M17 Effect of extended colostrum feeding on plasma glucagon-like peptide 1 concentration in newborn calves. Y. Inabu1,2, J. Pyo3, S. Plets2, M. Steele2, and T. Sugino1. 1The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan. 2Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

Glucagon-like peptide 1 (GLP-1) plays a role in the regulation of appetite and glucose homeostasis via the stimulation of insulin secretion. The objective of this study was to evaluate the effect of extended colostrum feeding on plasma concentrations of GLP-1. Holstein bull calves (n = 18) were fed pooled colostrum at 7.5% of BW at 2 h after birth, then fed mature milk (M), mixture at a ratio of 50:50 for pooled colostrum and milk (CM), or pooled colostrum (C; n = 6 for each treatment) at 5% of BW at 12 h after birth, and every 12 h thereafter until 72 h after birth. Blood samples were obtained before (1 and 2 h after birth) and after (until 75 h after birth) the first colostrum feeding, and plasma and milk (CM), or pooled colostrum (C; n = 6 for each treatment) at 18 h after birth, but further study is necessary to determine the effect on plasma insulin and glucose concentrations.

Key Words: adipokine, adipocyte, lipid mobilization

M18 Fetuin-A modulates lipid mobilization in bovine adipose tissue by enhancing lipogenic activity of adipocytes. C. Strieder-Barboza* and G. A. Contreras, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.

Fetuin-A (FetA) is an adipokine and free fatty acids (FFA) transporter linked to adipose tissue (AT) function in transition cows. Plasma and AT FetA decrease after parturition coinciding with reduced lipogenesis and increased lipolysis. In monogastrics, FetA enhances lipogenesis, but its role on lipid mobilization of ruminants is unclear. Our objective was to determine the effects of FetA on lipogenesis and lipolysis in bovine adipocytes. Preadipocytes from tailhead subcutaneous AT of dairy cows (n = 6) were induced to differentiate in a coculture system and used in the experiments. Lipolytic responses of adipocytes were evaluated after a 2-h β-adrenergic stimulation with 1 µM isoproterenol (ISO) alone or combined with 0.1 mg/mL of FetA (FETA+ISO). Medium alone (CON) or mixed with 0.1 mg/mL of FetA (FETA) served as controls. Lipogenic responses were assessed in adipocytes treated with CON or FETA for 48 h by quantification of FFA uptake (kinetic assay for 1 h) and triacylglycerol (TG) accumulation (Adipored) and gene (qPCR) and protein expression (Western blot) of lipogenic markers. Adrenergic stimulation with ISO increased lipolysis compared with CON, as reflected in the release of glucose (12 ± 0.04 vs 0.04 ± 0.02 nM/cell, P = 0.003) and FFA (15 ± 13 vs 6.2 ± 2.4 nM/cell, P = 0.04). Lipolysis induced by ISO was attenuated by FetA (FETA+ISO) as reflected by a lower glycerol (0.06 ± 0.04 nM/cell, P = 0.02) and FFA (5.7 ± 2.7 nM/cell, P = 0.01) release. The treatment with FetA enhanced lipogenic responses compared with CON as demonstrated by a 1.5 times increment in FFA uptake (P = 0.02) and TG accumulation (P = 0.05); and the upregulation of 1-acylglycerol-3-phosphate acyltransferase (AGAPT2) gene expression (P = 0.04) and protein content (P = 0.08). In conclusion, FetA attenuates lipolytic response and enhances lipogenesis in bovine adipocytes.

The upregulation of the rate-limiting lipogenic enzyme AGAPT2 by FetA suggests a potential pathway by which this adipokine promotes TG synthesis in adipocytes.

