981 Immunoglobulin binding in cows with Staphylococcus aureus mastitis. Amy Johnston-Ward*[1], Mulumebet Worku[1], Kevin Anderson[2], and Roberta Lymann[2], [1]*North Carolina Agricultural and Technical State University,* [2]*North Carolina State University College of Veterinary Medicine.*

Binding of immunoglobulins (Igs) to polymorphonuclear neutrophils (PMN) is important to the resolution of *Staphylococcus aureus* induced mastitis infection. The objective of this study was to assess the binding of Igs to PMN from cows with chronic *S. aureus* mastitis. Blood was collected from the jugular vein of six chronically infected and six healthy cows from the North Carolina Department of Agriculture- Caswell Dairy herd. The PMN were isolated by hypotonic lysis of red blood cells and differential centrifugation. PMN were then incubated with fluorescein-labelled bovine IgG1, IgG2 or IgM as sources of exogenous Igs. Neutrophils were also incubated with fluorescein-labelled goat anti-bovine IgG, IgG2 or IgM to assess endogenously bound Igs. Binding of Igs and level of Fc receptor expression were evaluated by flow cytometric analysis. The percentage of PMN from cows with chronic *S. aureus*infection that bound IgG1 was 29, IgG2 was 60, IgM was 52, anti-IgG was 74, anti-IgG2 was 73, and anti-IgM was 73. The percentage of Igs bound to PMN from healthy cows was 15, 54, 55, 72, 70, and 62 respectively. The level of receptor expression for cows with chronic *S. aureus* infection was 40 for IgG1, 90 for IgG2, 61 for IgM, 110 for anti-IgG, 73 for anti-IgG2, and 176 for anti-IgM as compared with the healthy cows with 30, 53, 106, 123, 75, and 159 respectively. Binding of endogenous Igs was not significantly different between healthy and chronically infected cows. Binding and Fc receptor expression for IgG1 was not significantly different between healthy and chronically infected cows. Although binding of IgG2 and IgM did not differ between the two groups, the level of expression of Fc receptors for IgM and IgG2 was significantly decreased in cows with chronic *S. aureus* mastitis. Both IgM and IgG2 are critical isotypes for PMN phagocytic function. These results may have significant implications for neutrophil function during mastitis infection, which may contribute to the chronic nature of *S. aureus* infection in these cows.

Key Words: Immunoglobulin, Mastitis, Fc receptor

982 Effect of time and frequency of administration of ketoprofen during surgical castration of beef cattle. S. T. L. Ting*[1,2], B. Earley[1], and M. A. Crowe[2], [1]*Teagasc, Grange Research Centre, Dunsany, Co. Meath,* [2]*Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4, Ireland.*

The effect of time and frequency of administration of ketoprofen (**K**) to surgically castrated calves on cortisol, acute phase proteins, immune function, and performance was determined. Fifty Holstein x Friesian bulls (11 mo of age; BW = 300 3.3 kg) were assigned to one of five treatments: 1) untreated control (**C**); 2) surgical castration (t = 0 min; **S**); 3) S following K dose at t = -20 min with 3 mg/kg BW i.v. (**S + K1**); 4) S following K at t = -20, and at 0 min with 1.5 mg/kg BW at each time point (**S + K2**); 5) as in (4), with K at 3 mg/kg at t = 24 h post treatment (**S + K3**). The area under the plasma cortisol against time curve was greater (P<.05) in all castrated calves than in controls; and was lower (P<.05) in S + K1 and S + K2, with intermediate levels in S + K3 compared with S calves. Peak cortisol levels were higher (P<.05) in all the castration groups than in C. There were no differences (P>.05) in interval to peak cortisol within the castration groups. On d 3, plasma haptoglobin and fibrinogen concentrations were higher (P<.05) in all the castrated animals than in C. There were no differences (P>.05) among treatments in KLH-induced interferon-γ (**IFN-**γ) production on d 0, 1, and 3. On d 1, Con A-induced IFN-γ production was lower (P<.05) in S and S + K3 than in C and S + K1, with intermediate levels in S + K2 calves. ADFI from d 1 to 33 was lower (P<.05) in S, S + K1, and S + K3, but not in S + K2 compared with C. ADG from d -1 to 35 was lower (P<.05) in S, S + K2 and S + K3, but not in S + K1 compared with C calves. In conclusion, surgical castration increased plasma cortisol and acute phase proteins, suppressed cell-mediated immunity, and reduced performance. K effectively reduced cortisol following castration, but there was no advantage in treating with split doses of K (S + K1 or S + K2 group). A repeated K dose 24 h post treatment (S + K3) failed to influence the changes in acute phase proteins and immune response.

