

**131 Studies to identify factors involved in the adherence of *Staphylococcus aureus* to bovine mammary epithelial cells.** M. Worku and A. J. Guidry\*, *Immunology & Disease Resistance Laboratory LPSI, ARS, United States Department of Agriculture.*

*Staphylococcus aureus* is a major cause of the \$2 billion loss due to mastitis in dairy cattle. *Staphylococcus aureus* have the ability to adhere and penetrate mammary tissue and form deep seated abscesses which cause chronic cases of mastitis that necessitate culling the cow. Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) on the surface of the *S. aureus* mediate the adherence to mammary tissue. *S. aureus* strain M60 was evaluated for its ability to adhere to collagen or fibronectin coated dishes. Recombinant collagen and fibronectin binding MSCRAMMs, an anti-MSCRAMM antibody and mutant strains of *S. aureus* that expressed or failed to express the fibronectin and/or collagen binding MSCRAMMs were also used to evaluate adherence of *S. aureus* to bovine mammary epithelial cell monolayers. *Staphylococcus aureus* strain M60 bound significantly less to collagen than to fibronectin ( $P < 0.05$ ). Collagen binding MSCRAMMs, had no significant effect on adherence ( $P < 0.05$ ). Fibronectin binding MSCRAMMs significantly blocked adherence of *S. aureus* ( $P < 0.01$ ). Anti-MSCRAMM antibody blocked adherence ( $P < 0.05$ ). Normal rabbit gamma globulin had no effect on adherence ( $P < 0.05$ ). Deletion of the gene for the fibronectin binding MSCRAMM significantly reduced adherence of *S. aureus* ( $P < 0.05$ ). Deletion of the gene for the collagen binding MSCRAMM had no significant effect ( $P < 0.05$ ). The fibronectin MSCRAMM appeared to play an important role in adherence of *S. aureus*. These data suggest that recombinant fibronectin binding MSCRAMMs and rabbit anti-MSCRAMM antibody have promise as a means of preventing *S. aureus* adherence. These data suggest that immunization of cows with fibronectin binding MSCRAMMs could aid in the prevention of *S. aureus* mastitis in cattle

**Key Words:** Adherence, *Staphylococcus Aureus*, Mastitis

**132 Protective effects against adhesion of enterotoxigenic *Escherichia coli* to immobilised intestinal mucosal preparations by egg-yolk antibodies.** L. Z. Jin\*, R. R. Marquardt, S. K. Baidoo, and A. A. Frohlich, *University of Manitoba, Winnipeg, Canada.*

Chicken egg yolk antibodies against enterotoxigenic *Escherichia coli* infection in piglets have been successfully developed recently. Few studies have directed attention to interference with the adhesion of the pathogen to host epithelial mucosa. The objective of the study was to determine if the adhesion of *E. coli* K88 to piglet intestinal mucus could be inhibited *in vitro* by spray-dried egg-yolk anti-K88 antibodies. Four 14±2-day-old healthy piglets were used for the preparation of mucus from the small intestine. Competition and displacement phenomena were investigated by incubating (a) egg-yolk antibodies and *E. coli* together prior to adding to the mucus and (b) *E. coli* and mucus, followed by egg-yolk antibodies. The results demonstrated that egg-yolk antibodies inhibited ( $P < 0.001$ ) adhesion of [<sup>3</sup>H]-labeled *E. coli* K88 to piglet small intestinal mucus by 85-97% when the egg-yolk antibodies were diluted 10, 20, 40 or 100 times. The adhesion inhibiting effects of egg-yolk antibodies declined ( $P < 0.001$ ) dramatically when the antibody dilution was more than 250 fold. A similar adhesion inhibiting effect was observed when egg-yolk antibodies were incubated with *E. coli* K88 for 15, 30 and 60 min prior to the adhesion test. Egg-yolk antibodies when diluted 50 and 100 fold had a very strong inhibiting ability against *E. coli* K88 at a concentration of 10<sup>9</sup> colony forming units (CFU)/ml (adhesion was < 6%). However, dilution of 100 times for egg yolk antibodies was insufficient to inhibit the adhesion of *E. coli* to intestinal mucus when the concentration of *E. coli* K88 was 10<sup>10</sup> cfu/ml. The displacement test indicated that there was no significant ( $P > 0.05$ ) reduction in the adhesion of *E. coli* K88 to the small intestinal mucus when egg-yolk antibodies were added after adhesion of the organism to the mucus. It is concluded that chicken egg yolk antibodies can inhibit but not displace the adhesion of *E. coli* K88 to the small intestinal mucus of piglets.

**Key Words:** Egg yolk antibodies, intestinal receptors, *Escherichia coli*

**133 Effects of bovine lactoferrin in combination with usual antibiotics on *Staphylococcus aureus*.** M. S. Diarra\* and P. Lacasse, *Dairy and Swine R&D Centre, Lennoxville, Quebec Canada.*

Lactoferrin (LF) is a glycoprotein naturally found in milk which appears to have antibacterial and anti-inflammatory activities. The objective of the present study is to evaluate the therapeutic potential of bovine apo-LF in combination with traditional antibiotic against reference strain of *Staphylococcus aureus* (SA) ATTC 25923 and SA strains SHY97-3906 and SHY97-4320 isolated from clinical bovine mastitis cases. Minimal inhibitory concentrations (MICs) of LF, Penicillin G (PG), Novobiocin (NB), erythromycin (ER), PG + NB, LF + PG, LF + NB and LF + ER were determined by microdilution broth technique in two duplicate separates experiences for the 3 above mentioned strains of SA. All strains were relatively more sensitive to usual antibiotic alone with MICs ranging from 0.023 to 0.125 µg/ml except strain SHY97-4320 which was highly resistant to PG (MIC = 32 µg/ml). LF demonstrated some inhibitory activity against SA strains with MICs ranging from 9.275 mg/ml for ATTC strain to greater than 12.5 mg/ml for the two clinical strains. LF increased the inhibitory activity by two-fold for PG whereas activity of NB and ER were not affected. PG, NB and ER strongly reduce the MIC of LF in varying degrees (20 to 1000-fold). Postantibiotic effect (PAE), is the suppression of bacterial regrowth that persist after a short exposure of microorganisms to an antibiotic. PAE was studied in two separates experiences, by exposure of bacteria to PG and LF + PG at 0, 1 and 5 times MIC. After 2-h of incubation cells were washed and incubated with constant agitation and the regrowth were monitored by standard count of cfu/ml. Duration of PAE increased with rising concentration for tested drugs. PG showed longer PAEs than PG + LF. The PAE of PG ranged from 1.05 ± 0.05 to 1.95 ± 0.15-h while PG + LF showed PAEs of 0.85 ± 0.05 to 0.9 ± 0.1-h on strain ATTC 25923. These data established a potentiation effect of LF with certain antibiotics against SA.

**Key Words:** Bovine Lactoferrin, *Staphylococcus Aureus*, Antibiotic Activities

**134 The Effect of Supernatant from Staphylococcal Enterotoxin-C Treated Lymphocytes on the Bactericidal Ability of Bovine Blood Neutrophils.** T. L. Ebling and L. K. Fox\*, *Washington State University, Pullman.*

The purpose of this experiment was to determine the effects of culture supernatant, derived from lymphocytes treated with staphylococcal enterotoxin-C, on the bactericidal ability of peripheral bovine neutrophils. *Staphylococcus aureus*, Newbould 305 (ATCC 29740), was grown in skim milk broth, rinsed and diluted in HBSS, plated on blood agar plates, cultured overnight, and counted to calculate CFU/ml. Bovine neutrophils were isolated from peripheral blood and incubated with different media for 1 h. The media types were antibiotic, antibiotic with supernatant, and control (without antibiotics or supernatant). After incubation the neutrophils were both rinsed and resuspended in control media or simply resuspended in the incubation media. Thus, we compared neutrophil bactericidal activity following 6 treatment regimens: T1, antibiotic media with supernatant and neutrophils rinsed after incubation; T2, antibiotic media with supernatant and neutrophils not rinsed after incubation; T3, antibiotic media with supernatant only; T4, antibiotic media and neutrophils rinsed after incubation; T5, antibiotic media only; and T6, control media and neutrophils not rinsed after incubation. Percentage *Staph. aureus* killed were: T1, 33.9%; T2, 63.4%; T3, 66.5%; T4, 33.0%; T5, 67.8%; and T6, 37.5%. Results indicated that supernatant from lymphocytes treated with staphylococcal enterotoxin-C did not affect the bactericidal ability of bovine blood neutrophils. Additionally, exposure to antibiotic media did not impair neutrophil function.

**Key Words:** Neutrophils, Staphylococcal enterotoxin, Killing

**135 Antibacterial Activity of Interleukin-2 Stimulated Bovine Lymphoid Cells is Mediated by the Release of a Soluble Factor.** K. A. Shafer-Weaver\*, Y. Z. Cao, and L. M. Sordillo, *The Pennsylvania State University, University Park.*

Host defense against bacterial pathogens requires the participation of various leukocyte subpopulations and humoral factors. Recently, much attention has been focused on the role of natural killer (NK) cells in the outcome of bacterial infections. NK cells are very heterogeneous, widely dispersed within the host, and their activity does not depend on prior sensitization or recognition of specific antigen. As such, NK cells provide essential host defenses against bacterial pathogens at the initial stage of bacterial invasion. Upon stimulation with interleukin-2 (IL2), NK cells have been shown to mediate nonspecific antibacterial activity that differs from both ADCC and phagocytosis. We have seen that isolated bovine lymphoid cells exhibit IL2 mediated antibacterial activity against *S. aureus* and that the specific subpopulation responsible for this activity are NK cells. Further research demonstrates that these cells mediate their antibacterial activity by the release of a soluble factor. Thus far, we have shown that the soluble factor is heat labile, inactivated by trypsin treatment, and precipitates at 80% ammonium sulfate suggesting it is protein in nature. Molecular weight fractionation demonstrated that our factor is between 50 and 10 kD. Purification of the factor over a DEAE anion exchange column has yielded one fraction that exhibits antibacterial activity against *S. aureus*. Evaluation of this fraction using SDS-PAGE has identified two candidate proteins that could be mediating the lymphoid-antibacterial activity. Currently, the identification and function of these two proteins are being studied. Understanding the antibacterial protein's mode of action should provide new information to develop treatments to augment this important host defense.

**Key Words:** Natural Killer Cells, Antibacterial

**136 Relationship between lysozyme, NAGase and cells isolated from blood and milk after calving.** A. Zecconi\*, V. Bronzo, P. Moroni, A. Casula, C. Luzzago, and R. Piccinini, *Università di Milano, Italy.*

Lysozyme is an enzyme which is released during phagocytosis, while NAGase activity has been related both to milk SCC and to the activity of PMNs, even if the enzyme has been shown to be mainly stored in macrophages. Therefore their assessment could be helpful to evaluate cellular immunological status both in milk and blood. To evaluate the relationship between these two enzymes and blood and milk cells and, on the other hand, the dynamic of the enzymes during the first month after calving, 40 cows randomly chosen from 4 commercial dairy herds were considered. Samples were taken weekly starting the day next to parturition for one month. Lysozyme was assessed spectrophotometrically by the lysis of *Micrococcus lysodeicticus*, NAGase was assessed fluorometrically, while differential cell count was assessed by esterase stain. Statistical analysis was performed by SAS GLM procedure. Serum lysozyme levels among herds varied between 6.54  $\mu\text{g/ml}$  and 18.50  $\mu\text{g/ml}$ . Whey lysozyme levels among herds varied between 87.30  $\mu\text{g/ml}$  and 129.10  $\mu\text{g/ml}$ . The range of NAGase observed in blood was between 59.16 and 81.51  $\mu\text{moles/ml}$ , while the range observed in milk was between 22.85 and 38.63  $\mu\text{moles/ml}$ . The statistical analysis showed that only herd had a significant effect on serum lysozyme variance, while sampling and herd had a significant effect on whey lysozyme variance. Sampling had a significant effect on serum NAGase activity variance, while sampling, herd and PMN ratio had a significant effect on whey NAGase variance. The results of this study showed that factors other than cells (i.e. herd, days in milk) could influence both milk NAGase and lysozyme. Particularly, herd differences suggest that factors such as genetics or nutrition could influence the level of these immune parameters.

**Key Words:** Immunity, Milk, Cellular Enzymes

**137 Nitric oxide production during endotoxin-induced mastitis in the cow.** S. Blais<sup>1</sup>, L. Bouchard<sup>1\*</sup>, X. Zhao<sup>2</sup>, and P. Lacasse<sup>1</sup>, <sup>1</sup>AAFC, Dairy and Swine R&D Centre, Lennoxville, Canada <sup>2</sup>Department of Animal Sciences, McGill University, St-Anne de Bellevue, Canada.

The purpose of this work was to evaluate nitric oxide production during the endotoxin-induced inflammatory response. One hour after morning milking, we infused the right hind quarter of 15 cows with 10ml of saline containing 10 $\mu\text{g}$  of *E. coli* purified lipopolysaccharide (LPS, 055:B5). The left quarter was infused with 10ml of saline and used as control. We recorded signs of inflammation, rectal temperature, somatic cell counts, serum albumin, and NAGase activity in milk as diagnostic markers of mastitis. Nitric oxide production was evaluated by measuring nitrite/nitrate concentration (stable metabolites of nitric oxide) in the milk. In LPS-infused quarters, an important increase in nitrite/nitrate concentration was observed, peaking 3 hours post-infusion (p.i.) (24.4 vs 12.9 $\mu\text{M}$ ,  $P < 0.001$ ). Concentrations then gradually decreased back to pre-infusion level 48 h p.i. (6.9 $\mu\text{M}$ ). These variations coincided with the variations observed with other mastitis markers used in this system. In control quarters, a small increase in nitrite/nitrate level was seen 3 h p.i., but returned rapidly to pre-treatment level 6 h p.i. (12.9 vs 8.6 $\mu\text{M}$ ). At three different time points we harvested somatic cells from the milk of 7 cows. Cells were plated and maintained in DMEM-F12 media for 24 h. Nitric oxide production was evaluated by measuring nitrite/nitrate concentrations in DMEM-F12 media. Concentrations were the same in medium from both right and left quarters one hour prior to infusion as well as 96 h p.i. However, in cells harvested 12 h p.i., media content of nitrite/nitrate was increased (20.7 vs 8.2 $\mu\text{M}$ ,  $P < 0.05$ ) by LPS infusion. The presence of a specific inhibitor of nitric oxide synthase (L-NIL, 200mM) reduced the effect of LPS infusion in these cells. Our results indicate that important amounts of nitric oxide are released during mammary inflammation driven by mastitis.

**Key Words:** mastitis, nitric oxide, endotoxin

**138 Induction of arginase does not diminish the capacity of bovine or murine macrophages to synthesize nitric oxide.** J. M. Fligger\*<sup>1</sup>, J. W. Blum<sup>2</sup>, and T. W. Jungi<sup>2</sup>, <sup>1</sup>The Pennsylvania State University, University Park, <sup>2</sup>The University of Berne, Berne, Switzerland.