Key Words: adipokine, adipocyte, lipid mobilization

M19 Ruminal, diet, and environmental factors that affect dairy calf performance. C. A. Ceh*, R. R. White, and K. M. Daniels, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Although impacts of dietary and environmental factors on calf performance have been investigated, no studies have investigated how rumen development, independent of diet and environment, influences dairy calf performance. The objective was to summarize the literature on calf performance and derive equations that relate rumin (e.g., rumen pH, reticulo-rumen weight, papillae area) and non-rumen factors (e.g., feed composition, form of feed, housing) to animal performance [e.g., intake of milk replacer (MR), starter, and forage; average daily gain (ADG); and feed efficiency]. In total, 146 treatment means from 36 trials were obtained under the following selection criteria: study reported dairy calves only; calf between 0 to 24 wk of age; calves had to be fed MR for some part of the study; study reported one or more rumen variables; and ADG >0.2 kg/d. Forward selection, multiple regression was used to derive equations to estimate variables that influenced the response variable in each model; models were weighted by the inverse of the standard error of the mean. Models were evaluated based on root estimated variance and concordance correlation coefficients (CCC). A positive association was seen in ADG between final body weight, weaned calves, and total amount of starter intake, while negatively associated with calf age, Holstein breed calves, and initial body weight (CCC = 0.961). Feed to gain ratio was positively associated with the weight of the ruminal contents (CCC = 0.904). Daily forage intake was negatively associated with the percent of the diet that was starter or MR (CCC = 0.999). Daily starter intake was positively associated with higher acid detergent fiber in the starter, a pelleted starter, and diets including starter and forage (CCC = 0.986). Daily MR intake was negatively associated with the percentage of the diet that was starter and ruminal pH (CCC = 0.940). Although dietary and environmental factors are closely associated with calf performance, ruminal factors appear to have additional, additive influences on calf performance.

Key Words: rumen development, meta-analysis, calf
M20 Epigenetic regulation of nuclear factor erythroid 2 like 2 (NFE2L2) signaling pathway through methionine supply during the periparturient period in liver of dairy cows. F. Batistel*1, S. Moeoez1, L. Han3, C. Parys2, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

Nuclear factor erythroid 2 like 2 (NFE2L2) is a transcription factor that regulates the expression of antioxidant proteins. We investigated the effect of increasing Met supply during the periparturient period on NFE2L2, its targets and the potential for epigenetics in its regulation. Multiparous cows were assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH) to ensure a ratio of Lys to Met in the metabolizable protein close to 2.8:1. Mepron (0.09–0.10% of the dry matter intake) was fed from −28 to 30 d relative to parturition. Liver was sampled from 8 cows/treatment at −10, +10, and +30 d relative to parturition. NFE2L2 and DNA methyltransferases (DNMT) were analyzed by protein blotting, while NFE2L2 targets and NFE2L2 DNA methylation were analyzed by RT-PCR. Glutathione, global DNA methylation, and histone H3 lysine 4 tri-methylation (H3K4me3) were analyzed by colorimetric commercial kits. Data were analyzed using a Mixed model considering block as random effect and treatment, time and its interaction as fixed effect. Compared with control, MET-fed cows had greater (P = 0.04) protein expression of phosphorylated NFE2L2 and tended to have greater (P = 0.07) total NFE2L2. These results agree with the lower (P = 0.03) DNA methylation of the NFE2L2 promoter, which indicates a higher rate of NFE2L2 transcription. Among the 7 NFE2L2 target genes analyzed, TXNRD1, HMOX1, PIR, and NQO1 were upregulated (P ≤ 0.05) by MET supply. Expression of TNX, FECH, and FTH1 was not affected (P ≥ 0.10) by MET. Hepatic glutathione was greater (P = 0.01) and global DNA methylation lower (P = 0.04) in MET-supplemented cows. The protein expression of the de novo DNMT3A and the maintenance DNMT1 were not affected (P ≥ 0.10) by MET-supply; however, DNMT3A protein expression increased (P = 0.05) over time. A treatment × time interaction (P = 0.05) was observed for the protein expression of the de novo DNMT3B because of an increase over time. Increasing MET-supply in the diet enhanced (P = 0.001) liver glutathione. OMX2 was not affected (P = 0.05) by MET. Global DNA methylation in liver samples from 8 cows/pregnancy were increased by MET supplementation. The application of MET tended to increase the DNA methylation of the NFE2L2 promoter. Additionally, the application of MET tended to decrease the activity of the de novo DNMT3A, which is involved in DNA methylation. These results suggest that MET supplementation can improve the expression of antioxidant proteins and decrease DNA methylation of the NFE2L2 promoter, which may have a positive effect on liver health.