Key Words: Cattle, Castration, Ketoprofen

983 Effect of body condition loss on cholesterol concentration and occurrence of postparturient diseases in holstein cows. I. H. Kim*[1], G. H. Suh[2], and D. S. Son[2], [1]*Chungbuk National University, Chongju, Chungbuk, Korea,* [2]*National Livestock Research Institute, Cheonan, Chungnam, Korea.*

This study was to investigate the relationship between amount of body condition score (BCS) loss from dry to early lactation period and serum cholesterol concentration and occurrence of postparturient diseases in holstein cows. Body condition scoring (using a 5-point scale with quarter-point divisions) was performed on sixty pregnant holstein cows. They were maintained in free-stall facilities, fed a total mixed ration. Cows were scored once for body condition during the dry period, near calving, and then every 1 month for early lactating 3 months. At the same time, blood samples were collected to evaluate serum cholesterol concentration. Regular reproductive health examinations were conducted by 1 veterinarian twice a month. Cows were categorized by BCS loss from dry to early lactation period into 3 groups : modest (0 to 0.75 points, n=21), moderate (1.0 to 1.25 points, n=21), or marked (1.5 to 2.5 points, n=18). Cholesterol concentration was lower (P<0.05) in marked BCS loss group (159 $\pm$35 mg/dl) than in modest BCS loss group (196$\pm$45 mg/dl) on Month 1 after calving. Occurrence of postparturient diseases were greater (P<0.01) in moderate BCS loss group (66.7%) and marked BCS loss group (83.3%) than in modest BCS loss group (23.8%). It is concluded that serum cholesterol concentration and occurrence of postparturient diseases were related to amount of BCS loss from dry to early lactation period in holstein cows.

Key Words: Body condition score, Cholesterol concentration, Postparturient disease

984 Autoclaved ruminal fluid immediately after birth improves the growth and health of neonate dairy calves. J.B. Russell*[1], T.V. Muscato[2], and L.O. Tedeschi[2], [1]*ARS/USDA,* [2]*Cornell University.*

Recent work (Muscato, T. V., L. O. Tedeschi, and J. B. Russell. 2002. The effect of ruminal fluid preparations on the growth and health of new-born dairy calves. J. Dairy Sci. in press) showed that ruminal fluid (RF) supplements (8 ml per d) decreased (P <0.05) the incidence of scours in dairy calves that were consuming milk or milk replacer, and this decrease was accompanied by an increase in BW gain during the first 2 wk of life (P <0.05). Because autoclaved ruminal fluid was also effective (P < 0.05), RF was not acting as a probiotic. When the time of dosage was decreased from 42 to 5 days, the calves still responded. A new trial was conducted to determine more precisely the minimum dosage of autoclaved RF needed to improve the health and growth of dairy calves. New-born calves were randomly allotted to 2 treatment groups (n =12). One group served a control and it did not receive any RF. The treatment group received 4 ml of autoclaved RF in the first clostrum via stomach tube. Both groups were fed equal amounts of milk replacer (7.5 kg/d, 3 feedings/d). Calves were: 1) inspected 5 times per day for scours, 2) weighed at birth and at 2, 4 and 6 wks of age, and 3) weaned at 6 wks. Scours were defined as fresh fecal material that had a runny or watery texture and either a white or grey color. Calves given only a single does of autoclaved RF immediately after birth had fewer scours days (0.5 versus 1.81 d/calf, P < 0.05) and gained more weight in the first 2 wk of life (5.24 versus 2.84 kg, P < 0.05). Because only a single does was required, autoclaved RF is a practical tool for improving the health and growth of new-born calves.

Key Words: Calves, Rumen Fluid, Scours

985 Transition period in dairy cows: immune system, inflammatory conditions and liver activity. L. Calamari, F. Librandi, E. Trevisi, and G. Bertoni*, *UCSC, Facolta di Agraria, Piacenza, Italy.*

