetametophen, Dexamethosone, Sodium Salicylate, Nordihydroguaiaretic acid, Indomethosone, and NS-398 (30 minutes at 37°C, 5% CO2, and 88% humidity). Supernatants were collected and assayed for PGE_2 using a PGE_2 enzyme immunoassay kit (Cayman). COX-2 expression in cells was assessed using a simple dot blot assay. Bovine PMN express COX-2 following isolation and on exposure to LPS. This expression was inhibited by the presence of NS-398. With the exception of Indomethasone, all NSAIDS tested inhibited PGE_2 production. Initial studies indicate that common NSAIDS inhibit PGE_2 production. NS-398 has been reported to temporarily block the attachment site for arachidonic acid on the cyclooxygenase enzyme, preventing it from converting arachidonic acid to prostaglandin. On bovine PMN the expression of COX-2 at the protein level may also be inhibited by NS-398.

Key Words: COX-2, Bovine, NSAIDS

M11 Microarray analysis of immunorelevant gene expression in LPS-challenged bovine mammary epithelial cells. R. S. Pareek*[1], O. Wellnitz[2], J. Burton[3], and D. Kerr[1], [1]*University of Vermont*, *Burlington*, [2]*Technical University of Munich*, *Munich, Germany*, [3]*Michigan State University*, *East Lansing*.

The mammary epithelial cell plays a role in the host response to bacterial invasion of the gland by alerting the immune system of the presence and location of the infection. To better understand this process a cDNA microarray approach was used to search for potential signals produced by mammary epithelial cells in response to exposure to Escherichia coli LPS. Total RNA from separate cultures of epithelial cells from four Holstein cows was harvested 6 h after LPS challenge or control conditions. For each cow, RNA from control or LPS exposed cells was transcribed to cDNA, then labelled with Cy3 or Cy5, and then equal quantities applied to a bovine total leukocyte (BOTL) microarray slide containing 1278 unique transcripts. Dye reversal was used so that RNA from two of the control cultures was labelled with Cy3 while RNA from the other two control cultures was labelled with Cy5. From the resulting microarray data we selected four of the eleven genes significantly ($p<0.02$) induced (>1.2 fold) in response to LPS exposure for more detailed analysis. Two genes, RANTES/CCL5 and T-PA, were validated by quantitative real-time RT-PCR (Q-RT-PCR) analysis and revealed that they were induced by 208-fold and 3-fold, respectively. Two other genes, IL-6 and CXCL5, were detected by northern blot analysis that indicated inductions of 9-fold and 10-fold, respectively. This model system provides evidence for an important role of the mammary epithelial cell in the acute phase of the innate response to infection.

Key Words: Bovine total leukocyte (BOTL) microarray, Lipopolysaccharide (LPS), Mammary gland

M12 Parenteral administration of glutamine modulates acute phase response in postparturient dairy cows. A. Jafari*[2,1], D. Emmanuel[1], J. Bell[1], R. Christopherson[1], G. Murdoch[1], J. Woodward[1], C. Field[1], and B. Ametaj[1], [1]*University of Alberta*, *Edmonton, Alberta, Canada*, [2]*Isfahan University of Technology*, *Isfahan, Iran*.

The transition period is characterized by immune suppression and increased incidence of infectious diseases in dairy cows. Glutamine, the most abundant free amino acid in circulation that plays a key role in immune cell division and proliferation, has been shown to decrease (25%) immediately after parturition. The goal of this study was to investigate effects of parenteral administration of glutamine on mediators of acute phase response in transition dairy cows. Three groups (n = 8/group) of multiparous Holstein cows were randomly assigned to the following 3 treatments: intravenous infusion of 10 L of 0.85% NaCl (control), or intravenous infusion of 106 or 212 g/d of L-glutamine mixed with 10 L of 0.85% NaCl for 8 h for 7 consecutive days starting on the day of parturition.

Blood samples were drawn from the jugular vein 1-2 wk before the expected day of parturition as well as on days 0, 7, 14, and 21 after parturition and plasma concentrations of serum amyloid A (SAA) and haptoglobin (Hp) were measured by ELISA. Concentrations of SAA and Hp in plasma of control cows increased on the day of parturition as well as at 7 days postpartum and declined thereafter. Cows infused with 212 g/d of L-glutamine had greater concentrations of SAA and lower concentrations of Hp on days 7 ($P<0.05$) and 21 ($P<0.05$) postpartum compared to controls. Cows infused with 106 g/d of L-glutamine also had greater concentrations of SAA in plasma on day 21 ($P<0.05$) postpartum compared to controls. In conclusion our data indicate that parenteral administration of glutamine modulates acute phase response in dairy cows immediately after parturition. Further research is warranted to understand the mechanism by which glutamine affects immune response in transition dairy cows.

Key Words: Glutamine, Acute Phase Response, Dairy Cows

M13 Evaluation of two simple tests for the detection of cryptosporidium parvum oocysts in calf feces. L. Trotz-Williams[1], S. Martin[1], D. Martin[2], T. Duffield[1], K. Leslie*[1], D. Nydam[3], and A. Peregrine[4], [1]*University of Guelph, Guelph, ON, Canada*, [2]*Ontario Ministry of Health and Long-Term Care, Etobicoke, ON, Canada*, [3]*Cornell University, Ithaca, NY*, [4]*University of Guelph, Guelph, ON, Canada*.