Key Words: Nuclear factor erythroid 2 like 2, Methionine, Liver, Epigenetics

M21 Milking intervals of cows with contrasting production. F. Masias1,2, N. Lyons3, M. Piccardi1,2, M. Balzarini1,2, R. Hovey4, and S. Garcia4, 1Cátedra de Estadística y Biometría de la Facultad de Ciencias Agropecuarias of Universidad Nacional de Córdoba, Córdoba, Argentina, 2Intensive Livestock Industries, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle NSW, Australia, 3Consejo de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina, 4Department of Animal Science, University of California, Davis, CA, 5School of Life and Environmental Sciences and Sydney Institute of Agriculture, The University of Sydney, Camden, NSW, Australia.

In automatic milking systems (AMS), there is variability in milking intervals (MI) within and between cows. Extended MI (particularly greater than 16 h in pasture-based systems) have a negative effect on milk yield (MY). Having cows that tolerate extended MI without negative effects on MY could improve overall system performance. The aim of this study was to describe MI of cows with contrasting production milked in pasture-based AMS. A database containing records of milking events for 917 multiparous cows for one year (July 2015 – June 2016) from 2 AMS farms in Australia was used. Each record contained farm, cow, lactation, days in milk, MI and MY. Daily yields were then calculated as the sum of milking events. Lactation curves were adjusted with an incomplete gamma function (Wood, 1967) with a random intercept. Daily yields were expected to be auto-correlated. The adjustments were made with PROC NL MIXED from SAS. Predicted curves of average daily production according to lactation (2 vs 3 or more) and calving season (warm vs cool) were obtained. The best linear unbiased prediction (BLUP) allowed categorization of cows and lactations as having either high or low milk production (positive and negative BLUP, respectively). Then, each MI were categorized as belonging to short (<16 h) or long (≥16 h) MI. Differences between categories were compared using the binomial test of proportions. There were significant differences in the distribution of MI within each production level (P < 0.0001). High production cows had 34% of milking events with intervals greater than 16 h and were 4.13 times more likely than low production cows to have low MI (<16 h). Preliminary results also indicate that high production cows do not have such a negative effect of longer MI on MY as the low production cows do. This study indicates that there are cows that have long MI and still maintain high levels of production. Identifying and selecting for these cows should enable improve robot performance.

Key Words: non-linear models, BLUP, robotic milking

M22 Evaluating the effects of fibrolytic enzymes derived from Trichoderma reesei fungal extraction on rumen fermentation, omasal nutrient flow and production performance in dairy cows during early lactation. B. Refat1, D. Christensens1, J. McKinnon1, A. Beattie1, T. McAllister2, W. Yang3, O. Alzahabi3, and P. Yu1, 1Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada, 2Crop Development Center, Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada, 3Lethbridge Research and Development Centre, Lethbridge, AB, Canada, 4AB Vista, Marlborough, United Kingdom.