Some keys of success in transition period are linked to the energy metabolism, namely of lipids, and to the immune system. The immune

system cytokines seem to impair lipid metabolism in the liver. In order to improve the knowledge on this topic a trial was carried out on 10 Italian Friesian dairy cows checked from 30 days before calving to 60 DIM. DMI and milk yield were recorded daily; blood samples were collected daily around calving (-4 to 4) and twice weekly in the other phases. Piacenza metabolic profile, NEFA, BHBA, tryglicerides, creatinine, vitamin A and E, NO_3, NO_2, lysozyme, reactive oxygen metabolite substances (ROM), TBARS and total antioxidants (AT) were evaluated on plasma. According to an aggregate liver activity index (LAI), that includes liver synthesis parameters (albumin, total cholesterol and vitamin A as index of RBP) observed during the first 30 DIM, the cows were partitioned in 2 groups (S: satisfactory; U: unsatisfactory). Despite higher values of AT ($P<0.01$) and lysozyme (n.s.) before calving, the cows of U group have shown a slightly higher incidence of troubles after calving (retained placenta, uterine diseases and digestive upsets). According to this, higher values of haptoglobin, ceruloplasmin ($P<0.05$) and ROM ($P<0.01$) in the first 15 DIM were found in U. Lower DMI and plasma urea ($P<0.01$) in the first 30 DIM were also found in U. Furthermore, higher GOT values ($P<0.05$) in the first 15 DIM and lower of albumin, vitamin A ($P<0.001$) and cholesterol (n.s.) in the first 60 DIM were found in U group, confirming a liver impairment. Finally cows of U group have had a slightly lower milk yield. In conclusion quite common health problems of the transition period causing inflammation seem to play an important role in the liver activity and cow performances. Nevertheless it is difficult to discriminate the primary cause: i.e. is the immunity impairment first or is the stress disease that cause an impairment of liver and immune system? (Supported by MURST, Cofin. 2000).

Key Words: Dairy Cow, Liver Activity, Immune System

986 Testing measures of lameness: using behaviour to predict presence and severity of hoof lesions in dairy cattle. F Flower* and D Weary, *Animal Welfare Program, Faculty of Agricultural Sciences, Univeristy of British Columbia*.

Lameness is costly to production and compromises the welfare of dairy cows, but is often difficult to identify especially at the early stages. We determined how successful existing and novel lameness assessment methods were in predicting the presence and severity of sole lesions. Lactating Holstein cows (n = 46), trained to walk along a concrete alley, were assessed for lameness from video records over 7 consecutive days. Three types of methods were tested: a) composite subjective scores b) computerized analysis of cow movements and c) measures of free-stall use. Composite subjective scores were assigned to each cow using video records. Scores were based on the extent of back arch, tracking up, head bob, joint flexion and the ability to bear weight. Each of these components was also assessed separately using a visual analogue scale. Computerized movement data including heel strike, toe-off, stride length and duration, stance and swing period, gait velocity and cadence were calculated using Peak Motus motion analysis software. Vertical displacement of vertebrae were calculated to quantify back arch. Video records of free-stall use were collected over 6 weeks and used to measure time spent lying in the stall, standing in the stall, and standing with only the front two feet in the stall. The number and severity of hoof lesions were scored 10 weeks later. Of the subjective measures, the composite subjective score best predicted the presence of sole lesions, accounting for 33% of the variation in a step-wise multiple regression model ($P < 0.001$). Of the computerized movement measures, hoof speed and variation in stride height best predicted sole lesions, together accounting for 34% of the variation in lesion number ($P < 0.01$). Of the stall usage measures, standing with two feet in the stall accounted for 41% of the variation in lesion number ($P < 0.001$). Thus all three methods are able to predict the number of sole lesions, one important component of lameness in dairy cattle.

Key Words: Lameness, Behaviour Scoring, Locomotion

987 Determining the incidence of Johnes Disease in Maine dairy herds using three ELISA tests. D.P. Marcinkowski[1], G. W. Anderson[1], M. M. Bryant*[1], and D. E. Hoenig[2], [1]*University of Maine, Orono,* [2]*Maine Department of Agriculture, Food and Rural Resources, Augusta*.

The objectives of this study were to compare the results of 3 serologic ELISA tests for Johnes Disease and determine the extent of the disease in Maine dairy herds. Six practicing veterinarians identified 25 commercial dairy herds from throughout the state. Herds were divided into 2 groups by herd size; small herds (less than 200 cows) and large herds (more than 200 cows). In the 22 small herds, up to 30 multiparous cows were sampled while in the 3 large herds up to 125 multiparous cows were sampled. A total of 1017 plasma samples were collected, representing 2.4% of the total dairy cows in the state. Plasma samples from each cow were divided into 3 aliquots and frozen for subsequent analysis. The samples were analyzed using two commercially available tests; the Tip-Test: Johne's (ImmuCell Corporation, Portland, ME), and the HerdChek-Mycobacterium paratuberculosis Antibody ELISA (Idexx Laboratories, Inc. Westbrook, ME). Samples were also analyzed at the Cornell University Veterinary Medicine Diagnostic Laboratory using a Johne's Kinetic ELISA (KELA). Results showed the percent seropositive cows to be 16.9, 3.8 and 18.7 for the Tip-Test, HerdChek and KELA respectively. Comparisons of the HerdChek versus KELA, Tip-Test versus HerdChek and, Tip-Test versus KELA showed test results disagreed in 18%, 19% and 27% of the samples respectively. Herd size did not effect the percent positive cows. These tests are valuable tools for screening individual cattle and assessing the overall risk of Johne's Disease in a herd. More definitive tests should be used when making management decisions about individual cows.

