50 Osteopontin immunoreactivity in peripheral blood mononuclear cells, ileum, and ileocecal lymph node of dairy cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*. E. L. Karcher*[1], C. S. Johnson[1], J. P. Bannantine[2], D. C. Beitz[1], and J. R. Stabel[2], [1]*Iowa State University*, *Ames*, [2]*USDA-ARS, National Animal Disease Center*, *Ames, IA*.

Osteopontin (Opn), a highly acidic glycoprotein, plays a role in initiating the innate immune response to mycobacterial infections by promoting cellular adhesion and recruitment of inflammatory cells from the peripheral blood. The formation of granulomas at the site of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection is critical for the early control of infection. The objective of this experiment was to identify Opn in peripheral blood mononuclear cells (PBMCs), ileum and ileocecal lymph node (ICN) of dairy cows naturally infected with MAP and to compare the frequency and intensity of staining between noninfected healthy controls, subclinical, and clinical cows. For analysis of peripheral blood, PBMCs were isolated weekly from naturally infected periparturient dairy cows 3 weeks prior to calving through 4 weeks postpartum. Western blot detected Opn protein bands at 24-, 37-, 50-, and 62-kDa in the PBMC lysates of all animals. The densities of the 24-, 37-, and 62-kDa proteins varied extensively between cows and the variation did not seem to be related to infection group. Immunohistochemical analysis was used to determine the location and expression of Opn in the ileum and ICN. The frequency and intensity of staining also was reported. Confirmation of the acid-fast bacilli in the tissue sections was achieved by the Ziehl-Neelsen method. Within the ileum, macrophages, lymphocytes, and plasma cells stained positive for Opn. Clinical cows expressed Opn at a greater frequency in the lamina propria. Control and subclinical cows did not have areas of granulomatous inflammation, but cells staining for Opn were equally intense for the three groups. Osteopontin expression in the ICN was not affected by MAP infection. Results of this study confirm for the first time Opn localization in the peripheral blood and in the intestinal tract of MAP-infected cows and differences in Opn expression at the site of infection.

Key Words: Osteopontin, *Mycobacterium avium* subsp. *paratuberculosis*

51 Effect of linoleic acid and dietary vitamin E supplementation on sustained conjugated linoleic acid production in milk fat from dairy cows. A. M. O'Donnell*, N. S. Mittelman, J. L. Capper, and D. E. Bauman, *Cornell University*, *Ithaca, NY*.

Conjugated linoleic acid (*cis*-9, *trans*-11 18:2, CLA), a bioactive fatty acid (FA) found in milk and dairy products, has potential human health benefits due to its anticarcinogenic and antiatherogenic properties. Milk fat CLA concentrations can be markedly increased by dietary manipulation; however, high levels of CLA are difficult to sustain as rumen biohydrogenation shifts and milk fat depression (MFD) is induced. The objective of the present study was to feed a typical Northeastern corn based-diet and investigate whether vitamin E and soybean oil supplementation would sustain an enhanced milk fat CLA content while avoiding MFD. Holstein cows (n = 48) were assigned to a randomized complete block design for 28 d and received one of four treatments: 1) control (C), 2) 10,000 IU/d vitamin E (E), 3) 2.5% soybean oil (Oil), and 4) 10,000 IU/d vitamin E plus 2.5% soybean oil (Oil/E). A 2 wk pre-treatment control diet served as the covariate. Milk fat percent was reduced by both high oil diets (3.52, 3.55, 2.94, and 2.98% for C, E, Oil, and Oil/E, respectively). However, milk yield was increased by the Oil/E diet, therefore milk fat yield was lowest in cows fed the Oil diet (1.35, 1.35, 1.06, and 1.25 kg/d for C, E, Oil, and Oil/E). Milk protein percent was higher for cows fed the Oil diet (3.06, 3.06, 3.29 and 3.03% for C, E, Oil and Oil/E), implying that nutrient partitioning was altered in response to the reduction in milk fat. Milk fat concentration of CLA more than doubled in cows fed the oil diets, with concurrent increases in *trans*-10 18:1 and *trans*-11 18:1 FA. Moreover, milk fat from cows fed the two oil diets had 31.0% less *de novo* synthesized FA and 33.7% more long chain preformed FA. In conclusion, dietary supplements of soybean oil caused a reduction in milk fat percent and a shift in fatty acid composition characteristic of MFD. Dietary vitamin E did not overcome the oil-induced reduction in milk fat percent or changes in FA profile, but partially mitigated the reduction in fat yield by increasing milk yield.