This study was performed to evaluate the effects of pre-treating barley silage-based diet with a fermentation extract derived from Trichoderma reesei (FETR, mixture of xylanase and cellulase; AB Vista, Marlborough, UK) on lactation performance, omasal nutrient flow and digestibility, rumen fermentation characteristics, and rumen pH profile in Holstein dairy cows during early lactation. The dairy trial was conducted using 9 Holstein dairy cows (averaging 46 ± 24 DIM and 697 ± 69 kg BW; 6 cows were fitted with a rumen cannula and 3 were non-cannulated). Two groups of cows were randomly assigned to each of the dietary treatments in a crossover design: control (without FETR supplementation) and supplemented (with 0.75 mL of FETR/kg DM of diet based on our previous study). The pre-treatment was applied to barley silage-based diet one hour before feeding by mixing FETR with the diet. The experiment consisted of 2 consecutive experimental periods of 27 d each. Within each period, the first 18 d were used for adaptation to the treatments, followed by 3 d of milk sampling, 3 d for the collection of the ruminal, omasal, and fecal samples, and the last 3 d for measuring the pH profile using indwelling pH probes. The application of FETR tended to decrease the DM intake compared with control (32.8 vs. 33.7; P = 0.08). There was a significant decrease (P = 0.05) in milk urea nitrogen by 7% and a numerical decrease (P = 0.16) in ruminal ammonia concentration by 14% as a consequence of adding FETR to the diet. In conclusion, dairy cows fed FETR pre-treated barley
silage-based diet display enhanced milk yield with less feed. The positive effect of adding FETR could benefit the dairy industry in Western Canada where barley silage-based diets are common

**Key Words:** fibrolytic enzyme, milk yield, nutrient flow

### M23 Supplemental methionine and lipopolysaccharide alters galectin gene expression in polymorphonuclear leukocytes (PMNL) from Holstein cows in vitro

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Supplementation of methionine (Met) to dairy cows is effective in the optimization of milk production and improvement of health. Isolated PMNL from multiparous Holstein cows were used to evaluate the effect of methionine supplementation on mRNA expression of galectin genes (LGALS1, LGALS2, LGALS3, LGALS4, LGALS8, LGALS9, LGALS12, -12, -12, -12) in vitro and on the impact of stimulation with lipopolysaccharide (LPS). Galectins are important immunological mediators of homeostasis and disease regulation. PMNL was isolated from 10 Holstein cows and divided into 2 groups (Group 1: n = 5 and Group 2: n = 5). PMNL from Group 1 were incubated with 3 levels of lysine (Lys) to Met ratios of (3.6:1, 2.9:1, or 2.4:1). PMNL from Group 2 were also incubated with 3 levels of Lys to Met ratios of (3.6:1, 2.9:1, or 2.4:1) and 50 μg/mL of LPS. Cells were incubated at 37°C, with 5% CO2 for 4 h. Met had no effect on LGALS expression in Group 1 cows. In Group 2 cows, however, LGALS4 (P = 0.005) and tended to increase LGALS8 (0.08) in Group 2. These results shed light on the modulation of galectin expression in cows in response to methyl donors and LPS. The observed modulation of galectins expression by bacterial LPS and methionine may be exploited for the design of anti-inflammatory therapeutics.

**Key Words:** galectins, methionine, lipopolysaccharide

### M24 Aluminosilicate clay reduces the deleterious effects of an aflatoxin challenge on performance in lactating Holstein cows