Key Words: Johnes, ELISA

988 Retrospective associations of prepartum intake, body condition score, body weight, and blood chemistry with the occurrence of retained placenta in dairy cows. H. M. Dann*, J. K. Drackley, and D. E. Morin, *University of Illinois, Urbana*.

In a previous study, multiparous Holstein cows were used to determine the effects of prepartum dry matter intake (DMI) on postpartum DMI, milk yield, blood chemistry, and liver composition. Because a high incidence of retained placenta (RP) occurred, a retrospective analysis was conducted to determine associations between RP and prepartum variables such as DMI, body condition score (BCS), body weight (BW), blood chemistry, and gestation length. Cows were dried off 60 d before expected parturition. Cows were fed a diet (1.54 Mcal NE_L/kg, 14.1% CP) from dry off to parturition at either ad libitum (A; n=16) or restricted (R; 80% of calculated NE_L requirements; n=17) intake. After parturition, cows were classified as having either expelled the placenta within 12 h (EP; n=17) or retained the placenta (RP; n=16). Seven cows fed A had RP and nine cows fed R had RP. Prepartum DMI (1.9 vs 1.1% of BW), serum glucose (60 vs 57 mg/dl), and serum insulin (8.2 vs 4.6 μIU/ml) were higher ($P<0.05$), and serum nonesterified fatty acids (NEFA; 156 vs 300 μEq/L) were lower ($P<0.05$) for cows fed A compared to R. Prepartum intake did not affect ($P>0.05$) serum β hydroxybutyrate (BHBA; 4.1 mg/dl), BCS (3.09), BW (738 kg), or gestation length (calved 2.7 d before expected date). No prepartum differences were detected ($P>0.05$) for DMI (1.5% of BW), BW (738 kg), or concentrations of glucose (59 mg/dl), insulin (6.4 μIU/ml), NEFA (228 μEq/L), and BHBA (4.1 mg/dl) in serum between EP and RP cows. Cows with RP had a lower ($P<0.05$) prepartum BCS (2.74 vs 3.43) than EP cows. Cows with RP had a shorter gestation than EP (calved 4.6 vs 0.8 d before expected date; $P<0.05$). Five RP cows had twins whereas one EP cow had twins. There was no interaction of prepartum intake (A vs R) and the occurrence of RP for prepartum DMI, BCS, BW, serum glucose, serum insulin, serum NEFA, or gestation length. Cows with low BCS during the prepartum period may have more RP regardless of prepartum DMI and associated factors.

Key Words: Body Condition, Retained Placenta, Intake

989 Correlation between liver dry matter and liver lipid concentrations in periparturient dairy cows. O. Rosendo*[1], C. R. Staples[1], and L. R. McDowell[1], [1]*University of Florida*.

Liver dry matter percent (DM) has been used to estimate total lipid concentrations on liver wet weight basis (TLwet) in other species than ruminants. To develop lipid concentration predictive equations from liver DM in periparturient dairy cows, data from an experiment that evaluate the effect of supplemental biotin on performance of transition cows were used. Liver samples were obtained from 40 multiparous Holstein cows at # 17, + 2, + 14, and + 28 d, relative to calving. A total of 425 liver aliquots were analyzed for TLwet (100 mg) by solvent extraction followed by chemical determination of triacylglycerol concentrations on wet weight basis (TAGwet). Liver DM was determined on a separate aliquot (100 mg) after drying for 24 h at 55 °C in a forced air oven.