A sucrose wet mount test developed at the Ontario Veterinary College (OVC) for the detection of Cryptosporidium parvum oocysts in calf feces, and a lateral immunochromatography test (BioX Diagnostics, Jemelle, Belgium) for C. parvum in feces, were evaluated in terms of epidemiological sensitivity and specificity, cost, and utility. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) targeting the Cryptosporidium oocyst wall protein (COWP) gene locus was used as the gold standard for evaluation of the tests.

One hundred and ninety-nine fecal samples from naturally infected Holstein calves under 21 days old were used for the study. All samples were analyzed in a blinded manner. Cohen's kappa statistic of agreement (κ) between the OVC sucrose wet mount test and COWP PCR-RFLP was 0.82, showing good agreement, and the sensitivity and specificity of the OVC sucrose wet mount test were 88.6% and 93.8%, respectively. The sensitivity and specificity of the lateral immunochromatography test were 78.3% and 93.3% respectively, and agreement between this test and PCR-RFLP was also good (κ= 0.73). There was also substantial agreement between the OVC sucrose wet mount test and the lateral immunochromatography test (κ = 0.84). Both tests were inexpensive and easy to use. However, the lateral immunochromatography test was faster and simpler to perform than the sucrose wet mount test, and was generally more user-friendly. Both of these tests provide practitioners and researchers with cheap, quick and accurate methods of detecting C. parvum infection in young calves.

Acknowledgements: Thanks to Grazyna Adamska-Jarecka for diagnostic support, and to Dr. Frances Jamieson and Billy Yu, Laboratories Branch of the Ontario Ministry of Health and Long-Term Care, for collaborative assistance with this research. The lateral immunochromatography test kits used for this project were kindly supplied by BioX Diagnostics.

This study was funded by the Ontario Ministry of Agriculture and Food, the National Sciences and Engineering Research Council of Canada, Dairy Farmers of Ontario and Dairy Farmers of Canada.

Key Words: Cryptosporidium parvum, Diagnostic tests, Calves

Breeding and Genetics I

M14 Estimatives of heritability to time in different distances of race in Quarter Horse. S. Oliveira, M. Correa, and M. Mota*, *Unesp*, *Botucatu, SP, Brazil*.

The Quarter Horse are known by their versatility, they can be used for conformation, work and race modalities. This way, this work objectived to estimate the heritabilities to race time in distances of 301m (2,770 observations), 320m (2,039), 365m (3,739) and 402m (5,366), in Quarter horse races. The components of variance needed to the obtation of the heritabilities were estimated by MTGSAM program, under animal model. The Gibbs sampling considered 2,005,000 samples, excluding the first 5,000 and after these, one in each 1,000 samples were stored for inference, totalizing 2,000 samples for studying. The linear model used to information analise included the random effects of animal and permanent environment and the fixed effects of age (2, 3 and 4 years-old or more), sex (male and female) and race. The estimatives of heritability were, in average, 0.26, 0.15, 0.40 and 0.35, respectively to the distances of 301, 320, 365 and 402m. The biggest density intermission "a posteriori", with 90% of probability, following the previous presentation order were 0.25 to 0.34, 0.09 to 0.33, 0.33 to 0.58 and 0.34 to 0.38. The results indicate that longer distances present bigger heritability and, in consequence, they shall possible good replies to the masal selection.

Key Words: Heritability, Quarter horse, Race

M15 Estimatives of repeatibility to time in different distances of race in Quarter horse. M. Correa, S. Oliveira, and M. Mota*, *Unesp*, *Botucatu, SP, Brazil*.

The Quarter horse are admired by their versatility, they can be used for conformation, work and race modalities. Based on this, this work objected to estimate the repeatibilities to race time in distances of 301m (2,770 observations), 320m (2,039), 365m (3,739) and 402m (5,366) in Quarter horse races. The components of variance needed to obtain the repeatibilities were estimated by MTGSAM program, under the animal model. The Gibbs sampling considered 2,005,000 samples, excluding the first 5,000 and after them, one in each 1,000 samples were stored for inference, totalizing 2,000 samples for studying. The linear model used to information analise included the random effects of animal and permanent environment and the fixed effects of age (2, 3 and 4 years-old or more), sex (male and female) and race. The setimatives of repeatibility were, for 301m, in average, 0.36, with 90% of probability of the information appear between 0.29 and 0.36, for 320m, 0.27, with the informatinos between 0.19 and 0.45, for 365m, 0.48, between 0.43 and 0.61, and for 402m, 0.68, between 0.65 and 0.68. The estimated results suggest that for longer distances, just a result of performance can be enough to rule out the animal, while for shorter distances, more than one result of performance shall be done.

Key Words: Repeatibility, Quarter horse, Race

M16 Simulation model of cashmere goat production system: I. A dynamic herd simulation model & breeding strategies for fiber quality. B. Tseveenjav*[1,2], D. J. Garrick[1], S. LeValley[1], and Z. Yondon[2], [1]*Colorado State University, Fort Collins*, [2]*Cashmere Goat Association of Mongolia, Ulaanbaatar, Mongolia.*

Fiber diameter is the most important factor determining the value per unit weight of cashmere fleece. The need to consider fiber diameter in selection programs in addition to cashmere fleece weight has increased in the last decade, because fiber diameter in many Cashmere populations has deteriorated as a result of intensive selection on fleece weight. The objective of this study was to quantify the superiority of an economic selection index applied to simultaneously decrease fiber diameter and increase cashmere fleece weight in indigenous Cashmere goats. Throughout computer modeling the effects of different breeding