In macrophages ( $M\phi$ ), two inducible enzymes use L-arginine (L-arg) as a substrate. Inducible nitric oxide synthase (iNOS) cleaves L-arg to produce nitric oxide (NO)+citrulline; arginase cleaves L-arg yielding urea+ornithine. Based upon experiments with murine bone marrow-derived  $M\phi$  (muBMM), it is generally accepted that interferon- $\gamma$  (IFN- $\gamma$ ) and bacterial lipopolysaccharide (LPS) induce iNOS activity in  $M\phi$ , whereas interleukin-4 (IL-4) induces arginase. In this study, the hypothesis that arginase regulates NO synthesis by competing with iNOS for substrate was tested. Given the potential for species variability in arginase regulation, we tested both muBMM and bovine monocyte-derived  $M\phi$  (boMDM). As expected, arginase activity in muBMM was increased ( $P < .10$ ) 2-fold by treatments containing IL-4. In contrast, the arginase activity of boMDM was increased 85% ( $P < .10$ ) only by treatments containing LPS. In muBMM cultures, both IFN- $\gamma$  and IL-4+IFN- $\gamma$  treatments resulted in a similar level of NO production despite the fact that the arginase content of cells receiving these two treatments differed ( $P < .01$ ) 3-fold. Although LPS was the major up-regulator of arginase in boMDM, this  $M\phi$  type also released maximal quantities of NO in response to treatments containing LPS with the exception of the IL-4+LPS treatment. In this case, the down-regulation of NO synthesis by IL-4 could not have been due to induction of arginase since IL-4 did not up-regulate arginase in boMDM. Regardless of the  $M\phi$  type studied, there was no consistent inverse relationship between intracellular arginase activity and NO synthesis, even in experiments where the L-arg concentration of the culture medium was reduced to physiological levels. In conclusion: (i) Arginase activity in  $M\phi$  is not universally up-regulated by IL-4; (ii) NO synthesis by  $M\phi$  is highly correlated ( $r^2 > .75$ ) with iNOS expression; (iii) Arginase does not compete with iNOS for substrate over a wide range of extracellular L-arg concentrations.

**Key Words:** Macrophage, Nitric Oxide, Arginase

**139 Effects of dietary vitamins A and E on nitric oxide (NO) production by mononuclear leukocytes from calves fed milk replacer.** V. Rajaraman<sup>1</sup>, B. J. Nonnecke<sup>1\*</sup>, S. T. Franklin<sup>2</sup>, D. C. Hammell<sup>2</sup>, and R. L. Horst<sup>1</sup>, <sup>1</sup>USDA, ARS, National Animal Disease Center, Ames, IA and <sup>2</sup>South Dakota State University, Brookings.

Production of NO by phagocytic leukocytes is required for microbial killing and signal transduction; however, excessive production is cytotoxic. Because the immune system of the neonate is generally hyporesponsive relative to that of the adult, and fat-soluble vitamins are known modulators of immune function, this study evaluated the capacity of blood mononuclear leukocytes from calves to produce NO and the effects of dietary vitamins A and E on NO production. Milk-replacer fed calves, 0 through 5 wks of age, received orally 100 IU/d of vitamin E as d- $\alpha$ -tocopherol or d- $\alpha$ -tocopheryl acetate and 0, 1,700 (NRC daily requirement), 34,000 or 68,000 IU/day of vitamin A as retinyl acetate. Cells from 1 wk-old calves produced less ( $P=.02$ ) NO than cells from older calves, a possible consequence of suppressive factors present in the circulation at birth or acquired through ingestion of colostrum (1L) fed within 6 h after birth. In general, calf leukocytes produced greater ( $P=.0001$ ) amounts NO than adult leukocytes, which may be characteristic of the neonatal immune system. Cells from calves fed d- $\alpha$ -tocopherol produced less ( $P=.03$ ) NO than those from calves fed d- $\alpha$ -tocopheryl acetate, regardless of level of vitamin A supplementation. Nitric oxide production by cells from calves fed d- $\alpha$ -tocopherol with 1,700 or 34,000 IU/day of vitamin A was less ( $P<.05$ ) than NO production by cells from calves fed 0 or 68,000 IU/d of vitamin A and was not different ( $P>.05$ ) from amounts produced by adult cells. In conclusion, dietary vitamin A and vitamin E altered the capacity of calf mononuclear leukocytes to produce NO and suggests that these vitamins influence maturation of this aspect of the neonatal immune system.

**Key Words:** Calf, Vitamin A, Nitric oxide

**140 Influence of the mammary gland on interferon- $\gamma$  (IFN- $\gamma$ ) and immunoglobulin M (IgM) secretion by blood mononuclear leukocytes from periparturient dairy cows.** B. J. Nonnecke<sup>1\*</sup>, K. Kimura<sup>2</sup>, and J. P. Goff<sup>1</sup>, <sup>1</sup>USDA, ARS, National Animal Disease Center, Ames, IA, <sup>2</sup>Iowa State University, Ames.

We have shown that compositional changes in circulating T cell populations and reduced functional capacity of circulating neutrophils from periparturient dairy cows are, in part, due to metabolic demands imposed by the presence of the mammary gland. To further characterize effects of the mammary gland on the immune cell function during the periparturient period, we evaluated, *in vitro*, the capacity pokeweed mitogen (PWM)-stimulated peripheral blood mononuclear leukocyte (PBML) from intact ( $n=6$ ) and mastectomized ( $n=6$ ) Jersey cows to secrete IFN- $\gamma$  and polyclonal IgM. Data were summarized and analyzed so that these functions could be examined within 5d periods beginning at 15d prepartum and concluding at 14d postpartum. The capacity of PBML from intact cows to secrete IFN- $\gamma$  and IgM changed significantly ( $P=.0002$  and  $P=.0007$ , respectively) during the periparturient period. For this group, the nadir of IFN- $\gamma$  and IgM secretion occurred at 0 to 4d postpartum and -5 to -1d prepartum, respectively. Although PBML from mastectomized cows showed a similar but less severe decline in function with approaching parturition and subsequent restoration of function approximately 5d to 14d after parturition, effects of parturition on PBML from mastectomized cows were not significant ( $P>.05$ ). Differences between IFN- $\gamma$  secretion by PBML from intact and mastectomized animals were significant only from birth to 4d postpartum. (for PWM at  $.2\mu\text{g/ml}$ ,  $P<.001$ ; PWM at  $2\mu\text{g/ml}$ ,  $P<.01$ ; and PWM at  $20\mu\text{g/ml}$ ,  $P<.05$ ). Similarly, differences between IgM secretion by intact and mastectomized cows were significant only from -5 to -1d prepartum (for PWM at  $.12\mu\text{g/ml}$ ,  $P<.05$ ). In both cases, PBML from intact cows were less ( $P<.05$ ) responsive than those from mastectomized cows. These results indicate that parturition causes a pronounced reduction in the functional capacity of bovine PBML and suggest that the metabolic demands placed on the periparturient dairy cow by the mammary gland contribute to the reduction in function.

**Key Words:** Parturition, Immunosuppression, Interferon

**141 Elucidation of bovine CD4+ lymphocytes role in postpartum associated immunoregulation.** C. M. Corl<sup>\*</sup>, K. A. Shafer-Weaver, and L. M. Sordillo, *The Pennsylvania State University, University Park.*

Dairy cows are more susceptible to mastitis during certain times of the lactational cycle including the postpartum period. Increased incidence of mastitis during the postpartum period has been correlated to diminished host immune responses. We hypothesize that one underlying mechanism of diminished immune functions is due to changes in T-lymphocyte subpopulations. Data from our laboratory has already demonstrated that CD8+ suppressor cells are more predominant during the postpartum compared to the mid-late lactating period. The objective of this study was to determine if CD4+ subpopulation also vary with respect to lactational stage. More specifically, we evaluated if these subpopulation are mainly T<sub>H</sub>-1 or T<sub>H</sub>-2 during the postpartum period based on cytokine profile expression. Peripheral blood mononuclear cells were isolated from dairy cattle during the postpartum or mid-late lactation periods. Cells were magnetically separated into purified (CD4+) or depleted (CD4-) populations using monoclonal antibodies specific for bovine leukocyte antigens and the VarioMacs cell separation system. Cultures were confirmed to be enriched (CD4+) or depleted (CD4-) by flow cytometric analysis. Cultures also were evaluated by competitive quantitative RT-PCR for interleukin (IL)-4, IL-10, IL-12 and interferon (IFN)- $\gamma$  mRNA. The cytokine profile of the isolated cell cultures will be compared with respect to stage of lactation. Studies are in progress to define CD4+ lymphocytes as predominantly T<sub>H</sub>-1 or T<sub>H</sub>-2 in order to elucidate their role in postpartum associated immunoregulation.

**Key Words:** T-lymphocytes, Cytokines, Immunoregulation

**142 Detection of protein tyrosine phosphorylation and L-selectin expression on bovine neutrophils around parturition.** E. Monfardini<sup>1</sup>, M. J. Paape<sup>\*2</sup>, Y. Wang<sup>2</sup>, M. Worku<sup>2</sup>, D. L. Wood<sup>2</sup>, and C. Burvenich<sup>1</sup>, <sup>1</sup>University of Ghent, Belgium, <sup>2</sup>Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD.

Reduced neutrophil (PMN) chemotaxis occurs during the periparturient period. Rolling, attachment, and chemotaxis of PMN are mediated by adhesion receptors such as L-selectin and integrins. Protein tyrosine phosphorylation (PTP) is important in regulation of integrin mediated cell adhesion. Binding of ligands to L-selectin and cellular stress regulate expression of mitogen activated protein kinase (MAP-K). The objectives of this study were to assess changes in PTP and expression of L-selectin using a monoclonal antibody (MAB) to bovine L-selectin. Blood PMN were isolated ( $n = 8$  cows) on d -14, -7, 0 (calving), +1, +2, +7, +14, and +28. Protein tyrosine phosphorylation of PMN lysates was detected by electrophoresis and Western blotting using anti-phosphotyrosine MAB. Several phosphorylated proteins were detected. Two bands (42-43 and 90 kD) varied in intensity over time. The intensity of the 42-43 kD band gradually increased from d -7, peaked at d +7 ( $P<0.05$ ) and steadily decreased to d +28 ( $P<0.05$ ). Antibodies to MAP-K reacted with the 42-43 kD band. Studies are underway to identify the 90 kD band which is similar in molecular weight to bovine L-selectin. Binding of MAB to L-selectin and evaluation of receptor expression was carried out on C5a activated and resting PMN. Flow cytometric analysis was used to measure % PMN fluorescing and receptor expression (log mean fluorescence channel, LMFC). Both groups showed similar trends in % fluorescence and LMFC. A decrease was observed at parturition, followed by an increase which peaked between d +7 and d +28 ( $P<0.05$ ). Defective chemotaxis observed during the periparturient period may be related to modulation of PTP in MAP-K and other PMN proteins and to alteration in L-selectin expression on bovine PMN.

**Key Words:** Neutrophils, L-Selectin, Phosphorylation

**143 Production of bispecific antibodies to bovine polymorphonuclear neutrophils and to *Staphylococcus aureus* capsular polysaccharide type 5.** Y. Wang<sup>\*1</sup>, M. J. Paape<sup>2</sup>, D. M. Segal<sup>3</sup>, P. Rainard<sup>4</sup>, B. Poutrel<sup>4</sup>, and Y. Nakamura<sup>5</sup>, <sup>1</sup>University of Maryland, College Park, MD, <sup>2</sup>USDA-ARS, Beltsville, MD, <sup>3</sup>NCI, NIH, Bethesda, MD, <sup>4</sup>INRA, Nouzilly, France, <sup>5</sup>Ajinomoto Co. Inc, Tokyo, Japan.

Polymorphonuclear neutrophil (PMN) phagocytosis is a major defense against mastitis pathogens. Mammary PMN respiratory burst activity (RBA), a measure of the bactericidal activity of PMN, is decreased compared to RBA of blood PMN. The current study was designed to produce bispecific antibodies (BsAb) to enhance the bactericidal activity of PMN. Initially, three anti-bovine PMN monoclonal antibodies (MAB) (11G10, 6C6 and 36H10) were tested for their effect on PMN RBA. The MAB 6C6 enhanced the RBA continuously over the 120 min ( $P < 0.05$ ). The MAB 11G10 and 36H10 induced a peak response at 30 min that persisted for 90 min ( $P < 0.05$ ). Three BsAb were developed for each of the three anti-PMN MAB and anti-*S. aureus* capsular polysaccharide type 5 (Cp5) MAB, using the heterobifunctional compound N-succinimidyl-3-(2-pyridyldithiol)propionate. The BsAb were purified by FPLC with a Superose 12 column. Dual specificity of the BsAb was confirmed by indirect immunofluorescence using flow cytometry. The BsAb recognizing *S. aureus* at one pole and PMN at the other should enhance phagocytosis of the organism and increase the bactericidal activity of PMN.

**Key Words:** Bispecific Antibodies, Polymorphonuclear Neutrophils, *Staphylococcus aureus*

**144 Relationship between plasma ascorbic acid concentration, metabolic parameters and milk somatic cell count in Holstein cows.** F. R. Lima<sup>\*1</sup>, M. V. Santos<sup>1</sup>, L. F. Laranja da Fonseca<sup>1</sup>, P. H. M. Rodrigues<sup>1</sup>, and S. M. B. Barros<sup>2</sup>, <sup>1</sup>Faculdade de Medicina Veterinária e Zootecnia, <sup>2</sup>Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Brazil.

The objective of the present study was to establish the association between plasma ascorbic acid level and glucose, insulin, non-esterified fatty acids (NEFAs); beta-hydroxybutyrate (BHBA) aspartate-amino transferase (AST) and milk somatic cell count (SCC) in Holstein cows. One hundred seventy six Holstein cows from 3 different herds were used in this study. Animals were randomly assigned in 5 groups according to the stage of lactation (Group 1: 1–28 days; Group 2: 29–56 days; Group 3: 57–140 days; Group 4: 141–280 days and Group 5: dry cows) and the number of lactation (primiparous or multiparous). Ascorbic acid determination was performed by HPLC technique. Statistical analysis was performed using the program SAS. Statistical significance was declared at 5% level. A significant correlation was observed between ascorbic acid and both glucose and NEFAs. However, a non-significant correlation was found between ascorbic acid and BHBA, insulin, CCS and AST, according to the table below.

	Glucose	NEFA	BHBA	Insulin	AST	CCS
Ascorbic acid	r = - 0.264	r = - 0.201	r = - 0.0812	r = - 0.0067	r = - 0.0408	r = - 0.0674
	P=0.0005	P=0.0086	P=0.2853	P=0.930	P=0.6006	P=0.5502

**Key Words:** Ascorbic acid, cow, nutrition

**145 Effects of stage of lactation and number of lactation on plasma ascorbic acid concentrations in Holstein cows.** M. V. Santos<sup>\*1</sup>, F. R. Lima<sup>1</sup>, L. F. Laranja da Fonseca<sup>1</sup>, P. H. M. Rodrigues<sup>1</sup>, and S. M. B. Barros<sup>2</sup>, <sup>1</sup>Faculdade de Medicina Veterinária e Zootecnia, <sup>2</sup>Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Brazil.

Dairy cows are totally dependent of endogenous synthesis of ascorbic acid to meet their requirements. Therefore, any condition that decrease the availability of ascorbic acid precursors like glucose and galactose, may result in insufficient synthesis of ascorbic acid. High producing dairy cows may be predisposed to subclinical ascorbic acid deficiency due to the elevated demand for glucose to lactose synthesis by mammary gland. The purpose of this study was to determine the effects of stage of lactation and number of lactation on plasma ascorbic acid concentration in Holstein cows. One hundred seventy six Holstein cows from 3 different herds were used in this study. Animals were randomly assigned in 5 groups according to the stage of lactation (Group 1: 1–28 days; Group 2: 29–56 days; Group 3: 57–140 days; Group 4: 141–280 days and Group 5: dry cows) and the number of lactation (primiparous or multiparous). Blood samples were taken for ascorbic acid determination by HPLC technique. Statistical analysis was performed using the program SAS. Statistical significance was declared at 5% level. Average plasma ascorbic acid concentration (mg/L) for Group 1 to 5 were 2.67; 2.60; 2.46; 2.63 and 2.60, respectively and for primiparous and multiparous were 2.63 and 2.52. Results of this study demonstrated that plasma ascorbic acid concentration did not change in response to stage of lactation and number of lactation.