A total of 150 TLwet, TAGwet, and DM averages were analyzed using correlation, regression and general linear model procedures of SAS. Concentrations of TLwet (range = 2.9-14.9%) and TAGwet (range = 0.71-10.41%) were highly correlated ($P < 0.001$) with liver DM percent (range = 20.0-36.2%). The regression coefficients for the TLwet and TAGwet simple linear equations as a function of DM (over the entire ranges of DM) were 0.42 and 0.66, respectively. The best-fitting models to describe the relationship between DM and liver lipids using the entire data set were the following second-order polynomial derived equations. For TLwet = 44.268 - (3.3625 x DM) + (0.0717 x DM^2)(R^2 = 0.53%; $P < 0.0001$) and for TAGwet = 39.983 - (3.2370 x DM) + (0.0678 x DM^2)(R^2 = 0.58%; $P < 0.0001$). Above liver DM of 25%, the third-order polynomial equations that follow: TLwet = 681.635 - (67.3989 x DM) + (2.2029 x DM^2) - (0.0235 x DM^3) (R^2 = 0.56%, $P < 0.0001$) and TAGwet = 639.039 - (63.6490 x DM) + (2.0857 x DM^2) - (0.0223 x DM^3) (R^2 = 0.61%, $P < 0.0001$) were the best-derived models. The use of these predictive equations for estimation of liver lipid concentrations may contribute to examine fatty liver problems in dairy herds while decreasing greatly the amount of time spent and cost of analysis involved in.

Key Words: Liver dry matter, liver lipids, correlation

990 Influence of *Lactobacillus brevis*1E-1 on the gastrointestinal microflora of pre-weaning and weaning pigs. S Banach*[1], T Rehberger[1], T Parrott[1], C Maxwell[2], J Coalson[3], and K Touchette[3], [1]*Agtech Products, Inc.*, [2]*University of Arkansas*, [3]*Merrick's, Inc*.

Maintaining a normal healthy intestinal microflora during the profound environmental and nutritional changes at weaning is critical to ensure optimal performance for pigs. The objective of this study was to determine the effects of feeding *Lactobacillus brevis* 1E-1 on the gastrointestinal microflora of pre-weaning and weaning pigs. Sows and gilts were randomly assigned to one of three treatments. Four litters received no milk replacer (control), five litters received milk replacer and five litters received milk replacer supplemented with 1E-1. Coliforms and *E. coli* were enumerated from esophageal, duodenal, jejunal, and ileal regions of intestinal tracts from one pig per litter at 9-13 (pre-weaning) and at 19-23 (weaning) days of age. *E. coli* and coliform populations in esophageal, duodenal and ileal regions of pre-weaning pigs were not significantly different. Pigs receiving 1E-1 had significantly lower jejunal *E. coli* populations compared to control (P< .02) and milk replacer (P< .05). Jejunal coliform populations tended to be lower for pigs receiving 1E-1 compared to control pigs (P= .11) but were not significantly different compared to pigs receiving milk replacer. There were no treatment effects on populations of coliforms and *E. coli* in the esophageal and duodenal regions for pigs at weaning. Pigs receiving 1E-1 had significantly lower *E. coli* populations in the jejunal region compared to control (P< .01) and milk replacer (P< .10). There were no significant treatment effects on jejunal coliform populations for pigs at weaning. In the ileal region of weaning pigs, the coliform populations neared significance for pigs receiving 1E-1 when compared to control (P= .07). *E. coli* populations were significantly lower for pigs receiving 1E-1 compared to control pigs (P< .05) and pigs receiving milk replacer (P< .02). These results suggest that feeding 1E-1 may provide a healthier intestinal microflora at weaning.

Key Words: Pigs, Weaning, Lactobacillus

991 Endotoxin (LPS) challenge increases plasma xanthine oxidase (XO) activity in cattle: effect of growth hormone (GH) and vitamin E (E) treatment. S. Kahl* and T.H. Elsasser, *USDA, Agricultural Research Service, Beltsville, MD*.