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Adsorbent use in aflatoxin (AF) contaminated diets is critical in alleviating detrimental effects of AF on dairy cattle performance. The objective of this study was to determine the effects of a commercially available aluminosilicate clay in a traditional lactation diet during an AF challenge on the presence of AF in milk, urine, and feces, and performance parameters of multiparous lactating Holstein cows. Sixteen multiparous, lactating, Holstein cows [BW (mean ± SD) = 758 ± 76 kg; DIM = 157 ± 43 d] were assigned to 1 of 4 treatments in a replicated 4 × 4 Latin Square design: no adsorbent and no AF challenge (CON), no adsorbent and AF challenge (POS), 113 g of aluminosilicate clay top-dressed on the ration (adsorbent; PMI Nutritional Additives, Arden Hills, MN) with AF challenge (F4), and 227 g of adsorbent with AF challenge (F8). For each period, milk was sampled 3 times daily from d 14 to 21, while feces and urine were sampled on d 14, 18, and 21. Statistical analysis was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Fat-corrected milk (POS = 37.2, F4 = 39.2, F8 = 38.9 kg/d) and ECM (POS = 37.3, F4 = 39.3, F8 = 38.9 kg/d) increased (P = 0.05 and 0.04, respectively) as concentration of adsorbent in the diet increased. Lactose yield (kg/d) increased as concentration of adsorbent in the diet increased (POS = 1.67, F4 = 1.89, F8 = 1.74 kg/d; P = 0.05). A quadratic treatment effect was present for protein yield (POS = 1.20, F4 = 1.28, F8 = 1.24 kg/d; P = 0.01). There was a decrease in milk AFM1 concentration (POS = 0.33, F4 = 0.32, F8 = 0.27 μg/kg; P = 0.001) as concentration of adsorbent in the diet increased. A quadratic treatment effect was present for AFM1 transference (POS = 0.45, F4 = 0.49, F8 = 0.39%; P = 0.03). There was a decrease for AFM1 concentration in urine (POS = 2.10, F4 = 1.89, F8 = 1.78 μg/kg; P = 0.04) and feces (POS = 4.68, F4 = 3.44, F8 = 3.17 μg/kg; P = 0.05) as concentration of adsorbent in the diet increased. In conclusion, the adsorbent used in this study had a positive effect on milk production, milk components, and AF excretion in milk, urine, and feces.

Pathogen-associated molecular patterns (PAMP) are highly conserved structural motifs that are recognized by pathogen recognition receptors (PRR) to initiate immune responses. Treatment of cells with double-stranded RNA (dsRNA) mimics viral infection and regulates expression of various genes. Polyinosinic-polycytidylic acid (Poly I:C) is a PAMP for viral infections. It is established that Poly I:C mediates the production of cytokines by binding to its PRR, toll-like receptor 3 (TLR3) on gamma-delta T cells. Because galectins are also involved in immune responses and the outcome of microbial infections, it was important to evaluate whether its production is affected by poly I:C stimulation in whole blood. The effect of Poly I:C, on the expression of galectins in cow blood was assessed in this study. Galectins are multipotent, evolutionarily conserved, carbohydrate-binding proteins that trigger a cascade of transmembrane signaling events such as cell activation, cytokine secretion, migration, and apoptosis. Blood was taken from 5 multiparous Holstein cows and incubated with 12.5 μg/mL of Poly I:C at 37°C, with 5% CO2 for 4 h. Met had no effect on LGALS expression in Group 1 cows. In Group 2 cows, however, LGALS8 transcription was increased when PMNL were treated with 2.9 Lys:Met and LPS (P = 0.0005). LGALS8 was increased in PMNL treated with 3.6 Lys:Met (P = 0.05). LPS increased the expression of LGALS4 (P = 0.005) and tended to increase LGALS12 (0.08) in Group 2. These results shed light on the modulation of galectin expression in cows in response to methyl donors and LPS. The observed modulation of galectins expression by bacterial LPS and methionine may be exploited for the design of anti-inflammatory therapeutics.

**Key Words:** galectins, methionine, lipopolysaccharide

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**M25** A double-stranded RNA, polyinosinic-polycytidylic acid (Poly I:C) enhances the expression of galectins-1, -3, -4, -8, and -9 in cow blood