**Key Words:** Ascorbic acid, cow, nutrition

**146 Adhesion molecules integral to neutrophil infiltration into intestinal mucosa: A double-edged sword.** M. R. Ackermann<sup>\*</sup>, Iowa State University, Ames.

Neutrophil infiltration into intestinal mucosa involves at least three families of adhesion molecules which mediate adherence to vascular endothelial cells and extracellular matrix proteins. The three families include: selectins,  $\beta_2$  integrins, and the immunoglobulin gene superfamily. Although there is redundancy in the process, each adhesion molecule family can be vital for infiltration. Neutrophil infiltration is essential for host defense against bacterial pathogens and this is underscored by work demonstrating that calves ( $n = 8$ ) with impaired expression of the  $\beta_2$  integrin family of adhesion molecules by neutrophils frequently develop enteric ulcers that, histologically, lack neutrophil infiltration. These calves respond poorly to antibiotic therapy and often die. On the other hand, excessive neutrophil infiltration during acute bacterial infections such as those caused by the food-borne pathogen *E. coli* O157:H7, are associated with mucosal damage. Preliminary findings suggest that inhibition of selectin-mediated adherence reduces the severity of *E. coli* O157:H7 enteritis and also the number of neutrophils in the mucosa. Thus neutrophil infiltration and the acute inflammatory response is a finely regulated process that, when inhibited temporarily, may reduce neutrophil-mediated mucosal damage. However, prolonged loss of neutrophil infiltration into the mucosa leads to impaired host defense and mucosal ulceration.

**Key Words:** inflammation, enteritis

**147 Respiratory immunity in health and disease.** F. Blecha\*, *Kansas State University, Manhattan.*

Defense mechanisms are essential to maintain the integrity and function of the lungs. Consequently, the respiratory tract is well-equipped to protect the lungs with a full complement of innate and acquired immune defense mechanisms. One important component of innate immunity is the elaboration of antimicrobial peptides. These natural antibiotics are a fundamental defense mechanism with potent activity against Gram-negative and Gram-positive bacteria. Epithelial cells of the bovine respiratory tract contain lingual and tracheal antimicrobial peptides, which are upregulated in response to inflammation and infection. Macrophages are the principle immune cell of the respiratory tract and several different classes are present including, airway, alveolar, interstitial, and intravascular macrophages. Unlike humans and rodents, where blood clearance of bacteria and particulates occurs primarily in the liver, pulmonary intravascular macrophages are responsible for blood clearance in ruminants and pigs. Thus, it is clear that these cells play a critical role in respiratory inflammation. Understanding the mechanisms that regulate the expression of antimicrobial peptides in the respiratory tract and a thorough understanding of the complete complement of pulmonary macrophages may provide new insights into respiratory inflammation and methods of disease prevention.

**Key Words:** Lung, Immunity, Antimicrobial Peptide

**148 Studies of host-pathogen interactions at an epithelial surface using experimental bovine mastitis as a model.** D. E. Shuster\*, *Fort Dodge Animal Health, Princeton, NJ.*

Experimental bovine mastitis provides a useful model to study the early interactions between host and invading pathogen. This presentation reviews published research to provide a molecular perspective on the early interactions between host and pathogen in bovine mastitis and more generally. The host-pathogen interaction involves bacterial replication within the host, bacterial detection by the host, production of inflammatory mediators, recruitment of host defenses, and elimination of the invading pathogen. Following penetration of the teat canal, bacteria multiply in milk to achieve concentrations as high as  $10^4$  cfu/ml with no apparent response by the host. The infection is then detected by the host, but our understanding of the molecular basis of bacterial recognition remains speculative. However, the process must involve passage of a signal across the impermeable epithelial barrier. Once the infection has been detected, the host response can be sudden and intense. The initial response is a breakdown of the epithelial barrier separating the site of infection from humoral defenses. This initial response is followed within a few hours by a sudden and intense influx of neutrophils. These host responses can rapidly eliminate mammary pathogens from the milk. Temporal studies of inflammatory mediators suggest that the complement system may play an important role in initial pathogen detection and recruitment of host defenses. Inflammatory cytokines, chemokines, and arachidonic acid metabolites are not produced early enough to account for the initial host defense responses, but may be important in sustaining leukocyte recruitment. These host-pathogen interactions will be reviewed to try to gain a better understanding of severe cases of coliform mastitis and ineffective therapy of staphylococcal mastitis.

**Key Words:** *Escherichia coli*, *Staphylococcus aureus*

**149 Hypersensitivity-like reaction in uterine inflammation.** R. H. BonDurant\*, *University of California, Davis.*

In the ruminant reproductive tract, the mucosal immune system must be able to distinguish allotypic pregnancy from pathogen, and to mute its responses to the former while mounting an effective "clearing" response to the latter. In non-pregnant females, the uterine defense is generally brisker in the follicular (estrogen-dominated) phase of the cycle, and more sluggish during the luteal (progesterone-dominated) phase. Progesterone appears to induce the formation of uterine proteins that have immune-suppressive functions. In the immediate post-partum period, the spectrum of intrauterine organisms is broad, but only a few (e.g. *A. pyogenes* and a few anaerobic species) are associated with significant inflammation of the endometrium. That inflammation is acute, catarrhal and self-limiting, with resolution occurring soon after a return to estrus. Infections that persist beyond the time of the first post-partum ovulation may become chronic, as the progesterone-dominated uterine defenses weaken. When pregnancy and infection coexist, as in venereal trichomoniasis, the uterine inflammatory response is mild (muted?) for the first 7–10 weeks, by which time a significant and specific IgG1 and IgA response can be measured in uterine secretions. Clearance of the non-invasive organism is associated with peak antibody activity. Although IgA is locally secreted, the means by which IgG1 arrives in the lumen is not certain; it seems likely that an inflammatory response induces vascular leakage and disruption of epithelial integrity in the endometrium sufficient to allow "oozing" of circulating IgG1 into the lumen. This inflammatory response may be mediated by endometrial mast cells and eosinophils. We have shown distinct concentrations of sup-epithelial eosinophils and mast cells in weeks 3, 6, and 12 following infection, but a marked disappearance (degranulation?) of mast cells at week 9, which is when IgG1 levels and pregnancy losses peak. In this protozoal infection, the uterine response shows many similarities to a hypersensitivity reaction.

**Key Words:** Uterus, Inflammation, Hypersensitivity

**150 Characterization of naturally occurring coliform mastitis.** J. R. Wenz\*, G. M. Barrington, F. B. Garry, G. M. Goodell, and R. P. Dinsmore, *Colorado State University, Fort Collins.*

Current knowledge of coliform mastitis pathophysiology derives largely from studies of experimentally induced disease. We have recently reported findings from naturally infected cows that differ from those in experimental disease and could substantially influence disease identification and therapy. Specifically, more persistent clinicopathologic abnormalities, a high prevalence of bacteremia and prolonged recovery of bacteria from the milk were identified. The present study was designed to further characterize naturally occurring coliform mastitis based on physical examination, clinical pathology and blood culture findings. Cows with suspected coliform mastitis were sampled from five farms. Cases were classified based on physical examination as mild (signs restricted to the gland), moderate (few systemic signs) and severe (extensive systemic signs). Blood (30ml) and milk (5ml) were collected aseptically along with blood for complete blood count and biochemical analysis from each cow at initial assessment (0hr) and 24 hr. Blood and milk samples were obtained at 0hr from control cows matched for lactation and days in milk. Preliminary findings revealed bacteremia in 13.6% (6/44) of cows. None of the control cows were bacteremic. Bacteremia was identified in 0, 9.1 and 45.5% of mild (n=22), moderate (n=11) and severe (n=11) cows respectively. *E. coli* was isolated from the blood of one cow in the moderate and one cow in the severe group. *Pasteurella* spp. were isolated from three and *Salmonella* type D from one cow in the severe group. *E. coli* isolates from blood and milk were determined to be of the same genotype by PCR analysis. There was a trend of increasing leukopenia with increasing severity of disease. Moderate and severe cows were more neutropenic (410 and 560 cells/ml) than those in the mild group 2740 cells/ml ( $p < 0.005$ ). Our preliminary results suggest bacteremia is an important sequela to naturally occurring coliform mastitis and is more likely to occur in moderate and severely affected cows. Based on these results, severity of disease based on physical examination may be an indicator of cows at risk for bacteremia. Systemic antibiotic therapy may be indicated in moderately and severely affected cows with coliform mastitis.

**Key Words:** Coliform Bacteria, Mastitis, Sepsis

**151 Efficacy of granulocyte-colony stimulatory factor (G-CSF) or G-CSF plus granulocyte macrophage-CSF in protection against *Escherichia coli* mastitis in postpartum dairy cows.** M. E. Kehrli\*, *National Animal Disease Center-USDA-ARS, Ames, IA.*

Neutrophils are the primary cellular defense mechanism that protects the mammary gland from infection. In this study, the ability of a cytokine which induces bone marrow production of neutrophils (G-CSF) was evaluated alone and in combination with another related cytokine (GM-CSF). Fifteen cows were divided into 3 treatment groups and administered treatments daily for 5 days via subcutaneous injections beginning 3 days after calving (each at 1 µg/kg). Cows were challenged in 1 quarter with 30 colony forming units of *E. coli* 6 days after calving. Five cows were removed from the study due to collateral disease. Two of 4 cows treated with G-CSF cleared the *E. coli* prior to the next milking. The 2 remaining G-CSF-treated cows that did exhibit *E. coli* in the challenged quarter at subsequent milkings, had improved clinical responses to challenge relative to saline controls and more rapid bacterial clearance rates (30.0 ± 14.5 h). Bacterial clearance rates of cows treated with both cytokines (98.0 ± 30.5 h) were indistinguishable from those of controls (93.1 ± 30.5 h). Animals treated with G-CSF exhibited normal rectal temperatures throughout the course of the study. Clinical condition of the mammary gland and milk from infected quarters was significantly improved for animals treated with G-CSF. Animals treated with G-CSF exhibited less milk production loss relative to controls and maintained feed intake. Animals treated with both cytokines exhibited decreased milk production at a level between that of the cows treated with G-CSF alone and the saline controls. Systemic administration of G-CSF alone or in combination with GM-CSF was associated with induction of a significant increase in the number of circulating leukocytes. Neutrophil iodination function was increased following treatment. Results indicate systemic administration of G-CSF can reduce the clinical severity and incidence of coliform mastitis by 50%.

**Key Words:** Cytokine, Mastitis, Coliform

**152 Immunization of cows with ferric enterobactin receptor from coliform bacteria.** J. Lin, J. S. Hogan\*, M. Aslam, and K. L. Smith, *The Ohio State University/OARDC, Wooster.*

The serum and milk IgG responses of lactating dairy cows were determined following immunization with ferric enterobactin receptor FepA. *Escherichia coli* 471 was cultured in iron-depleted medium, and outer membrane proteins were extracted by 2% N-lauroylsarcosine sodium salt and 2% Triton X-100. The FepA was isolated from the outer membrane proteins by ion-exchange chromatography. Twenty cows were assigned to four treatment groups of 5 cows each blocked by breed and days in milk. Treatment groups were vaccinated with 100 µg of FepA, 500 µg of FepA, *Escherichia coli* J5 bacterin, or sterile phosphate-buffered saline. Primary immunization was at approximately 200 days in milk and booster immunizations were given 14 and 28 d later. Serum and whey IgG titers to FepA from cows vaccinated with FepA were significantly higher than those from cows immunized with either *E. coli* J5 bacterin or phosphate-buffered saline. Serum and whey IgG titers to FepA were elevated by 14 d in the cows immunized with FepA. Significant differences were not observed between doses of FepA. Polyclonal antiserum from cows immunized with FepA reacted with FepA that was expressed by each of isolate of *Escherichia coli* (n = 25) and *Klebsiella pneumoniae* (n = 22). Immunization with FepA elicited an immunological response in serum and milk.

**Key Words:** Coliform Mastitis, Ferric Enterobactin Receptor, Vaccine

**153 Inhibition of in vitro growth of coliform bacteria by purified bovine IgG directed against ferric enterobactin receptor.** J. Lin\*, J. S. Hogan, and K. L. Smith, *The Ohio State University/OARDC, Wooster.*

The ability of purified bovine IgG, from cows immunized with ferric enterobactin receptor FepA, to inhibit the growth of coliform bacteria derived from bovine IMI was investigated in iron-restricted medium. Bovine IgG was stepwise purified from serum by ammonium sulfate precipitation and protein G-sepharose affinity chromatography. All isolates of *Escherichia coli* (n=21) and *Klebsiella pneumoniae* (n=21) were tested for growth in chemically defined medium containing 0.5 mg/ml of apolactoferrin. Addition of 4 mg/ml of purified bovine IgG directed against FepA in sythetic medium resulted in significant growth inhibition for both *E. coli* and *K. pneumoniae* isolates. Growth reduction of *E. coli* was greater than that of *K. pneumoniae*. Purified bovine IgG from cows immunized with *E. coli* J5 has minimal inhibitory effect on the growth of both *E. coli* and *K. pneumoniae* isolates. Supplementation of 50 µM of ferric chloride to the medium completely reversed the inhibitory effects of the antibodies. This suggested that bovine IgG directed against FepA inhibited the growth of coliform bacteria by interfering with the binding of ferric enterobactin complex to its cell surface receptor FepA.

**Key Words:** Coliform Bacteria, Ferric Enterobactin Receptor, Vaccine

**154 Intrinsic specificity of plasminogen activator induction in bovine mammary cells by *Staphylococcus aureus*.** B. Zavizion, I. Kanevsky, and A. J. Bramley\*, *University of Vermont, Burlington.*

As we reported earlier (J.Infect.Dis.,1997;176:1637-40), bovine strain of *Staphylococcus aureus* (M60) and its culture filtrate stimulate urokinase-type plasminogen activator (u-PA) expression in cultured bovine mammary cells. In order to investigate the nature of bacterial product(s) responsible for this effect, isogenic mutants of *Staph. aureus*, in which alpha-, beta-, and both toxins have been inactivated, were employed. Bacterial culture filtrates were prepared after 18 h growth in cell culture medium without serum. Bovine mammary cells at the stage of early logarithmic growth were incubated for 4 h in complete growth medium without serum supplemented with 30% (V/V) of bacterial culture filtrates. Indirect chromogenic substrate assay of cell-conditioned medium and cellular lysates revealed that inactivation of alpha-toxin has no effect on u-PA induction by bacterial culture filtrate. Mutant with inactivated beta-toxin exhibited significantly less u-PA stimulatory activity both in cell lysates and conditioned medium. Addition of purified beta-toxin (sphingomyelinase) at concentration 0.01-0.5 U/ml also caused an increase in u-PA activity in cell-conditioned medium although to a significantly lesser extent than bacterial culture filtrates. This confirms the contribution of sphingomyelinase to the stimulatory activity of bacterial culture filtrate. Concentrations of sphingomyelinase of 1 U/ml and above caused toxic effects on mammary cells. Further investigations with conditionally pathogenic *Staph. xylosum* and environmental *Staph. simulans* demonstrated that bacterial culture filtrates from *Staph. xylosum* stimulated u-PA activity mainly in conditioned medium while *Staph. simulans* filtrate caused an increase only in cell-associated u-PA. Taken collectively, these data led us to hypothesize that two different mechanisms may be involved in bacterial induction of u-PA production: first - on the level of cell-associated u-PA, and second - on the level of secreted u-PA. The results also suggest that the level of bacterial pathogenicity may correlate with the ability to increase host u-PA activity using both mechanisms.