In addition to its basic role in the metabolism of purine nucleotides, XO is involved in the generation of oxygen-derived free radicals and production and metabolic fate of nitric oxide (NO), an important component and regulator of the immune response to infection. Our objective was to determine the effect of LPS challenge (3.0 μg/kg BW, i.v. bolus, *E. coli* 055:B5) on plasma XO activity. We also studied the modifications of this response by daily treatment with recombinant GH (0.1 mg/kg BW, i.m., for 12 d) and E (mixed tocopherol, 1000 IU/d, i.m., for 5 d). Sixteen heifers (348.7 ± 6.1 kg) were fed a forage concentrate diet (15% CP) to appetite, and synchronized to a similar stage of the estrous cycle with two injections of $PGF_{2\alpha}$. Heifers were assigned to control (C, daily corn oil and saline-bicarbonate injections), GH, or GH + E treatments. All heifers were challenged with LPS 8 d after the last injection of $PGF_{2\alpha}$ (LPS1) and again 2 d later (LPS2). Blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 24, and 48 h relative to LPS injections. After LPS1, plasma XO activity (mU/mL) increased ($P < 0.001$) from 7.2 at 0 h to 28.1 at 4 h, reached peak (38.2) at 24 h and returned to basal level by 48 h after LPS2. LPS1 XO responses, measured as area under the time × concentration curve (AUC), were greater than those in LPS2 ($P < 0.001$). Total plasma XO responses to LPS (AUC, LPS1 + LPS2) were augmented over C with GH treatment (2202 vs 1412 mU/mL × h, SEM = 226, $P < 0.05$) but diminished to C responses in GH + E. There was a linear relationship ($r^2 = 0.605$, $P < 0.001$) between total response (LPS1 + LPS2) in plasma XO activity and plasma nitrate + nitrite (stable end products of NO) concentration. Results indicate that LPS-induced increases in plasma XO activity could be amplified by previous GH treatment but attenuated by E administration. The data also suggest that E may be effective in controlling some mediators of immune response associated with increased production of NO.

Key Words: Endotoxin, Xanthine oxidase, Vitamin E

992 Measurement of Bovine inflammatory cytokines by RT-PCR using an ex-vivo whole blood assay: Relevance to endotoxin levels in animal pharmaceuticals. M. L. Scott* and M. J. Myers, *U.S. FDA, CVM, Division of Animal Research*.

FDA requires that sterile products meet the guidelines for pyrogen levels. However, due to a lack of adequate information in food animals a very conservative approach has been set for animal drugs. The objective was to develop an in vitro method to generate data to help refine these guidelines. Blood collected from ten Holstein cows were cultured at 37°C in Ultraculture media for 0, 1, 3, 6, 12, 24, 48, and 72 hours with 1 ug/ml LPS. RNA was extracted and expression by RT-PCR used to analyze cytokine genes. Time course studies indicated that IL-6 and TNF-α hit a plateau between 1 and 3 hours. Blood from nine Holstein cows were cultured for 3hrs with LPS concentrations of 0, 1ug, 100ng, 10ng, 1ng, 100pg, 10pg, 1pg, and 100fg. Surprisingly, IL-6 at 100fg were 7x higher than no LPS, providing evidence that very low concentrations of endotoxin can provoke an inflammatory response. These values are greater than 10-fold more sensitive than current FDA approved pyrogen testing kits. In conclusion, the novel bioassay developed within are very sensitive and provide evidence that inflammatory responses can be triggered by very small quantities of LPS.

Key Words: Endotoxin, RT-PCR, Cytokine

993 Preparation and characterization of monoclonal antibodies to recombinant bovine CD14. E. J. Sohn*[1], M. J. Paape[1], and R. R. Peters[2], [1]*Immunology and Disease Resistance Laboratory, USDA-ARS, Beltsville, MD*, [2]*Department of Animal and Avian Sciences, University of Maryland, College Park*.

Lipopolysaccharide (LPS) is the predominant factor causing pathogenesis in intramammary infections in dairy cows by Gram-negative bacteria. Cluster of differential antigen 14 (CD14) mediates cellular responses to LPS. Information on the functional role of CD14 in the intramammary response to Gram-negative bacterial infection is limited. We have previously cloned and expressed recombinant bovine CD14 (rbosCD14) in a baculovirus/Sf-9 insect cell system. The objective of this study was to produce anti-rbosCD14 mAb in order to characterize the role of CD14 in intramammary infections by Gram-negative bacteria. Ten murine mAb reactive with rbosCD14 were produced, and an ELISA using rbosCD14, anti-rbosCD14 and goat anti-mouse IgG conjugated to horseradish peroxidase was developed. The mAb were further characterized by Western blot and flow cytometry. The mAb bound specifically to CD14 derived from SF-9 cells and identified a 55 kDa polypeptide band by Western blot. Flow cytometric analysis revealed that the mAb derived from cell lines 6-6-1(IgG_{2b}) and 1-54-2(IgG_1) bound to 80% of the leukocytes in a monocyte enriched preparation from bovine blood. The anti-rbosCD14 mAb generated in this study will provide useful reagents for studies on LPS-CD14 interrelationships during experimentally induced mastitis by Gram-negative bacteria and LPS.