Key Words: Conjugated Linoleic Acid, Milk Fat, Vitamin E

52 $CD4^+$ and $CD8^+$ T cell response in neonatal calves fed *Morinda citrifolia* (Noni). V. J. Brooks*[1], R. G. Godbee[2], S. F. Peek[1], and B. J. Darien[1], [1]*University Wisconsin*, *Madison*, [2]*University Nevada*, *Reno*.

Developmental immaturity of the immune system renders neonatal calves vulnerable to high rates of morbidity and mortality. Ingesting colostrum containing maternal immunoglobulins, leukocytes and cytokines is critical in ensuring calf health and survival. Juice made from the *Morinda citrifolia* fruit (noni) reportedly has immune enhancing effects, including anti-inflammatory activity and inhibition of tumorigenesis. The objective of this study was to evaluate the immune modulating effects of feeding neonatal calves noni puree by measuring lymphocyte proliferation and CD25 expression on $CD4^+$, $CD8^+$ and γδ T cells. Eighteen newborn Holstein bull calves were acquired in pairs from local dairies. All calves received 4.0 L of pooled colostrum by 12 h of age and were confirmed to have adequate passive transfer (>1200 mg/dL IgG). The calves were divided into two groups. Group 1 was comprised of control calves, while Group 2 received 30 mL of noni puree twice daily in milk replacer. Day 0 samples were obtained between 36 and 48 h of age and before the first feeding of puree. Peripheral blood mononuclear cells were collected and isolated from each calf on days 0, 3, 7, and 14. To measure lymphocyte proliferation, a mitogen induced Lymphocyte Blastogenesis Test (LBT) was performed. Mitogen induced activation of $CD4^+$, $CD8^+$ and γδ T cells was evaluated by the up-regulation of the IL-2 receptor, CD25, on these cells with two-color flow cytometry. For both tests concanavalin A (ConA) and phytohemagglutinin were used as global mitogens. Results showed a significant increase in CD25 expression on $CD8^+$ T cells in noni puree fed calves on day 3 of the study or approximately 5 days postpartum. Similarly, CD25 expression on $CD4^+$ T cells was also higher in noni puree fed calves on day 3. Both findings were in response to ConA stimulation, whereas the LBT did not show a significant difference between the two groups in their response to either mitogen. The precise mechanism and impact on long term health are not explained by the current study and are important areas deserving further study.

Key Words: *Morinda citrifolia*, Calves, Immunomodulation

53 Effects of alfalfa inclusion rate on productivity of lactating dairy cattle fed wet corn gluten feed based diets. C. R. Mullins*[1], K. N. Grigsby[2], and B. J. Bradford[1], [1]*Kansas State University*, *Manhattan*, [2]*Cargill, Inc.*, *Blair, NE*.

An experiment was conducted to evaluate the effects of varying alfalfa inclusion rate in diets containing 31% (DM basis) wet corn gluten feed product (Sweet Bran, Cargill, Inc.). Eighty primiparous and multiparous Holstein cows averaging 178 ± 90 DIM (mean ± SD) were randomly assigned to one of four sequences in a 4 × 4 Latin Square with 28-day periods. Treatments were diets containing 0, 7, 14, and 21% alfalfa on a dry matter basis, with corn silage, corn grain, soybean meal, expeller soybean meal, and mineral supplements varying across diets to maintain uniform nutrient densities. Diets were formulated for similar crude protein, neutral detergent fiber, and non-fiber carbohydrate concentrations. Feed intake, milk production, body weight, and body condition score were monitored, and linear and quadratic effects of increasing alfalfa inclusion rate were assessed using mixed model analysis. As the inclusion rate of alfalfa increased, dry matter intake increased linearly (P = 0.05; 26.7, 27.3, 27.4, and 27.5 kg/d for 0, 7, 14, and 21% alfalfa, respectively), and solids-corrected milk (P = 0.07; 29.9, 30.2, 30.8, and 30.5 kg/d) and energy-corrected milk production (P = 0.09; 32.9, 33.3, 33.8, and 33.6 kg/d) tended to increase linearly. Body weight gain decreased linearly (P = 0.02; 22.9, 18.0, 11.2, and 9.5 kg/28 d) with increasing alfalfa inclusion rate. Although increasing the inclusion rate of alfalfa increased the proportion of large particles in diets, treatments had no effect on milk fat yield or concentration. Feeding more alfalfa (up to 21% of DM) tended to increase milk yield while decreasing body weight gain, suggesting that metabolizable energy utilization shifted from body weight gain to milk production in these treatments. However, removing alfalfa from the diet had only minor effects on productivity.

Key Words: Dairy Nutrition, Byproduct, Forages

54 Diet does not affect putative mammary epithelial stem cells in pre-weaned Holstein heifers. K. M. Daniels*[1], A. V. Capuco[2], R. E. James[1], M. L. McGilliard[1], and R. M. Akers[1], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg*, [2]*USDA-Agricultural Research Service*, *Beltsville, MD*.