**Key Words:** Plasminogen Activator, Bacteria, Mastitis

**155 Proteolytic activity in endotoxin-induced mastitic milk.** C. Beaudry\*, G. Robitaille, and P. Lacasse, *Agriculture and Agri-food Canada, Dairy and Swine R&D Centre, Lennoxville, QC, Canada.*

Mastitis is associated with proteolysis of milk proteins. Although, the serine protease plasmin participates to this activity, the contribution of other proteases remains to be established. Therefore, the purpose of this work was to look for non-plasmin proteolytic activity in mastitic milk. One hour after morning milking, we infused the right hind quarter of 20 cows with 10ml of saline containing 10µg of *E.coli* purified lipopolysaccharide (LPS, 055:B5) to mimic mastitis. The left quarter was infused with 10 ml of saline and used as control. The proteolytic activities (PA) of these milks were studied using the zymogram technique with casein as substrate. Caseolytic activity of milk increased dramatically with the LPS infusion. Several bands of caseolysis did not match with those of plasmin. In order to characterize the enzymes involved, we added a series of protease inhibitors to the zymograms. Among the 13 different inhibitors assayed, the most potent inhibitors of caseolysis were the serine protease inhibitors Pefabloc (Boehringer Mannheim) and aprotinin. In addition to casein, we used collagen, gelatin and membrane proteins from mammary gland cells as substrates in zymograms. We compared PA of mastitic and normal milk and serum before and after heat inactivation. Mastitic milk was able to hydrolyze gelatin, collagen and, to a lesser extent, membrane proteins. Surprisingly, the pattern of PA of mastitic milk on the different substrates did not match very well. In non reducing and non denaturing conditions, the estimated molecular weight of the proteolytic enzymes ranged from 38 to more than 200 kDa. After carefully cutting the bands showing PA in each zymogram and eluting proteins, we performed a classical SDS-PAGE electrophoresis. We detected 9 different peptides: 5 ranging from 18 to 40 kDa and 4 between 75 and 225 kDa. Our results indicate that, in addition to plasmin, mastitic milk contains several other active proteases.

**Key Words:** Protease, Milk, Mastitis

**156 Effects of a natural teat canal insert during temporary cessation of milking on udder health.** T. Geishauser<sup>1\*</sup>, C. Seeh<sup>2</sup>, H. Bostedt<sup>2</sup>, and M. Shoukri<sup>1</sup>, <sup>1</sup> *University of Guelph, Canada* <sup>2</sup> *University of Giessen, Germany.*

Temporary cessation of milking in a single quarter has often been used to immobilize injured teats and to enhance wound healing. The objective of this study was to evaluate the effect of a natural teat canal insert (NIT) during temporary cessation of milking in a single quarter on udder health. Ten healthy dairy cows were followed over a 15 day period. From day 6 to 10 two quarters were not milked. The teat canal of a not milked quarter was administered with a NIT and the other not milked quarter was used as untreated control. The NIT significantly increased milk somatic cell count (SCC), but did not significantly affect udder infection risk. It kept the teat canal wide for five days, thereby adjusting to the teat canal. Cessation of milking for five days significantly increased SCC and udder infection risk. Five days after milking recommencement SCC had returned to normal whereas udder infection risk was still significantly higher. The NIT might be useful to prevent teat canal adhesions during immobilization of injured teats by temporary cessation of milking.

**Key Words:** teat canal insert, temporary cessation of milking, teat injury

**157 Effects of teat dilators and teat canulas on udder health.** K. Querengaesser<sup>1</sup>, T. Geishauser<sup>2\*</sup>, C. Hoepfner<sup>1</sup>, M. Medl<sup>1</sup>, <sup>1</sup> *Tieraerztliche Klinik Babenhausen, Germany* <sup>2</sup> *University of Guelph, Canada.*

The objective of this study was to evaluate the effects of teat dilators and teat canulas on udder health. Over a period of 15 days the teats of four dairy cows were inserted with teat dilators, teat canulas or were left untreated (controls). Cows were milked twice daily. Teat dilators were removed for milking. During milking time the plugs of the teat canulas were removed and milk was drained. All teats were examined endoscopically before the start and after the end of this experiment. The California Mastitis Test (CMT) and a bacteriological examination was performed with the morning milk before start, on day 5, on day 11 and after the end of this experiment. The use of teat dilators and teat canulas was associated with endoscopically visible injuries. The character of these injuries indicated, that teat dilators and teat canulas stab into the teat cistern. In three of seven teats the removal of the teat canula caused a circular separation and eversion of Fuerstenberg Rosette and teat canal skin. The use of teat dilators and teat canulas significantly increased CMT findings and the odds of a positive bacterial result. We conclude, that the use of teat dilators and teat canulas may harm udder health.

**Key Words:** teat dilators, teat cannulas, udder health

**158 Relationship between daily milk electrical conductivity, parity, and daily milk yield in dairy cattle.** G. M. Goodell\*, R. P. Dinsmore, and P. Chard, *Colorado State University, Fort Collins.*

The objective of this study was to explore the relationship between milk electrical conductivity (MEC) in mastitis-free cows, parity, and daily milk yield. The study was conducted in a 550 cow herd in Northern Colorado. The herd was milked 3 times a day through a double eight herringbone parlor which was equipped with milk flow and inline conductivity meters. Conductivity measurements were captured in a microcomputer during each milking session for the first 120 days of lactation. Other parameters captured included session milk yield, parity, and days-in-milk (DIM). Duplicate quarter milk samples were cultured at parturition and at monthly intervals. Cows were enrolled in this portion of the study only if both quarter samples were negative. Composite somatic cell counts (SCCs) were also collected at each monthly sampling. Cows with clinical mastitis during the 120-day period were excluded from the trial. Thirty-four cows were enrolled, including 16 of parity 1, 6 of parity 2, and 12 of parity 3 or greater. Mean SCC was 52,000 cells per ml. Somatic cell counts did not vary significantly with MEC. There was a significant (p=0.003) association between parity and milk electrical conductivity by one-way ANOVA; parity 3+ accounted for the largest difference in MEC. As expected, level of milk production was the most different between parity groups indicating analysis based on production was necessary. Cows were stratified into 3 groups by daily milk yield: <34 kg/day (n=12); 34–41 kg/day (n=10); and >41 kg/day (n=12). The average daily MEC in the 3 milk yield groups were found to differ significantly by one-way ANOVA (p=0.041), with the higher production group accounting for most of the variability. The data indicates that one must adjust for level of production when using milk electrical conductivity to predict changes in udder infection status.

**Key Words:** Mastitis, Milk Conductivity

**159 Milk electrical conductivity in cows with subclinical mastitis.** R. P. Dinsmore\*, G. M. Goodell, and P. Chard, *Colorado State University, Fort Collins.*

The objective of this study was to characterize the change milk electrical conductivity (MEC) in cows developing subclinical mastitis. The study was conducted in a 550 cow herd in Northern Colorado. The herd was milked 3 times a day through a double eight herringbone parlor which was equipped with milk flow and inline conductivity meters. Conductivity measurements were captured in a microcomputer during each milking session for the first 120 days of lactation. Duplicate quarter milk samples were cultured at parturition and at monthly intervals, and composite milk samples were collected monthly for somatic cell counts (SCC). To be enrolled in the study, cows with all quarters free of IMI at parturition had to develop a subclinical IMI in at least one quarter. Twenty cows met these criteria and were enrolled in the study. The mean daily MEC of cows with and without subclinical mastitis was stratified by lactation. There were trends toward increased MEC in parity 3+ cows with subclinical mastitis, but statistical significance was not achieved. Other preliminary analyses indicate that the session conductivity 24h preceding the mastitis event may be more predictive than the mean daily MEC. Cows with subclinical mastitis were also stratified by organism group. Mean daily MEC in 12 cows infected with major pathogens (environmental streptococci, *Arcanobacterium pyogenes*, and coliforms) was 6.45 mSiemens (mS), while in cows infected with minor pathogens (coagulase negative *staphylococci* and *Bacillus* sp.) the mean daily MEC was 6.00 mS.

Parity	1	2	3 +
Subclinical mastitis	5.95 <sup>1</sup>	6.27	6.39
No mastitis	5.61	5.66	5.90

<sup>1</sup>Mean MEC (mS)

**Key Words:** Mastitis, Milk Conductivity

**160 Sensitivity and specificity of milk electrical conductivity and somatic cell counts for the detection of subclinical mastitis.** P. Chard\*, G. M. Goodell, and R. P. Dinsmore, *Colorado State University, Fort Collins.*

An automated milk electrical conductivity (MEC) system was evaluated for the detection of subclinical mastitis. Specifically, milking session MEC and mean daily MEC were compared with individual cow composite somatic cell counts for the detection of IMI. The study was conducted in a 550 cow herd in Northern Colorado. The herd was milked 3 times a day through a double eight herringbone parlor which was equipped with milk flow and inline conductivity meters. Conductivity measurements were captured in a microcomputer during each milking session for the first 120 days of lactation. Other parameters captured included session milk yield, parity, and days-in-milk (DIM). Duplicate quarter milk samples were cultured at parturition and at monthly intervals, and composite milk samples were collected monthly for somatic cell counts (SCC). Subclinical mastitis was defined as the presence of the same species of bacterium in both of the duplicate samples at one or more monthly sampling. Sensitivity and specificity of session MEC, mean daily MEC, and SCC were calculated for cows with or without subclinical mastitis. Session MEC appeared to perform better as a test for infection status on all 3 days before culture sampling than did mean daily MEC.

Days prior to sampling	Sensitivity		SCC	Specificity		
	SMCEC <sup>1</sup>	MDMEC <sup>2</sup>		SMCEC	MDMEC	SCC
3	.58	.55	NA	.43	.34	NA
2	.61	.53	NA	.41	.34	NA
1	.66	.63	NA	.36	.33	NA
0	.61	.61	42	.64	.33	90

<sup>1</sup>Session MEC

<sup>2</sup>Mean daily MEC

**Key Words:** Mastitis, Milk Conductivity

**161 Breeding and calving management on U.S. cow-calf operations.** D. A. Dargatz, *USDA-APHIS Centers for Epidemiology and Animal Health, Fort Collins, CO.*

Efficient reproduction is critical to the profitability of the beef operation. Management during the breeding and calving periods on beef operations can have major impacts on the production endpoints (e.g. calves weaned per cow exposed) for the beef operation. The objectives of this study were to describe breeding and calving management on cow-calf operations from throughout the U.S. As part of the USDA's National Animal Health Monitoring System (NAHMS) Beef '97 Study a stratified random sample of cow-calf producers with 1 or more beef cows from 23 states were visited to collect data on breeding and calving management. Data from the 2,713 participating operations were weighted to account for their selection probabilities. Population estimates for the proportion of operations employing various management practices were made. The Beef '97 results showed that 53.6% of operations had no set breeding season. These operations accounted for 35.3% of the beef cows in the reference population. For operations that had a single breeding season (36.6% of all operations), 26.3% had a breeding season of 150 days or more. On average, producers observed heifers 3.6 times per 24 hour period during the calving period. Cows were observed less frequently (2.5 times per 24 hour period). Over half (55.7%) of producers observed heifers 2 times per day or less frequently. On average, heifers were allowed to labor 2.8 hours prior to giving assistance compared to 3.5 hours for cows. Over one-third (39.3%) of producers allowed heifers to labor for more than 2 hours prior to giving assistance. These data suggest that opportunities exist to improve reproductive management and production efficiency on U.S. cow-calf operations.

**Key Words:** Calving Management, Breeding Management

**162 Simulation study of the incidences of dairy cattle production diseases when extending lactations by 100 days with recombinant bovine somatotropin.** H. G. Allore\*<sup>1</sup>, H. N. Erb<sup>1</sup>, and C. Haferkamp<sup>2</sup>, <sup>1</sup>*Cornell University, Ithaca, NY,* <sup>2</sup>*Free University, Berlin, Germany.*

A dynamic stochastic discrete-event simulation model, SIMHEALTH, that focuses on mastitis, metabolic (milk fever, ketosis, displaced abomasum) and reproductive (dystocia, retained placenta, metritis, cystic ovary, abortion and embryonic death) disorders in dairy herds was used to investigate the impact of recombinant bovine somatotropin (rbST). SIMHEALTH accounts for the risk factors of parity, season of calving, stage of lactation and previous diseases. This research explored how extending lactation length with rbST would change annual and lactational disease incidences. The null hypothesis was that extending lactation length would not change the disease incidences. The simulation experiment compared the use of rbST for lactation length extended from 340 d (50-d voluntary waiting period; VWP) to 440 d (150-d VWP). Fifty replicates of each lactation length were simulated with herds of 100 cows for 4 years. Individual cows were followed and disease occurrences were recorded. The culling rule for reproductive failure was open 100 d before end of lactation or six services and not confirmed pregnant. Preliminary results of the annual and lactational incidence for completed lactations are in the table below. Reproductive culling decreased from 14.5% to 12.9% when VWP was increased from 50 d to 150 d.

Lactation length	Milk fever	Ketosis	Displaced abomasum	Dystocia	Retained placenta	Metritis	Cystic ovaries
Annual incidence (%) over 4 yr							
340 d	6.70	6.48	2.18	4.38	7.83	9.83	9.75
440 d	6.58	6.53	2.28	4.23	8.83	9.28	9.40
Lactational incidence (%) over 4 yr							
340 d	6.68	6.50	2.17	4.37	7.83	9.82	9.77
440 d	6.60	6.54	2.27	4.21	8.83	9.27	9.38

**Key Words:** Disease Incidence, Simulation, Extended Lactation Length

**163 Prevalence and risk factors for *Salmonella* shedding on U.S. dairy operations.** S. J. Wells\*<sup>1</sup>, P. J. Fedorka-Cray<sup>2</sup>, and E. K. Kabagambe<sup>3</sup>, <sup>1</sup>USDA-APHIS-VS Centers for Epidemiology and Animal Health, Ft. Collins, Colorado, <sup>2</sup>USDA-ARS, Athens, Georgia, <sup>3</sup>Louisiana State University, Baton Rouge, Louisiana.

*Salmonella* is one of the most common human foodborne pathogens found in foods of animal origin, and is pathogenic to cattle as well. One source of human exposure is food products from dairy cattle. The objectives of this study was to compare the fecal shedding prevalence of *Salmonella* in milk cows on-farm to that in cull dairy cows at markets and to evaluate herd-level risk factors for *Salmonella* shedding. As part of the USDA's National Animal Health Monitoring System (NAHMS) Dairy '96 Study, fecal samples from dairy operations with at least 50 milk cows across 19 states were collected in 1996 and sent to the USDA's National Veterinary Services Laboratories for testing. Samples were cultured from 91 dairy operations (3,640 milk cows and 668 cows to be culled in the next 7 days) and 97 cull dairy cow markets (2,287 cull dairy cows). NAHMS Dairy '96 Study results showed that fecal shedding of *Salmonella* in milk cows on farm (5.4%) is similar to that in feedlot cattle (5.5%). Fecal shedding, however, was higher in dairy cows on-farm designated for culling within the next 7 days (18.1%) and in culled dairy cows at markets (14.9%). 27.5% of dairy operations and 66.7% of markets had at least one dairy cow shedding *Salmonella*. Season of sample collection and herd size was related to fecal shedding of *Salmonella*. In addition, specific management data was evaluated for association with *Salmonella* shedding. A multivariable model fitted to obtain adjusted odds ratios (OR) and 95% confidence limits (CL) included herd size (OR=5.8, CL 1.1-31.3), region (OR=5.7, CL 1.4-23.5), use of flush water systems for handling manure (OR=3.5, CL 0.9-14.7), and feeding brewers byproducts to lactating cows (OR=3.4, CL 0.9-12.9). These data suggest that fecal shedding of *Salmonella* by dairy cows is dependent upon culling status and that management practices can be manipulated to reduce fecal shedding.