Key Words: Mastitis, CD14, LPS

994 Comparisons of functional capacities of blood mononuclear leukocytes (MNL) and neutrophils (PMN) from calves and heifers vaccinated with attenuated *Mycobacterium bovis* (BCG). M. Foote*[1], B. Nonnecke[1], W. Waters[1], T. Rahner[1], M. Palmer[1], W. Miller[2], M. Fowler[2], T. Johnson[2], B. Perry[2], and D. Hammell[2], [1]*National Animal Disease Center, USDA, ARS, Ames,* [2]*Land O'Lakes, Inc., Webster City.*

Development and modulation of immune competency in calves during the first months of life is not well described. The purpose of this study was to characterize age-related changes in the functional capacities of PMN and MNL populations from young calves. Milk replacer-fed calves were nonvaccinated (NVAC, n=12) or vaccinated subQ (VAC, n=12) with BCG at 1 and 7 wk of age. Functions of PMN and MNL populations from blood samples collected at 0 (vaccinated), 2, 5, 6 (boosted), 7, 8 and 11 wk of the study period were evaluated *in vitro*. Yearling heifers (n=4) were vaccinated and sampled concurrently with the calves. DNA synthesis by nonstimulated calf MNL exceeded (P<.05) synthesis by nonstimulated adult MNL from wk 2-11. Pokeweed mitogen-induced DNA synthesis by calf MNL was lower (P <.05) than adult MNL at wk 0 only. Responses of VAC MNL to eliciting antigens (PPD and *M. bovis* whole cell sonicate) were evident at >2 wk after primary vaccination and frequently were not different from adult MNL demonstrating competency of adaptive-arm of the neonatal calf's immune system. Quantification of CD4, $\gamma\delta$ TCR+, and CD8 T cells in 48 h cultures by flow cytometry (wk 6 only) indicated that vaccination, age (calf vs. adult), and type of stimulation affected (P<.05) cell proliferation. Changes in cervical skin-fold thickness after intradermal administration of PPD (wk 11) were pronounced and comparable in VAC and adults. Nonvaccinated calves did not respond to PPD. PMN function (iodination and cytochrome C reduction) was affected by age (P<.05); but not vaccination. These results indicate that the calf vaccinated at 1 wk of age is capable of developing a vigorous response to antigenic challenge *in vitro* and *in vivo*. Hyporesponsiveness of the neonate's PMN and MNL populations to non-antigenic stimulation may be linked to its developmental immaturity.

Key Words: Calf, Lymphocyte, Neutrophil

995 DNA vaccination in dairy cows: I. Effect of targeting a DNA vaccine to professional antigen presenting cells using bovine CTLA-4 sequences. L. Shkreta*[1], B.G. Talbot[1], and P. Lacasse[2], [1]*Sherbrooke University, Sherbrooke, QC, Canada,* [2]*AAFC - Dairy and Swine R&D Centre, Lennoxville, QC, Canada.*

The objective of this study was to determine the immune response to a DNA vaccine targeted to professional antigen presenting cells. 36 Holstein cows, 60 d prior expected calving (d 0), were randomly assigned to a 3 X 4 factorial design where the main effects were the site of immunization and the type of plasmid injected. Cows were vaccinated with the plasmid expression vector pCI alone (control), a plasmid encoding the bacterial antigen β-galactosidase (pCI-βgal) or a plasmid encoding a fusion between bovine T lymphocyte antigen 4, human IgG hinge region and β-gal (pCI-bCTLA-βgal). Animals were needle injected either in the neck and gluteus muscles, in the skin of the ear, in the mammary gland or in the supra-mammary-lymph node. Cows were immunized three times at 21 day intervals, with 1 mg of DNA per injection. Indirect ELISA was used to monitor anti-β-gal antibody responses in serum and milk. Lymphoproliferation and lymphocyte phenotype profiles were analyzed to evaluate cellular immunity. Increased antibody responses in serum confirmed the induction of specific immune responses (P<0.05) against β-gal for both DNA vaccines with a predominance of the IgG2 isotype responses over the IgG, IgG1 and IgM isotype responses in decreasing order. The IgG1, IgG2 and IgM responses were similar, but cows injected with pCI-bgal tended to have a higher IgG response (P=.07) than cows immunized with pCI-bCTLA-βgal. No β-gal specific IgG, IgG1, IgG2 and IgA responses were clearly detectable in milk The lymphocyte proliferation index (LPI) indicated that both plasmids induced (P<0.001) a cellular response. The LPI was higher (P<0.01) with pCI-βgal (3.3) than with pCI-bCTLA-βgal (2.53; control=1.0). The ratio of CD4 to nave immune cells was increased by both vaccines (P<0.001) while the ratio of CD4 to CD8 lymphocytes was significantly increased (P<0.01) in pCI-bCTLA-bgal only. In conclusion, plasmid DNA vaccines encoding the bacterial antigen β-gal were able to elicit significant humoral and cellular responses in dairy cows. However, despite changes in the profile of the immune response, the overall effect was not clearly enhanced by fusing the antigen with the professional antigen presenting cell targeting sequence bCTLA-4.