E. Asiamah*, S. Adjei-Fremah, K. Ekewemalor, B. Osei, and M. Worku, North Carolina A&T State University, Greensboro, NC.

Pathogen-associated molecular patterns (PAMP) are highly conserved structural motifs that are recognized by pathogen recognition receptors (PRR) to initiate immune responses. Treatment of cells with double-stranded RNA (dsRNA) mimics viral infection and regulates expression of various genes. Polyinosinic-polycytidylic acid (Poly I:C) is a PAMP for viral infections. It is established that Poly I:C mediates the production of cytokines by binding to its PRR, toll-like receptor 3 (TLR3) on gamma-delta T cells. Because galectins are also involved in immune responses and the outcome of microbial infections, it was important to evaluate whether its production is affected by poly I:C stimulation in whole blood. The effect of Poly I:C, on the expression of galectins in cow blood was assessed in this study. Galectins are multipotent, evolutionarily conserved, carbohydrate-binding proteins that trigger a cascade of transmembrane signaling events such as cell activation, cytokine secretion, migration, and apoptosis. Blood was taken from 5 multiparous Holstein cows and incubated with 12.5 μg/mL of Poly I:C at 37°C, with 5% CO2 for 30 min. Total RNA was isolated, reverse-transcribed to cDNA, and then used in real-time PCR experiments with the use of commercially sequenced cow specific primers. GAPDH and β-actin served as internal controls. Fold change in transcript abundance was calculated using the Livak method. Where ΔCt = (Target genes treat – GAPDH/β actin treat) – ΔCt (Target genes PBS – GAPDH/β actin PBS). Fold change = 2(-ΔΔCt). Concentrations of Galectins in plasma were measured using bovine Enzyme-linked Immunosorbent Assay (ELISA). Galectin concentration was analyzed with Proc GLM in SAS 9.4. Poly I:C increased the transcription of LGALS1, LGALS4, and LGALS8 (1.78, 1.88, and 1.73 folds respectively). Secretion of Gal-1, -3, -8, and -9 were increased in treated samples compared with control (P = 0.001, P = 0.01, P = 0.0001, P = 0.0001, respectively). The results demonstrate that Poly I:C differentially modulates transcription of mRNA and secretion of galectins in cow blood. Elucidation of the relationship between PAMPS and galectin expression may help to define their roles in viral diseases as well as aid in drug design in the dairy industry.

**Key Words:** galectin, polyinosinic-polycytidylic acid (Poly I:C), pathogen-associated molecular patterns (PAMP)

Degradation of amino acids by rumen microbes poses a challenge as the true benefit of amino acid supplementation is only exploited when the amino acid is presented to the small intestine for absorption. Our laboratory has previously demonstrated that prepartum intravenous (IV) infusions of 5-hydroxytryptophan (5-HTP), the immediate precursor to serotonin, increases circulating total calcium concentrations. This suggests potential for 5-HTP as a therapeutic management strategy for hypocalcemia prevention. However, whether 5-HTP can escape the rumen and be absorbed is unknown. The objective of this study was to determine if ruminal administration of 5-HTP increases circulating serotonin concentrations. The experiment was conducted as a 4 × 4 replicated Latin square using 4 nonlactating, nonpregnant, ruminally fistulated multiparous dairy cows. Experimental dosings of 5-HTP were administered on 2 consecutive days and given on a mg/kg of body weight basis. The resulting 4 treatments were saline infusion of 0 mg/kg 5-HTP (CON), IV infusion of 1 mg/kg 5-HTP (IV), 1 mg/kg intraruminal 5-HTP, and 2 mg/kg intraruminal 5-HTP. Whole blood was collected relative to administration of the second experimental dose for 3 continuous days with a 7-d washout period between treatment periods. Data were analyzed using the MIXED procedure of SAS with repeated measures. The ruminal 2 mg/kg 5-HTP treatment increased (P = 0.03) serotonin compared with the 0 mg/kg 5-HTP dose for 8 h after the second treatment. However, on d 3 and 4 of treatment, there were no differences between the CON (P > 0.05) and the 2 mg/kg 5-HTP administered intraruminally. The IV dose increased serotonin concentrations on d 2 compared with the 0 mg/kg 5-HTP dose, the ruminally dosed 1 mg/kg and 2 mg/kg 5-HTP treatments (P < 0.0001, P < 0.0001, and P = 0.0016, respectively). These data reveal that 2 mg/kg 5-HTP administered intraruminally acutely increased circulating serotonin concentrations. Future feeding experiments need to be done to determine the optimal 5-HTP dose for potential commercial use as a mitigation tool for hypocalcemia.