**Key Words:** Salmonella, Dairy Cows, Risk Factors

**164 Survival of Intracellular *Mycobacterium paratuberculosis* after Pasteurization of Raw Milk.** J. R. Stabel\* and E. Steadham, USDA/ARS/National Animal Disease Center, Ames, IA.

*Mycobacterium paratuberculosis*, an acid-fast bacillus that causes enteritis (Johne's disease) in ruminants, has been suggested as an etiological agent of Crohn's disease in humans. The mode of transmission is unclear, however, some evidence suggests that humans may become infected via contaminated milk. Currently, it is not known whether commercial pasteurization effectively kills *M. paratuberculosis* in contaminated raw milk. We previously demonstrated in our laboratory, using a laboratory-scale pasteurizer unit designed to simulate the high-temperature, short-time method (72°C, 15 sec) currently used by commercial dairies, that treatment of raw milk inoculated with *M. paratuberculosis* resulted in killing of all the bacteria. However, *M. paratuberculosis* is an intracellular pathogen that resides within the macrophages of the host and evades destruction. It is unknown whether the macrophage would provide a protective environment during pasteurization of milk, which would enable the bacteria to survive. We evaluated this hypothesis by conducting studies in which we experimentally infected bovine mammary gland macrophages or a bovine macrophage cell line with *M. paratuberculosis* (100:1 bacteria to cell). Twenty-four hours after infection, macrophages were removed from flasks, washed, counted, and added to raw milk to achieve an infection level of 10<sup>6</sup> CFU of *M. paratuberculosis* per ml of milk. Milk was then treated with the pasteurizer unit at 65°C and 72°C for 15 seconds. Aliquots of treated milk were placed on ice, sonicated, diluted, and plated onto agar medium. Viable bacteria were counted after 12 weeks of incubation. Results from these experiments demonstrate that heat treatment at either temperature (65°C, 72°C) effectively killed all *M. paratuberculosis*. This would suggest that the macrophage does not protect the pathogen from penetration of heat during the pasteurization process.

**Key Words:** Milk, Johne's disease, Pasteurization

**165 Isolation and enumeration of *Campylobacter* species from commercial swine operations.** C. R. Young\*, R. B. Harvey, R. L. Ziprin, and D. J. Nisbet, USDA, ARS, FAPRL, College Station, TX.

Our studies have involved an ongoing epidemiological survey of *Campylobacter* incidence within three commercial swine farms in Texas. Samples of cecal contents were collected from 250 market pigs at slaughter and *Campylobacter* incidence determined using enrichment broth and *Campylobacter*-restrictive media. In five separate occasions from two growout operations, the incidence of *Campylobacter* isolations varied from 70-95%. Ninety-nine percent of all isolates, as determined by both metabolic fermentation and serological assays, were found to be *C. coli* with the remainder being *C. jejuni*. Cecal contents of 50 gilts from a breeding farm were collected at slaughter and *Campylobacter* incidence determined. Approximately 90% of gilts were positive for *C. coli*. Enumeration of *C. coli* showed 10<sup>3</sup> to 10<sup>4</sup> cfu per gram of cecal content. In a separate study, 40 piglets (d 9-14) were sampled and 80% were positive for *C. coli* by the time of weaning. These data are important in that *Campylobacter* species, recognized as foodborne pathogens, readily colonize a high percentage of commercial pigs by an early age and do occur in high numbers in the intestinal tract at time of slaughter.

**Key Words:** Swine, *Campylobacter*, Epidemiology

**166 Reduction of *Salmonella typhimurium* in continuous-flow competitive exclusion cultures derived from swine cecal bacteria.** M. E. Hume<sup>1</sup>\*, S. A. Buckley<sup>1</sup>, R. L. Ziprin<sup>1</sup>, R. C. Anderson<sup>2</sup>, L. H. Stanker<sup>1</sup>, and D. J. Nisbet<sup>1</sup>, <sup>1</sup>USDA/ARS, FAPRL, Food and Feed Animal Research Unit, College Station, TX <sup>2</sup>Milk Specialties Co., Dundee, IL.

*Salmonella* colonization and shedding in swine may result from various environmental and developmental stress factors. The incorporation in feed of subtherapeutic levels of selected antibiotics have been reported to influence *Salmonella* colonization and shedding. The application of competitive exclusion (CE) technologies has been used to reduce *Salmonella* spp. colonization in poultry. The concept of CE is the use of beneficial bacteria to reduce or eliminate colonization by enteropathogens. The objective of the present study is to determine the effect of the feed additive chlortetracycline (CTC) on the ability of CE bacteria to displace *Salmonella typhimurium* (St) from a continuous-flow (CF) chemostat culture. CF cultures of CE bacteria were derived from cecal microflora collected from swine maintained on feed which contain either no added CTC or on feed containing CTC. *In vitro* chemostat cultures were inoculated with St at 10<sup>2</sup>, 10<sup>4</sup>, or 10<sup>6</sup> cfu/ml. Samples were removed at twenty-four hour intervals and analyzed for St. Regardless of the dosage, St was reduced to undetectable levels in each chemostat vessel. Inoculation of the chemostat cultures with 10<sup>2</sup>, 10<sup>4</sup>, or 10<sup>6</sup> cfu/ml St resulted in elimination of St within 2 - 3, 3 - 4, or 4 days after inoculation, respectively. The CE culture derived from the swine given CTC-treated feed required one day longer to eliminate St than the culture derived from swine microflora not given CTC. These data suggest that CTC may impact the swine microflora in the gut and leads to decreased ability of the microflora to inhibit St colonization.

**Key Words:** *Salmonella*, Competitive exclusion, Chlortetracycline

**167 Influence of feed withdrawal on cecal environment and *Campylobacter* populations in a swine surgical model.**

R. B. Harvey<sup>\*1</sup>, C. R. Young<sup>1</sup>, R. C. Anderson<sup>2</sup>, M. M. Swindle<sup>3</sup>, S. A. Buckley<sup>1</sup>, and D. J. Nisbet<sup>1</sup>, <sup>1</sup>USDA, ARS, FAPRL, College Station, TX, <sup>2</sup>Milk Specialties Co., Dundee, IL, <sup>3</sup>Medical University of South Carolina, Charleston, SC.

Various stress factors have been proposed for increased shedding of enteropathogens in food producing animals. *Salmonella* numbers have been reported to increase in animals following feed withdrawal. The objective of this study was to evaluate the influence of feed withdrawal on cecal environment and *Campylobacter* populations in swine. Four Yucatan Miniature gilts (15 kg), naturally infected with *C. jejuni*, had cecal cannulas surgically implanted. Gilts were fed a corn/soybean meal/whey starter diet (22% protein) twice daily. Cecal samples were collected for 14 d prior to and for 14 d following feed withdrawal, and mean values were determined for cecal pH, volatile fatty acids (VFA), and colony forming units (cfu) of *C. jejuni*. Following a 48 h fast, cfu of *C. jejuni* increased from  $1.3 \times 10^5$  to  $2.9 \times 10^7$ /g cecal content, whereas cecal pH increased from 6.10 to 7.76. Acetic, propionic, butyric, and valeric acids decreased from 74.2 to 28.93, 61.85 to 17.63, 8.97 to 7.28, and 3.15 to 2.90  $\mu\text{mol/g}$  cecal content, respectively. Isobutyric and isovaleric acids increased from 1.92 to 3.30 and 1.37 to 2.72  $\mu\text{mol/g}$ , respectively. Within 24 h of full feed, differences were detected for cecal values of pH, cfu, and VFA, and by 7 d, values were similar to prefast levels. These data are important from a food safety aspect in that feed withdrawal, commonly associated with shipping and slaughter operations, induces increased gastrointestinal populations of *C. jejuni* in swine.

**Key Words:** *Campylobacter jejuni*, Feed Withdrawal, Cecum

**168 Effects of prenatal dietary copper level on immune function of calves at birth and 56 days of age.** J. C. Branum<sup>1\*</sup>, G. E. Carstens<sup>1</sup>, E. H. McPhail<sup>1</sup>, K. W. McBride<sup>1</sup>, and A. B. Johnson<sup>2</sup>, <sup>1</sup>Texas A&M University, College Station <sup>2</sup>Zinpro Corp., Eden Prairie, MN.

To determine the role of prenatal dietary Cu on immunocompetence of calves, 48 Simmental cows, previously fed a Cu-depletion diet, were blocked by liver Cu (avg = 8.5 ppm) and randomly assigned to treatments at 196 d of gestation. Treatment diets contained 0, 20, 40 or 80 ppm supplemental Cu as Cu-amino acid complex and were fed individually until parturition. The basal diet contained 4.5 ppm Cu, 5 ppm Mo and .6% S. Liver biopsies were performed on cows and calves at parturition and 56 d postpartum to assess Cu status. Calves were fed their dam's colostrum at 50 mL/kg BW and blood samples collected at 0, 6, 12, and 24 h of age to assess passive IgG<sub>1</sub> transfer. At 56 d of age, skin swelling responses to intradermal injections of phytohemagglutinin (PHA) were measured at 6, 12, 24, and 48 h postinjection to assess cell-mediated immunity. Cow and calf liver Cu concentrations at parturition increased ( $P < .05$ ) quadratically as dietary Cu increased (cows: 10, 122, 188 and 261  $\pm$  16.7; calves: 212, 323, 343 and 353  $\pm$  34.7 ppm Cu for 0, 20, 40 and 80 ppm Cu treatments, respectively). Colostral whey and calf serum IgG<sub>1</sub> concentrations were not affected by dietary Cu. However, colostrum somatic cell counts were higher ( $P < .05$ ) in 0 and 20 Cu cows than 40 and 80 Cu cows (1746, 1628, 565 and 379  $\pm$  340  $\times 10^3$ /mL, respectively). Cow and calf liver Cu concentrations at 56 d postpartum increased ( $P < .05$ ) quadratically as dietary Cu increased (cows: 16, 115, 164 and 229  $\pm$  13.3; calves: 47, 75, 100 and 97  $\pm$  9.7 ppm Cu, respectively). Calves born to 0 Cu cows tended to have higher ( $P < .1$ ) 56-d ADG than calves born to 40 and 80 Cu cows (.94, .89, .79 and .82  $\pm$  .04 kg/d, respectively). Average PHA-induced skin swelling was greater ( $P < .02$ ) in 80 Cu calves than all other treatments (1.89, 1.74, 1.93 and 2.85  $\pm$  .26 mm, respectively). Results indicate that prenatal dietary Cu did not affect passive IgG<sub>1</sub> transfer in newborn calves, but altered cell-mediated immunity of 56-d-old calves.

**Key Words:** Liver copper, Calf, Immunity

**169 Effects of dietary copper insufficiency and sources of dietary copper on copper status and intramammary infections at calving.** R. J. Harmon<sup>\*</sup>, D. S. Trammell, B. A. Smith, and R. W. Scaletti, University of Kentucky, Lexington.

Thirty-one primigravid Holstein heifers were maintained on a basal (6–7 ppm Cu; -CU) diet or diets supplemented (10 ppm) with either copper proteinate (CUP; Alltech, Inc.) or copper sulfate (CUS) beginning 120 d prepartum through about 60 d of lactation. Liver biopsies and blood samples were taken during the trial for liver and blood minerals and plasma ceruloplasmin (Cp), and milk samples were taken within 3 d of calving for bacteriology. The overall mean liver Cu contents were about two-fold higher ( $P < 0.01$ ) in both CUP and CUS groups than that in -CU animals. Liver Cu was higher ( $P < 0.07$ ) in the CUP group than in the CUS group at calving. Evaluation of overall mean liver and plasma Cu contents and plasma Cp activities tend to support the idea that organic Cu supplements have different bioavailability compared with that of inorganic forms in periparturient heifers. Plasma Cu was highest ( $P < 0.07$ ) in CUP animals, most notably at calving. In contrast, overall mean Cp activities were highest ( $P < 0.01$ ) in the CUS group, most notably postpartum. Cp is reported to be the major Cu transport protein produced in the liver. The blood Cu and Cp data suggest the CUP supplementation can affect plasma Cu levels without marked stimulation of Cp. A higher proportion ( $P < 0.01$ ) of quarters were confirmed uninfected (40% more) and fewer ( $P < 0.01$ ) were infected with coagulase-negative staphylococci (70% less) in CUP compared with -CU and CUS groups. However, CUP and CUS animals had higher ( $P < 0.05$ ) percentage quarters infected with major pathogens than the -CU group. The data suggest that CUP may be taken up or transported via a different mechanism than inorganic sources of Cu.

**Key Words:** Copper, Mastitis, Minerals

**170 Effect of mastectomy on steroid hormones, energy status, and lymphocyte function in periparturient dairy cows.** K. Kimura<sup>\*1</sup>, J. P. Goff<sup>2</sup>, B. J. Nonnecke<sup>2</sup>, R. L. Horst<sup>2</sup>, and M. E. Kehrli, Jr.<sup>2</sup>, <sup>1</sup>Iowa State University, Ames, <sup>2</sup>National Animal Disease Center, Ames, IA.

We have been studying the effect of milk production on periparturient immunosuppression. Using 6 intact (INT) and 6 mastectomized (MAST) multiparous Jersey cows, we analyzed plasma steroid hormones [estrone (E1), estradiol (E2), progesterone (P), and cortisol (C)], daily feed intake (DMI), plasma calcium (Ca) and non-esterified fatty acids (NEFA), and determined lymphocyte function [interferon- $\gamma$  (INF- $\gamma$ ) and immunoglobulin (Ig) M secretion in vitro] during periparturient period. All INT developed milk fever and 3 INT also developed ketosis and displaced abomasum. E1 and E2 showed a marked linear increase from day -10 to day 0 around parturition and dropped rapidly to low levels immediately post partum. Both E1 and E2 were significantly higher in MAST (Peak E1:  $0.8 \pm 0.06$  vs.  $1.5 \pm 0.06$  ng/ml, Peak E2:  $169 \pm 23$  vs.  $232 \pm 45$  pg/ml). P level remained high until 2 days before calving (6 to 7 ng/ml in both groups) and decreased precipitously prior to calving. C level increased at parturition (INT:  $19 \pm 4$ , MAST:  $13 \pm 4$  ng/ml) and decreased to prepartum level on day 2 after calving. Both P and C level showed no significant difference between cow groups. DMI declined before calving and increased after calving in the same manner in both cow groups. Ca dropped suddenly on the day of calving ( $4.2 \pm 0.3$  mg/dl) and NEFA increased significantly from day -1 to day 10 (peak on day 1:  $1.4 \pm 0.2$  mEq/L) in INT but there was no significant change in MAST (Ca:  $7.3$  to  $8.6$  mg/dl, NEFA:  $0.1$  to  $0.4$  mEq/L). Both INF- $\gamma$  and IgM secretion decreased significantly in INT cows at calving, but not in MAST. The difference between INT and MAST was significant for both lymphocyte assays (see companion abstract). We previously reported the decline in T cell subsets (CD3, CD4, CD8 positive and  $\gamma\delta$  T cells) at calving only in INT, and decline in neutrophil iodination ability both in INT and MAST before calving but quick recovery in MAST whereas no recovery in INT even at 20 days after calving. Milk production plays an important role in periparturient immunosuppression by diminishing lymphocyte function and recovery in neutrophil function. These effects do not appear to be the result of high levels of steroid hormones as we speculated. Significant differences in plasma estrogens, Ca and NEFA suggest that lower estrogens, hypocalcemia or negative energy balance may contribute to loss of immune function around calving in dairy cows.