Overfeeding prepubertal heifers can impair mammary epithelial growth and development, processes that depend on stem cells. We evaluated effects of milk replacer (MR) composition on putative bovine mammary epithelial stem cell populations using a 5-bromo-2-deoxyrudine (BrdU; a thymidine analog) label retention method. Stem cells retain BrdU over time through selective segregation of labeled template DNA strands during mitosis, whereas the label is diluted in non-stem daughter cells. Twenty-four newborn heifers were fed one of four MR diets (n=6/diet): CON (20% CP, 21% fat MR fed at 441 g DM /d), HPLF (28% CP, 20% fat MR fed at 951 g DM/d), HPHF (27% CP, 28% fat MR fed at 951 g DM/d), and HPHF+ (27% CP, 28% fat MR fed at 1431 g DM/d). Calves were fed twice daily; water and starter (20% CP, 1.43% fat) were offered free choice. At 30 d of age heifers were given 5 mg BrdU per kg BW daily for 4 d. Heifers were euthanized 29 d later. Mammary tissue was collected from two peripheral and two cisternal parenchymal regions in the left front quarter. Histological sections were prepared and processed for dual-label immunofluorescent detection of Ki67 and BrdU. Digital images were obtained at 40x magnification from 10 regions per slide. Labeled cells were counted manually and total number of epithelial cells determined using image analysis software. Diet had no effect on percentage of labeled mammary epithelial cells, nor was there a diet by region interaction. Percentage of BrdU-labeled epithelial cells was largest in cisternal regions of the gland (2.39 ± 0.44%) and decreased toward the periphery (0.73 ± 0.45%). The opposite was true for Ki67-labeled epithelial cells (8.97 ± 0.81 vs. 10.88 ± 0.77%). This is consistent with decreased proliferation and increased cell cycle arrest in cisternal regions. In peripheral regions, percentage of BrdU-labeled epithelial cells averaged 0.12 ± 0.11%. These data provide no evidence that putative mammary epithelial stem cells are affected by protein and fat differences in MR fed to pre-weaned calves.

Key Words: Heifer, Mammary, Stem Cell

55 Gene expression for enzymes involved with volatile fatty acid and glucose metabolism are affected by the dietary forage–to–concentrate ratio. G. B. Penner*[1], M. Taniguchi[1], L. L. Guan[1], K. A. Beauchemin[2], and M. Oba[1], [1]*University of Alberta*, *Edmonton, Alberta, Canada*, [2]*Agriculture and Agri-Food Canada*, *Lethbridge, Alberta, Canada*.

The objectives of this study were to determine the effects of the dietary forage–to–concentrate ratio on in vivo volatile fatty acid (VFA) clearance, and the expression of genes related to ruminal absorption and metabolism of VFA and glucose. Twelve ruminally cannulated dry Holstein cull cows were fed either a control diet (HF) or a diet to predispose cattle to a high VFA load (LF), which contained 92% and 36% forage on a DM basis, respectively. After a 28-d diet adaptation period, ruminal pH, ruminal VFA concentrations, and in vivo VFA clearance were measured. Cows were subsequently euthanized and ruminal tissue (ventral sac) was harvested for gene expression analysis using quantitative real-time PCR. Mean ruminal pH was 0.45 pH units lower (6.03 vs. 6.48; $P < 0.01$) and total VFA concentration was 25 m*M* higher (138 vs. 113 m*M*; $P < 0.01$) for the LF compared to the HF treatment. The fractional liquid passage, VFA clearance, and VFA absorption rates were not different between treatments and averaged 8.3 ± 0.8, 21.5 ± 0.9, and 13.2 ± 1.0%/h, respectively. With respect to butyrate metabolism, the LF treatment had a 1.34 ± 0.12 fold greater expression of β–hydroxybutyrate dehydrogenase ($P = 0.04$) and tended to have a 1.72 ± 0.35 fold greater expression of acyl CoA synthetase ($P = 0.09$). However, the expression of hydroxymethylglutaryl CoA synthase tended to be reduced (0.83 ± 0.07 fold; $P = 0.06$) for the LF relative to the HF treatment. Furthermore, the expression of hexokinase tended ($P = 0.08$) to be increased by 1.30 ± 0.13 fold but sodium glucose linked transporter and pyruvate dehydrogenase were decreased by 0.44 ± 0.11 ($P < 0.01$) and 0.70 ± 0.11 ($P = 0.04$) fold for the LF treatment relative to the HF treatment. These data indicate that the VFA load does not affect the rate of VFA absorption in vivo but may affect the pathway of butyrate metabolism and the utilization of glucose as an energy source.