Key Words: DNA vaccine

996 DNA vaccination in dairy cows: II. Effect of injection site on immune responses to plasmid DNA immunization. L. Shkreta*[1], B.G. Talbot[1], and P. Lacasse[2], [1]*Sherbrooke University, Sherbrooke, QC, Canada,* [2]*AAFC - Dairy and Swine R&D Centre, Lennoxville, QC, Canada.*

The site of immunization appears to influence the immune response to plasmid DNA vaccination. The objective of this study was to evaluate the effect of immunization site on immune responses to plasmid DNA immunization. Twenty-four Holstein cows 60 d prior to expected calving (d 0) were randomly assigned to a 2 X 4 factorial design where the main effects were the site of immunization and the plasmid injected. Cows were vaccinated by needle injection either in the neck and gluteus muscle (IM), in the ear skin (ID), in the mammary gland (IMGld) or in the supra-mammary-lymph node (ILN), with either pCI-βgal or pCI-bCTLA-4-IgG-βgal, encoding non-secreted and secreted forms respectively of the bacterial antigen, β-galactosidase. Animals were injected three times, at 21 day intervals, with 1 mg of DNA per injection. The level of β-galactosidase antibodies in the serum was evaluated at d 0 to d 110. As expected, both plasmids induced significant immune responses. Cows injected IM and IMGld tended to have higher humoral responses than cows immunized ID or ILN. For IgG and IgG-1 isotype responses, the area under the curve for IM, ID, IMGld and ILN, averaged 7.8, 2.7, 6.6 and 3.1 for IgG and 2.6, 1.4, 3.6 and 1.5 for IgG-1. The injection site of the DNA vaccine did not significantly affect the magnitude of the IgG2 and IgM antibody responses, although a similar trend to the IgG results was observed. The ratio of IgG2/IgG1 isotype responses indicated the predominance of IgG2 responses over IgG1 for each site of injection, being the highest for intramuscular and the lowest for intramammary gland injection. The lymphocyte proliferation index and lymphocyte phenotype profiles were not affected by the injection site. These results suggest that for DNA vaccination by needle injection in dairy cows the injection site does not appear to be a determining factor for the immune responses. Thus the vaccination site can be chosen for practical rather than immunological reasons.

Key Words: DNA vaccine

997 Recombinant bovine soluble CD14 reduces fatality of endotoxin challenged mice. J. W. Lee*[1], X. Zhao[1], and M. J. Paape[2], [1]*Department of Animal Science, McGill University, Quebec, Canada,* [2]*Immunology and Disease Resistance Laboratory, USDA-ARS, Beltsville, MD.*

Endotoxin, or lipopolysaccharide (LPS), has been demonstrated to be responsible for the pathogenesis of Gram-negative bacterial infections, such as bovine coliform mastitis. The cellular response to LPS is modulated by the interaction among LPS, LPS-binding protein and CD14. The production of inflammatory cytokines, including TNF-α , by LPS-activated monocytes/macrophages leads to an overwhelming systemic response and causes death in severe cases. Accumulated evidence shows that the soluble form of CD14 (sCD14) competes with membrane-bound CD14 for LPS and inhibits cell activation. To investigate the protective effect of sCD14, recombinant bovine sCD14 (rbosCD14) was produced by transfected insect sf/9 cells and its biological function was evaluated in mice. Eighty-one 8-wk old BALB/cj female mice were randomly assigned to two groups, and injected intraperitoneally with either LPS (8 μg/gBW, n = 41) or LPS plus rbosCD14 (6.8 μg/gBW, n = 40). Survival rate for LPS and LPS plus rbosCD14 injected mice at 24 h was 30 and 72%. At 48 h survival rates were 7 and 37%. Results indicated that rbosCD14 was able to decrease the fatality of LPS challenged mice. These results suggest use of rbosCD14 as a therapeutic agent for neutralizing LPS during acute endotoxin shock in ruminants.

Key Words: Endotoxin, CD14, Mastitis