**Key Words:** Mastectomy, Periparturient Immunosuppression, Dairy Cows

**171 Effect of mammary gland and milk production on vitamin A and E metabolism.** R. L. Horst\*, K. Kimura, and J. P. Goff, *National Animal Disease Center, USDA-Agricultural Research Service, Ames, IA.*

Plasma concentrations of vitamins A (retinol) and E (tocopherol) decrease precipitously at calving. This change is attributed to loss of these vitamins to the mammary gland and colostrum. Theoretically, if no milk production occurred there would be no decline in plasma retinol and tocopherol concentrations at calving. Using cows that were intact or mastectomized (MastX) blood levels of tocopherol and retinol and its retinoic acid (RA) metabolites (All-trans, 9-cis RA, 13-cis RA and 9,13-dicis RA) were monitored during the periparturient period. Retinol is the precursor to All-trans and 9-cis RA which bind specific cellular receptors to mediate biological actions attributed to vitamin A. 13-cis and 9,13-dicis RA can be isomerized to 9-cis RA in body tissues, thus acting as precursors to the active isomers. Two wks before parturition plasma retinol and tocopherol concentrations were 142 ng/ml and 1.84 µg/ml respectively and declined at parturition in both MastX and intact cows. However, the retinol and tocopherol nadirs were 32 and 57% of precalving levels in intact cows and 56 and 90% of precalving levels respectively in MastX cows. Plasma 9-cis RA concentrations were greater in intact cows than MastX cows during the periparturient period but were not affected by parturition in either group. Plasma All-trans and 13-cis RA concentrations were similar in both intact and MastX cows and were not affected by parturition. Plasma 9,13-dicis RA was generally < .5 ng/ml in both groups of cows prior to calving. However after calving 9,13-dicis RA concentration increased to 3.5 ng/ml in intact cows but did not increase in MastX cows. Milk production accounts for a major portion of the tocopherol, and about half of the retinol decline observed in the plasma of periparturient cows. The mammary gland seems to play a major role in the appearance of 9-cis RA and 9,13-dicis retinoic acid in the plasma of the post-parturient cow.

**Key Words:** Vitamin A, Vitamin E, Retinoic Acid

**172 Effect of transition rations on hoof sole hemorrhages, rumen pH and clinical lameness in Holstein cows.** G. M. DeChant\*, C. A. Risco, G. A. Donovan, T. Q. Tran, H. H. Van Horn, and D. R. Bray, *University of Florida, Gainesville.*

Objectives were to estimate the level of subclinical laminitis (SL) by observing solar hemorrhages and ulcers and monitor the level of ruminal acidosis and clinical lameness in periparturient dairy cows. Holstein cows (n=98) were assigned to treatments which consisted of a late dry ration fed for 3 weeks prior to calving, and a lactating ration fed for 3 weeks postpartum. Rations varied in levels of net energy of lactation (NEL), acid detergent fiber (ADF), and neutral detergent fiber (NDF), and were classified HOT (high NEL and low fiber) or COOL (lower NEL and higher fiber). For the prepartum rations levels of NEL (Mcal/kg), % ADF, and % NDF were 1.50, 30.7, 47.4 and 1.66, 23.2, 39.2 for the COOL and HOT rations, respectively, and 1.70, 22.4, 36.8 and 1.77, 17.5, 31.4 for the COOL and HOT lactating rations, respectively. Four treatment combinations resulted: HOT-COOL, HOT-HOT, COOL-COOL, and COOL-HOT. All cows were fed a neutral ration after 3 weeks in lactation. Solar hemorrhage and ulcer scores among treatments were similar at 25 to 35 DIM (p>0.05). The COOL-HOT group had significantly higher scores (p<0.05) than the HOT-HOT and the COOL-COOL groups from 55 to 75 DIM. By 85 DIM treatment had no effect on scores. Overall rumen pH values were not different (p>0.10) between treatments, however the rate of ruminal acidosis (pH≤5.8) at 8 and 22 DIM samples was significantly higher (p<0.05) in the postpartum HOT groups versus the COOL groups. No direct correlation between lowest postpartum rumen pH and hoof scores on an individual cow basis could be found. Clinical lameness was not affected by treatment (p>0.10). Transition rations which permit late dry dairy cows to acclimate to the high energy rations of lactation need to be formulated to minimize abrupt changes in energy and fiber in order to lower the risk of SL in the first 3 months of lactation.

**Key Words:** Subclinical Laminitis, Ruminal Acidosis, Rations

**173 Effect of time after feeding on urine pH determinations to assess response to dietary cation-anion adjustment.** J. P. Goff\* and R. L. Horst, *National Animal Disease Center, USDA-ARS, Ames, IA.*

Monitoring urine pH can prove a useful means of assessing the response of close-up dry cows to dietary anion addition as part of a program to reduce hypocalcemia and prevent milk fever. A common question that arises when urine pH monitoring programs are instituted is "When should urine be collected from the cows in relation to the time cows are fed?". This study attempts to answer that question. In the first study, 21 non-lactating, non-pregnant Jersey cows were fed corn silage, corn silage with 1 Eq/d added potassium carbonate (K carb), or corn silage with 1.5 Eq/d added hydrochloric acid (HCl) at 8 AM and 8 PM. On the 4th and 5th day of feeding each diet urine was collected from the cows just before feeding cows at 8 AM and again 3, 6, 9, and 12 hr after being fed. Average urine pH of cows across all sampling times was 7.33 ± .04 for cows fed corn silage alone, 8.22 ± .01 for cows fed diet w/K carb, and 5.92 ± .02 for cows fed diet w/HCl. There was no significant effect of time after feeding or day of collection (1st or 2nd collection day) on urine pH. In a second trial, 25 dry non-pregnant cows were offered their ration just once a day at 11 AM. Diets offered were corn silage or corn silage with 1.5 Eq / d added HCl. On the 4th day of feeding the diets, urine was collected from the cows at 8 AM and 11 AM (21 and 24 hrs after last meal offered) and at 3 and 6 hr after being offered their ration. As before, adding HCl to the diet significantly reduced urine pH. However in contrast to trial 1, urine pH was significantly affected by sampling time. Urine pH of corn silage diet cows was 7.90 ± .06 at the time of feeding and 7.13 ± .13 at 3 hrs after feeding. Similarly, cows fed corn silage with HCl had urine pH of 7.04 ± .11 at feeding time and 6.17 ± .07 at 3 hrs after feeding. This study suggests that sampling urine for monitoring DCAD adjustments will be difficult to interpret if the cows are fed just once a day.

**Key Words:** Urine pH, Milk Fever, Cation-anion

**174 Effect of intravenous dose of galactose on its urinary excretion in sheep.** M. L. Bruss\*<sup>1</sup> and J. J. Ceron<sup>2</sup>, <sup>1</sup>*University of California, Davis,* <sup>2</sup>*Universidad de Murcia, Murcia, Spain.*

Galactose is an important metabolic intermediate, and the elimination of an IV dose has been used as a measure of hepatic function. The objective was to evaluate the dose dependency of urinary loss of injected galactose. Six groups (4 sheep/group) of white-faced crossbred Rambouillet ewes were injected via an IV catheter with varying doses of sterile galactose. The average weight of the sheep was 53.6 kg (SE = 1.4), water and alfalfa pellets were provided ad libitum, and they were not pregnant or lactating. Urine was collected via previously inserted bladder catheters, and galactose concentration was measured. Total galactose excretion as percent of dose was calculated, and the results were:

Dose (mg/kg)	500	350	250	150	100	50
% of dose in urine	38.2	37.7	30.8	23.7	18.1	12.3
SE	1.8	2.5	2.6	3.6	4.3	1.3
Difference indicators	a	a	ab	bc	cd	d

Data was analyzed with one-way ANOVA, and P was < 0.0001; columns with no common difference indicators differed (SNK test; P < 0.05). There was a perfect positive rank correlation of dose with percent of dose excreted. A plot of the data indicated a hyperbolic relation of dose and percent excreted. A Lineweaver-Burk plot (r = 0.996) indicated maximal possible excretion would be 50% of dose, and the dose that would produce half-maximal excretion was 155 mg/kg. The saturation of renal excretion is probably due to a balance between renal filtration rate and uptake elsewhere (i.e. liver) of injected galactose. The urinary excretion of injected galactose is considerably greater in sheep than published values for humans.

**Key Words:** Galactose, Sheep, Urine

**175 Milking prior to parturition does not reduce liver triglyceride concentration at calving.** R. A. Hackbart\*, S. J. Bertics, and R. R. Grummer\*, *University of Wisconsin, Madison.*

The objective of this study was to determine if prepartum milking alleviates fatty liver at calving. Twenty multiparous Holstein cows were used in a randomized complete block design. Cows were blocked according to calving date and placed on trial 17 d prior to expected calving; measurements on d -17 served as covariables. Treatments were no prepartum milking (POST) or prepartum milking beginning at 10 d prior to expected calving (PRE). Mean ( $\pm$  SE) liver triglyceride and plasma glucose and NEFA on d -17 were  $3.3 \pm .2$  % (DM basis),  $65.6 \pm 1.5$  mg/dl, and  $204 \pm 15$  ueq/L. Milk yield (kg/d, mean  $\pm$  SE) for cows milked prepartum at -7, -3 and -1 d prior to parturition was  $1.5 \pm 1.3$ ,  $5.3 \pm 2.4$ , and  $9.9 \pm 2.9$ . Milk yield postpartum was not significantly affected by treatment. Plasma NEFA and glucose and liver triglyceride were not affected by treatment.

	POST		PRE	
	Mean	SE	Mean	SE
<u>Day 0 (d of calving)</u>				
Liver triglyceride, % DM basis	15.0	1.7	15.2	1.6
Plasma glucose, mg/dl	67.4	5.9	65.1	6.5
Plasma NEFA, ueq/L	1206	178	1195	196
<u>Day 35 postpartum</u>				
Liver triglyceride, % DM basis	5.5	.9	5.4	.9
Plasma glucose, mg/dl	63.0	2.5	60.6	2.3
Plasma NEFA, ueq/L	331	20	310	24

Results did not support the hypothesis that prepartum milking would reduce the severity of fatty liver at parturition and during early lactation.

**Key Words:** Prepartum Milking, Fatty Liver, Nonesterified Fatty Acids

**176 Effectiveness of calcium chloride in increasing blood calcium levels of periparturient cows.** T. R. Dhiman and V. Sasidharan\*, *Utah State University, Logan.*

Effectiveness of CaCl<sub>2</sub> supplements in increasing blood serum Ca in periparturient dairy cows was studied. Thirty-six multiparous pregnant cows were assigned to four treatments. After calving, cows received basal diet and two doses of either inert control gel (CON), gel containing CaCl<sub>2</sub> and B-complex vitamins (CVG), gel containing CaCl<sub>2</sub> plus 2.7 g of P and 2.9 g Mg (CMG), or CaCl<sub>2</sub> as drench containing B-complex vitamins (CDH). The first dose was given within 2 h of calving and the second dose 12 h after the first dose. Each dose contained 0.07, 54.5, 56.0, and 33.2 g of elemental Ca in CON, CVG, CMG, and CDH treatments, respectively. Blood samples were collected at 0, 15, 30, 60, 180 and 360 min after each oral dose. During pre-treatment the blood serum Ca concentrations were 6.26, 7.56, 6.20 and 5.96 mg/dl in CON, CVG, CMG and CDH treatments, respectively. The average increase in serum Ca from pre-treatment level was 0.4 mg/dl with oral supplementation of Ca as gels and 0.6 mg/dl as drench (Table below). Milk yield during the first 4 wk of lactation was same in all treatments. Three cases of clinical milk fever were observed in CON and one case in CDH treatment. Oral supplements of CaCl<sub>2</sub> as gel or drench increased the blood Ca levels in periparturient dairy cows ( $P = 0.03$ ). Increased supply of Ca though oral supplements of CaCl<sub>2</sub> may prevent milk fever in cows with marginal hypocalcemia.

Average change in blood serum Ca from pre-treatment level, mg/dl

Item	Treatment					P-value		
	CON	CVG	CMG	CDH	SEM	Trt	Time	Trt×Time
Dose 1	-0.06	0.30	0.31	0.34	0.10	0.03	0.20	0.31
Dose 2	-0.61	0.46	0.48	0.78	0.04	0.01	0.44	0.01

**Key Words:** Cow, Calcium, Milk Fever

**177 Field procedure for monitoring blood glucose in cattle.** T. S. Rumsey\*, S. Kahl, T. H. Elsasser, A. S. Kozak, and D. E. Carbaugh, *USDA, Agricultural Research Service, Beltsville, MD.*

A reliable and rapid field method for monitoring blood glucose during acute phase responses (APR) to pathophysiological challenges is sometimes needed for quickly determining glycemic status but not necessarily for absolute glucose concentration. This study compared the Accu-Chek® Easy™ (ACE) monitor (Boehringer Mannheim, Indianapolis, IN; one of several monitors available for human self-monitoring of blood glucose) and the YSI 2700 analytical method (Yellow Springs Instrument, Yellow Springs, OH). The study involved 434 jugular blood samples from beef steers immediately before and during the APR of 62 individual challenge injections of *E. Coli* endotoxin (LPS, Sigma, 055:B5) injected at either 0, .2, 1.0, or 3.0  $\mu$ g/kg BW. Typically, steers display hyperglycemia within 1 h after LPS injection followed by hypoglycemia by 3-4 h and a slow return to normal by 24 h. Mean glucose concentrations (n=62) for ACE and YSI before injection were 60.3 (CV=17.6) and 72.3 (CV=11.4) mg/dL. At peak (1 h post injection), mean concentrations were 57.5, 78.4, 94.8, and 83.8 mg/dL ( $P < .01$ , SEM=6.4) for ACE and 69.8, 81.9, 90.4, and 83.9 mg/dL ( $P < .01$ , SEM=4.0) for YSI following the injection of 0, .2, 1.0, and 3.0  $\mu$ g LPS/kg BW, respectively. During hypoglycemia (3-4 h post injection), respective mean concentrations were 59.0, 45.6, 42.0, and 37.3 mg/dL ( $P < .001$ , SEM=2.5) for ACE and 66.2, 51.7, 42.5, and 39.7 mg/dL ( $P < .001$ , SEM=2.6) for YSI. During both hyper- and hypoglycemic periods, mean glucose rank was the same for ACE and YSI. Regression of YSI on ACE values was linear from 25 to 80 mg/dL ( $YSI = 7.5 + .98ACE$ ,  $R^2 = .74$ ,  $S_{y.x} = 7.2$ ). Above 80 mg/dL, ACE increasingly overestimated glucose concentration suggesting a quadratic relationship ( $YSI = -33.3 + 11.9 \times ACE^{.52}$ ,  $R^2 = .98$ ,  $S_{y.x} = 7.4$ ). In conclusion, a field monitor such as the ACE can be used to monitor glycemic status in ruminants for determining hypo- and hyperglycemic responses to pathophysiological challenges. However, achieving analytical accuracy requires the monitor to be standardized against a calibrated laboratory procedure.