Key Words: serotonin, 5-hydroxytryptophan (5-HTP), rumen

M27  Effects of feeding more milk on periprandial glucagon-like peptide-2 (GLP-2) concentrations in dairy calves. J. L. Haisan*1, M. Oba1, and T. Sugino2, 1University of Alberta, Edmonton, AB, Canada, 2Hiroshima University, Higashi-Hiroshima, Japan.

The objective of this experiment was to determine the effects of providing an increased amount of milk on periprandial glucagon-like peptide-2 (GLP-2) concentrations in pre-weaned dairy calves. Nineteen female Holstein calves were randomly assigned to 1 of 2 treatments on d 2 after birth; HIGH (10 L/d; n = 9) or LOW (5 L/d; n = 10) amount of pasteurized whole milk. All calves were allowed 2.5 L of milk per meal until d 50 before a 10-d weaning transition began. Calves were housed in individual pens for the first 21 ± 3 d, before being moved to a group pen and fed using an automated calf feeder. Calf starter was provided ad libitum from d 21 ± 3 d. At wk 3 (before being moved to the group pen), wk 5, and wk 7 (before the weaning transition) of life, a series of blood samples were collected relative to their morning milk meal, which was at least 6 h after a previous milk meal. Overall, HIGH calves tended to have increased mean plasma GLP-2 concentrations on wk 3 (P = 0.10) and wk 5 (P = 0.08), but no difference was observed on wk 7. Similarly, pre-meal concentration of plasma GLP-2 tended to be greater for HIGH calves compared with LOW at wk 3 (1.05 vs. 0.77 ng/mL; P = 0.11), and was greater at wk 5 (0.76 vs. 0.44 ng/mL; P = 0.04), however no difference was observed at wk 7 (0.56 vs. 0.47 ng/mL; P = 0.77).

Although starter intake of HIGH calves tended to be lower than LOW calves (241 vs. 413 g/d; P = 0.06) for wk 4 to 7, there was a negative correlation between starter intake and pre-meal GLP-2 concentrations (r = 0.30; P = 0.02) at wk 5, indicating that plasma GLP-2 concentration of pre-weaned dairy calves can be increased to a greater extent by milk intake than starter intake. Plasma glucose concentrations were not different among treatments at any measured time point; however plasma insulin concentration tended to be higher for HIGH calves on wk 3 (P = 0.08). These results suggest that feeding more milk early in life can increase plasma GLP-2 concentrations in pre-weaned dairy calves.

Key Words: glucagon-like peptide-2 (GLP-2), starter intake, dairy calves

M28  Effects of citrus oil components on Escherichia coli P4 growth and on bovine neutrophils. C. M. Scholte*1, T. H. Elsasser2, S. Kahl2, D. Biswas1, and K. M. Moyes1, 1Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 2Animal Biosciences and Biotechnology Laboratory, USDA-Agricultural Research Service, Beltsville, MD.