Key Words: Real-Time PCR, VFA Metabolism, VFA Absorption

56 Lactation performance and amino acid utilization of cows fed increasing amounts of de-oiled dried distillers grains with solubles. K. Mjoun*[1], K. F. Kalscheur[1], A. R. Hippen[1], D. J. Schingoethe[1], and D. E. Little[2], [1]*South Dakota State University*, *Brookings*, [2]*DairyNet Inc.*, *Brookings, SD*.

As the ethanol industry continues to grow, innovative products are emerging; deoiled dried distillers grain with solubles (dDGS), a low fat (3.7% Ether Extract) product, is an example of this evolvement. The effects of feeding increasing quantities of dDGS on lactation performance and amino acid utilization were evaluated with 23 multiparous and 19 primiparous Holstein cows in a randomized complete block design for 8-wk including a 2-wk covariate period. The dDGS were included in diets at 0, 10, 20, and 30% of the diet on a DM basis. Increasing dDGS in diets tended to affect DMI in a quadratic manner ($P = 0.09$), such that cows fed the 20% dDGS had the greatest DMI (24.4 kg/d) and those fed the 30% dDGS had the least (22.1 kg/d). Milk production (34.9 kg/d) was not affected by the inclusion of dDGS. Milk fat percentage tended to increase linearly ($P = 0.09$) from 3.21 to 3.64% as dDGS increased in the diets. Similarly, milk fat yield tended to increase linearly from 1.09 to 1.29 kg/d as dDGS increased from 0 to 30% of the diet. Milk protein percentage (2.98, 3.08, 3.11, and 2.98%) and milk protein yield (1.02, 1.08, 1.10, and 1.03 kg/d) responded in a quadratic manner ($P < 0.01$). Milk urea N decreased linearly ($P < 0.01$) from 15.5 to 13.1 mg/dL. The efficiency of N utilization for milk protein synthesis was not affected by including dDGS and averaged 25.9%. The efficiency of milk production (ECM/DMI) increased linearly ($P < 0.05$) with increasing dDGS in the diet. Arterial Lys decreased linearly (66.0, 57.6, 51.9, 44.8 μM/L; ($P < 0.01$) whereas arterial Met increased linearly (16.5, 17.9, 22.5, 29.3 μM/L). Arteriovenous difference of Lys linearly decreased (42.6, 37.1, 36.6, 32.5 μM/L) while that of Met was unaffected. The extraction of Lys by the mammary gland increased linearly (64.3, 64.4, 70.7, 72.2%) while that of Met decreased linearly (71.6, 57.5, 50.8, 42.7%). These results indicate that addition of up to 30% of dDGS in mid-lactation diet supported lactation performance similarly to that of a control diet based on soybean products.

Key Words: De-Oiled Dried Distillers Grains with Solubles, Dairy Cow, Amino Acids

57 Development of a mechanistic model to predict feed intake in domestic and wild ruminants of various physiological states. T. Hackmann* and J. N. Spain, *University of Missouri*, *Columbia*.

Understanding the regulation of feed intake is important in nutritional management of domestic and wild ruminants. A mechanistic model of ruminant feed intake and digestion was formulated to investigate hypotheses of intake regulation and to serve as a predictive tool. The model is a compartmental model and represents fluxes of nutrients across pools in the reticulorumen, small intestine, and large intestine. Similar to a model published by D.S. Fisher, predicted intake is the level of intake that reaches an optimum balance among chemostatic, distention, and protein feedbacks. Unlike in previous models, the chemostatic setpoint is varied with the energetic demands of the animal in order to investigate how intake is affected by these demands, which vary with species, body weight, physiological state, and level and stage of production. Data from 15 studies that report ad libitum DMI of all-forage diets were used to compare model-predicted DMI with actual DMI for animals of a range of physiological states (gestation, lactation, growth, and no production), levels and stages of production, body weight (16 to 907 kg), and species (14 bovids, 4 cervids, and 1 giraffid). The coefficient of determination (R2) between actual and predicted DMI was 0.701 and 0.910 when DMI was expressed as percentage of BW and kg/d, respectively (n = 158). For domestic species (cow, sheep, goat) alone, agreement was higher, with values of R2 of 0.718 and 0.954 when DMI was expressed as percentage of BW and kg/d, respectively (n = 107). The good agreement between predicted and actual DMI and other considerations support the hypotheses of intake regulation embodied in the model, namely that energetic demands of the animal regulate intake. The model shows promise as a predictive tool for application to domestic and wild ruminant species and awaits further development to model non-all-forage diets.

Key Words: Intake, Model, Ruminant