**Key Words:** Cattle, Blood Glucose, Glucose Methods

**178 The analytical appraisal of four methods for evaluation of colostral immunity of calves.** R. Skrzypek\*<sup>1</sup>, W. Deptula<sup>2</sup>, W. Jarmuz<sup>3</sup>, R. Kliks<sup>1</sup>, and D. Stanislawski<sup>1</sup>, <sup>1</sup> *AU Poznan*, <sup>2</sup> *US Szczecin*, <sup>3</sup> *IGIHZ Jastrzebiec, Poland.*

The investigations were carried out on blood serum samples from 320 single-born Holstein x Friesian calves of both sexes. The samples were taken once, when the calves were at the age from 24 to 40 hours after birth. The immunological traits assayed were: total protein (TP, biuret method), globulins measured indirectly as a difference between TP and albumins determined by the method with bromocresol green (GLOB), globulins measured directly (SST, sodium sulphite test), and immunoglobulins (ZST, zinc sulphate turbidity test). Correlation coefficients between the traits assayed were significant ( $P < .001$ ) and ranged from .90 (TP and ZST) to .99 (TP and GLOB). Coefficients of variation for the particular traits were different, ranging from 21.3 % for TP to 76.4 % for ZST. This indicates that despite very high analytical correlation, usability of particular methods for evaluation of health status of the newborn calf can be different. Means and coefficients of variation for globulins measured indirectly and directly were almost identical (21.5 g/l and 21.7 g/l, 59.5 % and 62.7 %; respectively), but the former method appeared to be much less time-consuming. Therefore, from the analytical point of view we rank GLOB above SST.

**Key Words:** Calves, Colostral Immunity, Methods

**179 Effects of testing lab, counting method, storage and shipment on somatic cell counts in goat milk.** S. S. Zeng<sup>\*1</sup>, E. N. Escobar<sup>1</sup>, S. P. Hart<sup>1</sup>, L. Hinckley<sup>2</sup>, M. Baulthaus<sup>3</sup>, G. T. Robinson<sup>4</sup>, and G. Jahnke<sup>5</sup>, <sup>1</sup>E. (Kika) de la Garza Institute for Goat Research, Langston University, OK; <sup>2</sup>University of Connecticut, Storrs, <sup>3</sup>DQCI Services, Inc., St. Paul, MN; <sup>4</sup>Texas Milk Marketing Order, Carrollton, TX; <sup>5</sup>Dairy Laboratory Services, Dubuque, IA.

Somatic cell counts (SCC) in goat milk from different stages of lactation were determined at four laboratories using the pyronin Y-methyl green (PYMG) direct microscopic method and/or Fossomatic machines calibrated with either goat or cow milk standards. The effects of sample shipment and storage on SCC of goat milk were also determined. Results of this study indicated that the PYMG microscopic method and the Fossomatic machine calibrated with goat milk standards gave comparable estimates of SCC in goat milk ( $P > 0.05$ ). However, on average the Fossomatic machines ( $n=3$ ) calibrated with cow milk standards estimated SCC of goat milk to be 24.5% higher than a Fossomatic machine calibrated with goat milk standards ( $P < 0.05$ ). No significant difference in SCC of goat milk existed ( $P > 0.05$ ) between laboratories ( $n=2$ ) when the PYMG microscopic method was used. Shipping milk samples in an ice box (a 3-day round trip) and storing in a refrigerator (3 days) did not affect SCC results ( $P > 0.05$ ).

**Key Words:** Somatic Cell Count, Goat Milk

**180 In vivo effect of locoweed consumption on immunocompetence and serum swainsonine concentrations of sheep in a nutrient restricted state.** J. B. Taylor<sup>\*</sup>, J. R. Strickland, D. E. Hawkins, and T. May, *New Mexico State University, Las Cruces.*

The effects of locoweed consumption on immunocompetence and serum swainsonine (SW) concentration in sheep fed a nutrient restricted diet (1.4% BW) were investigated. Twenty-eight sheep (BW =  $48.7 \pm 6.7$  kg) were randomly assigned to one of five treatments and received either 0, .2, .4, .8, or 1.6 mg of SW/kg of BW provided by locoweed (*Oxytropis sericea*) for a 28 d treatment period. Diets were restricted to 1.4% BW and were formulated to be isonitrogenous and isocaloric. During the 14 d adaptation period animals received a basal diet of blue grama and alfalfa hay. During the treatment period, locoweed replaced alfalfa. Peripheral blood lymphocytes (PBL) were collected on d 0, 7, 14, 21, and 28 for lymphoblastogenesis assays (PBL were stimulated with mitogens concanavalin A, phytohemagglutinin-P, pokeweed mitogen, and lipopolysaccharide for 72 h) to evaluate the effect of locoweed consumption on proliferative responses. On d 0, 1, 7, 14, 21, and 28, blood samples were collected to determine serum SW concentration. Body weight stabilized during adaptation period. No treatment x sampling date interaction ( $P > .31$ ) or treatment effect ( $P > .24$ ) was observed for the lymphoblastogenesis assays. A treatment effect on serum SW concentrations among treatments within date was detected on d 1, 7, 14, 21, and 28 ( $P < .0009$ ). Serum SW in .8 and 1.6 mg of SW/kg BW treatments had greater serum SW compared to other treatments ( $P < .0001$ ) on d 1, 7, 14, 21, and 28. A linear dose response ( $P < .0006$ ) was detected at d 1, 7, 14, 21, and 28. No observed effect of locoweed consumption on lymphoblastogenesis assays indicates that locoweed did not adversely affect immunocompetence of nutrient restricted sheep. A linear response detected in serum SW concentrations establishes a dose response relationship of locoweed intake on serum SW concentrations.

**Key Words:** Sheep, Locoweed, Swainsonine

**181 In vivo effect of locoweed consumption on serum clinical profiles of sheep in a nutrient restricted state.** J. R. Strickland<sup>\*</sup>, J. B. Taylor, D. E. Hawkins, and T. May, *New Mexico State University, Las Cruces.*

The effects of locoweed consumption on serum clinical profiles of sheep in a nutrient restricted (1.4% BW) state were investigated. Twenty-eight sheep (BW =  $48.7 \pm 6.7$  kg) were randomly assigned to one of five treatments (TRT) and received either 0, .2, .4, .8, or 1.6 mg of swainsonine (SW)/kg of BW provided by locoweed (*Oxytropis sericea*) for a 28 d treatment period followed by a 21 d recovery period without locoweed. Jugular blood samples were collected 23 h post-feeding on d 0, 1, 7, 14, 21, and 28 to determine serum clinical profiles during the locoweed-fed period. Additionally, jugular blood was collected on d 35 and 49 of the recovery period to determine if serum clinical profiles returned to baseline (d 0). A treatment x sampling date interaction was detected for serum alkaline phosphatase (AP;  $P = .0001$ ), aspartate aminotransferase (AST;  $P = .0001$ ), triglyceride (TG;  $P = .02$ ), and cholesterol (CH;  $P = .02$ ). Serum AP was increased over the 0 mg TRT in the .8 and 1.6 mg TRT by d 1 ( $P = .001$ ). In addition, on d 7, 14, 21, and 28, .4 mg, .8 mg, and 1.6 mg TRT exhibited higher serum AP than 0 mg TRT. Serum AST was elevated ( $P = .0001$ ) in all treatment groups compared to 0 mg TRT on d 7, 14, 21, 28, and remained elevated in .4 mg, .8 mg, and 1.6 mg TRT at d 49. Serum TG was decreased ( $P < .05$ ) in .4 mg and .8 mg TRT on d 14 and in 1.6 mg TRT on d 21 compared to 0 mg TRT. On d 7, 14, 21, and 28, serum CH was depressed ( $P < .02$ ) in 1.6 mg TRT when compared to 0 mg TRT. A treatment effect ( $P = .04$ ) for serum lactate dehydrogenase (LDH) activity was detected. The .4 mg, .8 mg, and 1.6 mg TRT increased serum LDH compared to 0 mg TRT. A linear dose response was detected for serum LDH, AP, and AST. These data indicate a dose related effect of locoweed on serum constituents. Further, these data indicate that animals consuming .4 mg SW/kg BW or higher do not fully recover by 21 d after withdrawal from locoweed exposure.

**Key Words:** Sheep, Locoweed, Serum Profile

**182 Influence of ergotamine on metabolic hormones in follicular phase heifers.** R. Browning, Jr., S. J. Gissendanner, and T. Wakefield<sup>\*</sup>, *Tennessee State University, Nashville.*

Adrenal and thyroidal responses to ergotamine were examined in four cycling Holstein F<sub>1</sub> heifers (397 kg; SD = 21). Luteolysis for paired heifers was induced synchronously with two PGF<sub>2 $\alpha$</sub>  injections given 11 d apart on two occasions. Two days after the second PGF<sub>2 $\alpha$</sub>  injection, plasma was sampled every 15 min for 1 h before a bolus i.v. treatment of saline (SAL) or ergotamine tartrate (19  $\mu$ g/kg body weight; ET) and for 4 h after treatment. Heifers were then administered 100 USP units of oxytocin i.v. and bled every 30 min for another 3 h. Ambient temperature (31.4°C; SD = 1.3) and relative humidity (42%; SD = 11) were recorded hourly. Respiration rates were measured hourly by counting breaths taken in 15-s intervals. Each heifer received one treatment per synchronized period and both treatments during the study. Treatment x time affected respiration rate ( $P < .001$ ) and plasma concentrations of triiodothyronine ( $P < .01$ ), thyroxine ( $P < .09$ ), and cortisol ( $P < .001$ ). Respiration rates increased ( $P < .01$ ) from  $14.4 \pm 1.8$  breaths/15 sec before ET to  $33.3 \pm 1.8$  breaths/15 s by 6 h after ET. Respiration after SAL did not differ from the pretreatment rate of  $15.5 \pm 1.8$  breaths/15 s. Plasma triiodothyronine increased ( $P < .01$ ) transiently from .85 ng/mL before ET to  $1.01 \pm .03$  ng/mL at 2 h after ET. Triiodothyronine was unchanged from .86 ng/mL before SAL to  $.89 \pm .03$  ng/mL 4 h after SAL, then decreased ( $P < .01$ ) to  $.55 \pm .04$  ng/mL by 3 h after oxytocin. Thyroxine was higher ( $P < .01$ ) after both ET and SAL compared to pretreatment, but increased sooner after ET, 45 to 55.3 ng/mL in 1 h, than after SAL, 50.6 to  $59.8 \pm 2.1$  ng/mL in 2 h. Cortisol was higher ( $P < .01$ ) during the first 3 h after ET, 32.2 to 45 ng/mL, than before ET,  $15 \pm 2.6$  ng/mL, and was lower 6 h after SAL compared to pre-SAL,  $25.4 \pm 2.6$  vs.  $7.7 \pm 3.6$  ng/mL. Results further indicate that ergotamine can increase plasma concentrations of hormones important in regulating metabolic and thermoregulatory functions of cattle.

**Key Words:** Fescue Toxicosis, Ergotamine, Hormones

**183 Cytokine modulation by ergotamine in Balb/c male mice.** N. M. Filipov\*, F. N. Thompson, R. P. Sharma, and R. R. Dugyala, *The University of Georgia, Athens.*

The ergopeptine alkaloid, ergotamine (ET), mimics the effects of ergopeptine alkaloids found in endophyte-infected (E+) fescue forage considered causative for fescue toxicosis. Altered immune capacity, compromised intake and thermoregulation, and inflammatory changes (elevation of angiotensin converting enzyme and other molecules contributing to vascular stasis such as an adhesion factor and thromboxane B<sub>2</sub>) are observed in fescue toxicosis. Taken together, these suggest the cytokine pattern may be altered by ergot alkaloids. Thus, the objective of this study was to determine whether major splenocyte-derived cytokines, interleukin-2 (IL-2), interleukin-4 (IL-4), interferon-gamma (IFN-gamma), and macrophage-derived cytokines, interleukin-1 beta (IL-1 beta), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha) are affected by ergotamine exposure. Two sets of male BALB/c (n=5/trt) mice were treated with ergotamine tartrate (s.c.) for 10 days at doses of 0, 0.4, 2.0, 10.0, and 50.0 mg/kg BW. Twenty-four hours after the last treatment splenocytes (S) were isolated from one set of animals and macrophages (M) from the other set for determination of IL-2, IL-4, IFN-gamma, and IL-1, IL-6, TNF-alpha, respectively. Following activation with 5 µg/ml Con A (S) and 10 µg/ml LPS (M), cells were incubated for 48 and 24 h, respectively, supernatants collected and assayed for respective cytokines by ELISA. Additionally, differential white blood cell (WBC) counts were performed and the neutrophil (N): lymphocyte (L) ratio calculated. Ergotamine increased IL-6 levels at all doses and TNF-alpha at the highest dose. There was no treatment effect on IL-1 beta, IL-2, IL-4, and IFN-gamma. Also, no effect was observed upon total and differential WBC counts as well as N: L ratio. In summary, ergotamine appears to affect cytokines from the pro-inflammatory lineage, namely IL-6 and TNF-alpha; increased production of those cytokines may contribute for the manifestation of fescue toxicosis.

**Key Words:** Fescue Toxicosis, Ergotamine, Cytokines

**184 Bacterial endotoxin-induced suppression of insulin-like growth factor-I in growing pigs.** K. J. Wright\*, B. Ramanathan, C. M. Hill, S. S. Dritz, E. L. Knoppel, and J. E. Minton, *Kansas State University, Manhattan.*

Reduced growth performance is associated with disease processes in growing pigs and other livestock. However, the effects of disease processes on the somatotrophic axis have not been clearly elucidated. We designed an experiment to evaluate the effect of a model of gram-negative bacterial sepsis on the growth axis by evaluating the insulin-like growth factor-I (IGF) response to *E. coli* endotoxin in young growing pigs. In addition, we monitored feed intake, body temperature and plasma cortisol as well-documented physiological markers of the acute phase response to bacterial endotoxin. Thirteen weaned pigs were housed in individual pens with *ad libitum* access to feed and water. After an acclimation period, venous catheters were placed in all animals. Blood sampling began 72 h after catheter insertion. Pigs were treated (i.p.) with sterile saline (SAL; n=7) or 100 µg/kg endotoxin (END; n=6). Samples of plasma were obtained from -24 to 72 h relative to treatment. Daily feed intake also was monitored during this time. In addition, rectal temperatures (RT) were obtained with digital electronic thermometers from 24 h before to 36 h after treatment. As expected, endotoxin treatment produced an unmistakable febrile response and elevation in plasma cortisol. RT was elevated in END-treated pigs at 2, 4, 6, 8, and 12 h after treatment (P < .05), but was similar to SAL at other times. Similarly, plasma cortisol was increased from 2 to 12 h after treatment in END-treated pigs (P < .05), reaching a maximum of 160.9 ± 6.8 ng/mL at 2 h (vs 25.2 ± 6.3 ng/mL in SAL). In contrast, IGF was suppressed profoundly by endotoxin treatment. IGF generally was reduced in END-treated pigs from 2 to 44 h after treatment (P < .05), and was maximally suppressed at 6 h (38.9 ± 6.8 vs 7.5 ± 7.4 ng/ml). Daily feed intake also was reduced in END-treated pigs through 72 h after treatment (P < .05). The results suggest that gram-negative bacterial sepsis may be associated with reduced IGF.