Citrus oils (CO) have known antimicrobial properties and as such may serve as alternatives to conventional drug mastitis treatments; however, it is unknown how these oils affect environmental mastitis pathogens and the cow’s cellular immune response. The objectives of this study were to (1) determine the minimum inhibitory concentrations (MIC) of CO against Escherichia coli P4, and (2) evaluate CO cytotoxicity and their acute effect on oxidative response of bovine blood polymorphonuclear leukocytes (PMN). Citrus oils and its components, citral, linalool, valencene and limonlene, (0–10 µL/mL) were dissolved in ethanol and phosphate buffer solution with a 7:1 ethanol to oil ratio to maintain oil solubility. A control treatment of ethanol-only was also tested. Milk and blood were obtained from 12 healthy, mid-lactation dairy cows. Bacteriostatic MIC were determined through broth and milk microdilution. Bovine PMN were isolated from blood and incubated with varying citral, linalool, and ethanol concentrations. Following incubation, oil and ethanol cytotoxicity and PMN oxidative response were determined by quantifying PMN lactate dehydrogenase release into media and reactive oxidative species production, respectively. Cytotoxicity and oxidative burst response data were analyzed by ANOVA using the MIXED procedure of SAS 9.4. Of the CO components, citral and linalool had the lowest MIC and were unaffected by the presence of ethanol. Citral was the most effective at inhibiting (0.4 µL/mL in broth; 0.8 µL/mL in milk) E. coli P4 growth. No citral, linalool, or ethanol concentrations affected PMN oxidative burst response (P > 0.05); however, citral and linalool concentrations (0.1–0.8 µL/mL) were more toxic to PMN than control (0 µL/mL; P < 0.01). The ability of citral and linalool to inhibit proliferation of E. coli P4 highlight their potential as alternative antimicrobial therapies for bovine mastitis. Increased in vitro cytotoxicity suggests that further tests may be needed to optimize treatment strategy.

Key Words: mastitis, alternative therapy, Escherichia coli

M29  Evaluating the effects of a rumen and hindgut starch challenge on the inflammatory immune response in Holstein cows. A. M. Barnard*, M. Conklin, B. Aylward, R. Dyer, R. Arsenault, and T. F. Gressley, Department of Animal and Food Sciences, College of Agricultural and Natural Resources, University of Delaware, Newark, DE.
Grain induced subacute ruminal acidosis has been linked to systemic inflammation. We hypothesized that a rumen and hindgut starch challenge would stimulate an inflammatory response in intestinal tissue. Six rumen cannulated nonlactating nonpregnant Holstein cows were assigned according to a randomized block design to a control diet (CON) or CON top-dressed with 20% ground barley (STARCH). Diets were restricted fed to achieve weight gains of ~45 kg by the end of the 20-wk experiment. STARCH cows also received abomasal infusions of corn starch (4 g/kg BW per day) and CON cows received abomasal infusions of water pulse dosed twice a day during wk 8, 12, 16 and 19–20. Rumen fluid, feces and blood were collected weekly (non-infusion weeks) or 3 times weekly (infusion weeks). Rumen fluid and feces were analyzed for pH, lactate and VFA and blood samples were analyzed for haptoglobin (Hp) and serum amyloid A. At wk 20, cows were euthanized and mesenteric adipose, colon and jejunum were collected for determination of immune cell phenotype. Weekly data were analyzed using a repeated measures Glimmix model in SAS that included fixed effects of diet, month, infusion, and 2 way interactions and the random effect of cow. Immune cell phenotype data were analyzed with a model including effect of diet only. Diet × infusion tended to affect Hp ($P = 0.08$) and fecal pH ($P = 0.10$), lactate ($P = 0.08$) and butyrate ($P = 0.09$). Interactions were due to effects observed during infusion periods, when Hp and fecal pH tended to be lower in STARCH cows. Fecal lactate and butyrate tended to be higher in STARCH cows. Diet did not affect immune cell phenotype. Overall means (% total) of dendritic cell markers MHCII, CD40, CD80 and CD86 were 25.1%, 1.1%, 0.9%, 0.2% (adipose), 25.2%, 9.3%, 26.4%, 3.3% (colon) and 13.7%, 3.3%, 14.2%, 1.7% (jejunum), respectively. Means of effector T-cell markers CD4 and CD8 were 14.6%, 22.6% (colon) and 4.2%, 12.2% (jejunum), respectively. Although replication was low, the rumen and hindgut starch challenge did not appear to induce systemic inflammation or inflammatory cell infiltration into the intestines.

**Key Words:** hindgut, starch, inflammation