**Key Words:** Bacterial Endotoxin, IGF-I, Pigs

**185 A method for the detection of the red maple toxicant utilizing washed equine erythrocytes.** D L Brown, *Cornell University - Animal Science Department.*

The ingestion of 1.5 to 3.0 g red maple (*Acer rubrum*) leaves per kg body weight by horses can result in hemolytic anemia, methemoglobinemia, rapid heartbeat, dehydration, icterus, eye hemorrhage, oliguria, discolored urine and death. The objective of this study was to test the ability of washed horse erythrocytes to detect the presence of the unknown toxicant. This toxicant was detected by incubating 4ml 50% washed equine erythrocytes from 18 different horses in buffered saline (110mM NaCl, 20mM Na<sub>2</sub>HPO<sub>4</sub>, 4mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) at 37 C with 800 microliters aqueous extract (filtrate from 1g leaf per 50ml distilled water) or proportional aqueous suspensions of chloroform or ethanol extracts. Oxidation challenge: Aliquots of 200 microliters washed cells were removed from incubation vessel and dissolved in 10ml hemolytic buffer (20mM KH<sub>2</sub>PO<sub>4</sub>, 16mM Na<sub>2</sub>HPO<sub>4</sub>, .05% Triton X, pH 7.8) and vortexed. Methemoglobin % was determined as the reduction in absorbance at 635nm (A<sub>635nm</sub>) caused by adding KCN divided by the reduction caused by adding KCN to solution treated with KFeCN. Cell fragility challenge: Aliquots of 60 microliters washed cells were removed after 120min and suspended in 10ml of 0, .40, .45, .50, .55, .60, .65, .70, .75, .90 % NaCl(aq), incubated for 1hr at room temperature, vortexed, then centrifuged 15min at 1500g. The absorbance at 540nm by the supernatant relative to that from the 0% NaCl mixture was taken as at the fraction of cells hemolyzed at a given NaCl concentration. Results: When vitamin E deficient (<.80 micrograms/dl blood) horses were used as the source for washed red cells, the aqueous extract of red maple leaves reliably elevated methemoglobin from an initial 1-3% to an final 10-152hr. Chloroform and ethanol extracts had no effect and horses with >.80 micrograms/dl vitamin E provided less reliable substrate for oxidation. Cell fragility was not increased by any extract. These results indicate that oxidation of equine erythrocyte hemoglobin could be used to aid the identification and isolation of the toxicant responsible for disease in horses.

**Key Words:** Red maple, Horse, Methemoglobinemia

**186 Cow preference to filler materials in stall mattresses.** E. H. Shim\*, R. D. Shanks, and G. C. McCoy, *University of Illinois, Urbana.*

The preference of cows to different filler materials and sand in free stalls may indicate greater comfort, which may contribute to improved health and productivity. Double-layered mattresses were randomly installed in 123 of the 164 free stalls at the University of Illinois Dairy Unit. The remaining 41 stalls, randomly distributed throughout the five barns, used sand as the stall surface. Three types of filler materials were used in the mattresses: rubber, plastic, and mixed (50% rubber, 50% plastic). Thirteen 24-hour watches (approximately every 2 to 3 weeks), between August 1996 through May 1997, were conducted to determine cow preference. Each stall was recorded hourly as empty, cow lying, cow standing, cow backwards, or cow half in stall. Cows significantly differentiated between materials when lying down and choosing which stalls to occupy. Cows were lying down in stalls with rubber filled mattresses 43.3 ± 1.3% of the watch period, 39.5 ± 1.3% on mixed, 33.5 ± 1.3% on plastic, and 26.4 ± 1.3% on sand. Stalls with rubber filled mattresses were occupied 55.1 ± 1.4% of the watch period, 51.9 ± 1.5% on mixed, 45.3 ± 1.5% on plastic, and 35.3 ± 1.5% on sand. Cow preference ranked rubber filled mattresses first, then mixed, followed by plastic, with mattresses preferred over sand.

**Key Words:** Mattress, Cow Comfort, Filler

**187 Hoof dimensional dynamics and incidence of lameness in Holstein dairy cows before and after turnout to pasture from concrete cubicles or straw yards.** K. Meyer, J. R. Scaife\*, and H. Galbraith, *The University of Aberdeen, Scotland.*

Lameness in dairy cows results in large financial losses to the dairy industry and severely compromises the welfare of affected cows. The causes of lameness are multifactorial and may be of genetic, nutritional, and environmental origin. The purpose of this study was to investigate the effect of housing on claw dimensions, rates of hoof growth and wear and the incidence of lameness. Sixty cows were selected randomly from each of two dairy herds and were observed in three visits six weeks apart. The herds were under the same management and of similar genetic merit, but one was housed in concrete cubicles (HC) while the other in straw yards (HS). Cows were turned out to pasture between the first and second observation, after which they had to walk on farm tracks to and from the milking parlor. At the onset of the study, a higher incidence of lameness (0=sound, 1=lame) was shown by HC vs. HS cows (.43±.1 vs. .19±.1,  $P<.01$ ), but this difference disappeared after turnout. The dorsal claw angle of HS cows was smaller ( $P<.05$ ), i.e., shallower claws, than for HC cows at the first two observations while the angle decreased in both groups over this time ( $P<.01$ ). At the first visit only, lame cows in both herds had a smaller dorsal angle than sound cows ( $P<.05$ ). Before turnout, HC cows showed less wear of the dorsal hoof border than HS cows ( $3.0\pm.4$  vs.  $4.6\pm.3$  mm/month,  $P<.01$ ). After turnout, there was no difference in wear of the dorsal hoof border between herds, however, wear was greater in both herds after turnout ( $P<.01$ ). Hoof growth tended to be greater for HC cows. In conclusion, hoof growth rate was not affected by housing system, but dorsal border wear increased in both herds probably due to walking on roads. Housing cows in straw yards significantly reduced the incidence of lameness, however, turning out cows to pasture ameliorated negative effects of previous housing.

**Key Words:** Housing Systems

**188 Characterization of keratin proteins in bovine solar horn.** K. Meyer\*, H. Galbraith, M. A. Lomax, T. J. Wester, and D. G. Hazlerigg, *The University of Aberdeen, Scotland.*

Lameness in dairy cows results in large financial losses to the dairy industry and severely compromises the welfare of affected cows. A large proportion of lameness cases involves hemorrhage and lesions of the solar horn of the hoof. Deterioration of hoof horn quality may involve inadequate keratinization with differences in amounts of specific keratins present in the horn. We characterized keratin proteins of the bovine solar horn using SDS-PAGE and Western-blotting. Proteins from samples of cornified *stratum corneum* taken from four sites along the sole of hooves obtained in abattoirs from four adult cows were extracted in 8M urea. The extracted proteins were separated by SDS-PAGE and transferred to PVDF membranes. The membranes were incubated with an antibody which detects most acidic type I and all basic type II keratins (AE1/AE3) or an antibody which detects cytokeratin 9 (CK9), an acidic keratin not detected by AE1 and a major constituent of hardened epidermal structures. Immunoreactive proteins were visualized on x-ray film using a chemiluminescent secondary antibody system. Protein bands were quantified from dried, Coomassie blue stained gels and exposed x-ray film using laser densitometry image analysis. Comparisons of keratin expression among sampling sites showed no gross differences in size of keratins or amounts of the different keratins present. The CK9 antibody revealed a single band at approximately 68 kDa. Cytokeratin 9 has not been previously identified in bovine solar horn but the size of protein detected by the CK9 antibody corresponds to CK9 reported from other sources and species. In conclusion, keratin expression as detected using AE1/AE3 and CK9 antibodies does not vary in the bovine solar horn. However, the occurrence of CK9 in bovine solar horn further indicates that its expression may be functionally related to weight-bearing sites subject to mechanical stress and abrasion.

**Key Words:** Cytokeratin 9, Western-blotting, Sodium Dodecyl Sulfate Polyacrylamide Electrophoresis

**189 Evaluation of a decoquinate compound for ameliorating natural infections of *Cryptosporidia spp.* in neonatal dairy calves.** R. B. Thornton\*<sup>1</sup>, W. A. Greene<sup>1</sup>, D. R. Redman<sup>1</sup>, and J. E. Fox<sup>2</sup>, <sup>1</sup>*The Ohio State University, Wooster,* <sup>2</sup>*Rhone-Poulenc, Atlanta, GA.*

Thirty-one calves (17 Holstein, 14 Jersey) were alternately assigned to treatment groups at birth to determine the effectiveness of decoquinate (DECCOX<sup>®</sup>) for controlling natural infections of *Cryptosporidia spp.* (C). Twice daily for 28 d, 15 calves received a lactose-based premix containing 0.5% decoquinate (D) for a decoquinate dosage level of approximately 5 (3-6) mg/kg body weight/day and 16 calves received a similar amount of non-medicated lactose premix (L). Premixes were mixed in colostrum and milk replacer prior to feeding. Birth, weekly, and 30 d body weights were recorded. Fecal observations and samples were collected daily within 2 h after the morning feeding starting on d 3. Each sample was processed using an acid-fast stain to identify C oocysts, verified by a fluorescent antibody technique. Random samples were processed using a fluorescent antibody technique to identify *Giardia spp.* (G). Average daily gain (kg) was .45 for D calves and .48 for L calves ( $P=.55$ ). There was no difference ( $P=1.0$ ) in the prevalence of C between D (28.6%) and L (26.7%) groups. Days of abnormal stools, days to first C shedding, and total days of C shedding were similar ( $P>.05$ ) between groups: 3.9, 17.8, and 3.8 for D, respectively, and 4.0, 16.2, and 2.3 for L, respectively. The prevalence of G was 0% for D calves and 26.7% for L calves ( $P=.10$ ). With a low incidence rate of C, the addition of decoquinate to the milk diet of calves appeared to have nominal benefit.

**190 Determination of the effect of monensin on coccidiosis prevention and control in young calves.** D. L. Hancock\*<sup>1</sup>, E. E. Thomas<sup>1</sup>, J. T. Symanowski<sup>1</sup>, R. R. Marks<sup>1</sup>, K. A. Conrad<sup>1</sup>, T. H. Ferguson<sup>1</sup>, J. I. Wilkinson<sup>1</sup>, D. R. White<sup>1</sup>, J. A. Edwards<sup>2</sup>, K. F. Lechtenberg<sup>3</sup>, and C. A. Speer<sup>4</sup>, <sup>1</sup>*Elanco Animal Health and* <sup>2</sup>*Lilly Research Labs, Greenfield, IN,* <sup>3</sup>*Midwest Veterinary Services, Inc., Oakland, NE,* <sup>4</sup>*Montana State Univ., Bozeman.*

Ninety one Holstein male calves were purchased at 1 to 2 d of age and fed non-medicated milk replacer (weaned at 42 d) and starter feed until 9.5 wks of age (average 69 kg). Feeding of treatment diets (0, 11.1, 22.2 or 33.3 g/t monensin (DM basis) which resulted in monensin intakes of 0, 0.31, 0.61, and 0.93 mg/kg bw/d) was then initiated. Seven days later calves were challenged orally with ~300,000 oocysts (~231,000 *E. bovis*, 54,000 *E. zuernii* and 15,000 other *E. species*). Calves remained on monensin treatment for 28 d post challenge. Weights were determined weekly and intake, oocysts counts, and fecal scores were determined daily and averaged to weekly values prior to statistical analysis. These data were analyzed with a repeated measures analysis. Values in the table below are LS means averaged over the 4 weeks.

Item	Monensin intake (mg/kg bw/d)			
	0	0.31	0.61	0.93
Feed (DM) intake, kg/d	2.02	2.10 <sup>.447</sup>	2.07 <sup>.649</sup>	2.10 <sup>.443</sup>
Weight gain, kg/d	0.62	0.85 <sup>.004</sup>	0.75 <sup>.093</sup>	0.72 <sup>.173</sup>
Gain Efficiency, gain/feed	0.25	0.39 <sup>.009</sup>	0.38 <sup>.020</sup>	0.35 <sup>.060</sup>
Total oocysts, log <sub>10</sub> /g feces	.774	.253 <sup>&lt;.001</sup>	.197 <sup>&lt;.001</sup>	.151 <sup>&lt;.001</sup>
<i>E. bovis</i> oocysts	.712	.195 <sup>&lt;.001</sup>	.143 <sup>&lt;.001</sup>	.097 <sup>&lt;.001</sup>
<i>E. zuernii</i> oocysts	.452	.071 <sup>&lt;.001</sup>	.048 <sup>&lt;.001</sup>	.047 <sup>&lt;.001</sup>
Fecal score*	.222	.135 <sup>.111</sup>	.157 <sup>.239</sup>	.125 <sup>.076</sup>

Superscripts are the P-value of the treatment LS mean compared to controls. \* 0=Normal, 1=Slight diarrhea, 2=Diarrhea, 3=Diarrhea with blood, 4=Diarrhea with mucous/tissue. Analyzed to a square root basis. Total, *E. bovis*, and *E. zuernii* oocyst counts were significantly less while fecal scores were numerically less in all monensin treated groups relative to controls. Gain efficiency was greater than control at the point .31 dose and remained relatively constant at the higher monensin doses. Weight gain was increased by all monensin treatments with the 0.31 and 0.61 mg/kg bw/d intake levels being significant. Results show that monensin was effective for prevention and control of coccidiosis in calves at intakes ranging from 0.31 to 0.93 mg/kg bw/day.

**Key Words:** Coccidiosis, Monensin, Calves

**191 Feed related anemia in channel catfish fed *Bacillus thuringiensis*.** G. J. Burtle<sup>1\*</sup>, J. R. Cole, Jr.<sup>3</sup>, and G. W. Lewis<sup>2</sup>, *The University of Georgia*, <sup>1</sup>Tifton and <sup>2</sup>Athens, <sup>3</sup>*Vet. Diagnostic & Investigational Lab., Tifton, USA.*

Acute anemia in channel catfish (*Ictalurus punctatus*) has been attributed to bacterial contamination of feed. Cases of diet-related anemia are characterized by catfish populations with hematocrits averaging 20% and moribund individuals with pink blood and hematocrits below 10%. The disease has been theorized to occur after bacterial degradation of folate in catfish feed or in catfish digestive tracts. Diets containing *Bacillus thuringiensis* subsp. *kurstaki* (Bt) and diets made in part of feed from a diet-related anemia case were fed to channel catfish fingerlings in an attempt to produce acute anemia in the catfish. Treatments were replicated three times with tanks of 12–20 catfish fingerlings (10–15 g initially). After two weeks of feeding either 5,000 or 50,000 colony forming units/g diet, hematocrit (relative red blood cell volume) was significantly ( $P < .05$ ) lower (20–25%) in catfish fingerlings than hematocrits (25–35%) in catfish fed the control diet. Diets containing feed from the diet-related anemia case did not have significantly ( $P > .05$ ) lower hematocrits than controls after four weeks of feeding. However, *Bacillus* sp. were isolated from the internal organs of catfish fingerlings fed either Bt or case feed. Weight gain after 24 days was significantly ( $P < .05$ ) less (191 g) for tanks of catfish fed Bt compared to either those fed case feed (210 g) or control feed (212 g). Although Bt caused observable anemia, significant weight gain reduction, and became systemic in catfish fingerlings, the acute anemia observed in diet-related anemia of catfish was not observed. Case feed added as 25% of the diet was not enough to cause anemia.

**Key Words:** Anemia, Channel Catfish, *Bacillus thuringiensis*