Late-Breaking Original Research

**LB1** Composition, nitrogen fraction, and amino acid profile of mare's milk produced in the mountains and highlands. A. T. Mazhitova,* and A. A. Kulymyrzaev, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan.

The study was carried out to determine the effect of 2 ecological regions and lactation period on chemical (P < 0.01) and amino acid (P < 0.01) composition and nitrogen fraction (P < 0.01) of mares grazing on pastures at 1700 m (vegetation is dominated by shrubs and sub-shrubs) and 2200 m (vegetation is dominated by grasses, forbs, sub-shrubs) above sea level. The animals were kept under extensive pasture conditions and received no additional feed supplements. Milk samples were collected monthly from May to July and from May to August from mares grazing at 1700 m (Mountains) and 2200 m (Highlands), respectively. Total solids (11.39–10.47% and 11.56–10.86%), milk fat (1.80–1.33% and 1.83–1.60%) and ash (0.48–0.26% and 0.55–0.30%) content of milk for the Mountain and Highland regions respectively were decreased to the end of lactation period. The average percentages of casein to whey protein ratio were 52:47 and 50:42 for milk obtained from Mountain and Highland pastures. The highest content of essential amino acids were obtained in May milk for both regions (1251 and 1284 mg/100 g of milk) and the lowest content were in July (852 mg/100 g of milk) and August milk (880 mg/100 g of milk), but the percentages of essential amino acids in protein of mare's milk were the highest in June (49%) and July milk (49%) produced at 1700 m and 2200 m respectively. The results of the study have shown that the changes in chemical composition, nitrogen fraction, and amino acid profile of the mare's milk during lactation period are influenced by geographical location of pastures.

**Key Words:** mare's milk, amino acid composition, pasture

**LB2** Colorimetric detection of volatile organic compounds for shelf-life monitoring of milk. M. Ziyain,* Washington State University, Pullman, WA, USA. Colorimetric nanosensors for monitoring food quality and shelf life provide an exciting development with obvious economic benefits. In this study, a colorimetric sensor based on silicon dioxide (SiO$_2$) nanoparticles and Schiff’s reagent to detect volatile organic compounds (VOCs) generated by the growth of spoilage bacteria in pasteurized whole milk stored at 7, 13, 15, and 19°C was developed. Volatile organic compounds formed from microbial growth were detected using solid-phase microextraction (SPME) and gas chromatography. Volatile organic compounds (VOCs) generated by the growth of spoilage bacteria in pasteurized whole milk stored at 7, 13, 15, and 19°C was developed. Nanosensor response correlated well with microbial growth during ESBM and true ileal digestibility (TID) of protein and AA in milk replacers (MR) containing all milk proteins (CON) or an enzyme-treated soybean meal (ESBM) protein. A T-cannula was placed in the ileum of 9 Holstein calves at 15 d of age. After 2 wk post-surgery, calves were randomly assigned to a 3 × 3 replicated Latin square with 5-d periods. Calves were fed 2× daily at a rate of 2% (DM) of BW, adjusted weekly. No starter was offered to minimize rumen development. Digesta samples were collected continuously during 12 h on d 4 and 5 of each period. Basal endogenous losses of AA (AA$_{endo}$) and CP (CP$_{endo}$) were estimated by feeding a nitrogen-free MR to each calf during 1 period. Total (basal + specific) AA$_{endo}$ and CP$_{endo}$ were estimated by multivariate regression of the χ distances between digesta and reference protein AA profiles. Ileal digesta pH with the ESBM (7.27) diet was lower (P < 0.01) than with CON (7.51). According to the piecewise nonlinear model of pH fluctuation, digesta pH during ESBM decreased slower after feeding and reached its nadir later than with the CON diet. Diet did not affect (P = 0.45) ADG, but calves on the ESBM diet showed a bigger increment of withers height (0.89 vs 1.61 cm) and lower mean fecal scores (2.81 vs 1.71). Basal ΣAA$_{endo}$ and CP$_{endo}$ were 13.9 and 22.4 g/kg of DM, respectively. Total ΣAA$_{endo}$ (24.6 vs 32.1 g/kg DM) and CP$_{endo}$ (28.2 vs 37.1 g/kg DM) were higher (P < 0.05) with ESBM than with CON. Accordingly, AID and SID of most AA, CP, and ΣAA were lower or tended to be lower with ESBM. However, TID did not differ between diets for CP and all AA except Ala and Ile; TID for Arg tended for the following outcomes: antibiotic (ABX) use at dry-off; risk of new and cured infections (IMI) during the dry period; 120-d clinical mastitis (CM) and culling risks; milk yield and somatic cell count (SCC) in the first 120 d in milk. Seven herds were recruited from 4 study sites (CA, IA, MN, and NY). Cows (n = 1,275) were randomly allocated to BDCT, Culture-SDCT, and Algorithm-SDCT. All quarters of the Blanket group were treated with intramammary ABX. Quarters of Culture cows received ABX if any growth was observed after milk culture using the MN Easy 4Cast plate. Algorithm cows received ABX in all quarters if they met any of the following criteria: ≥ 2 cases of CM during lactation, CM during the 14 d before dry-off, or any test day SCC > 200,000 cells/ml during lactation. All quarters were treated with an internal teat sealant. Risk differences (RD), Hazard ratios (HR) and adjusted means were estimated using marginal standardization, Cox proportional hazards and linear mixed models, respectively. Quarter-level ABX use was reduced by 55% in each SDCT group. IMI cure risk was similar in Blanket (89.8%), Culture (90.0%, RD = +0.2%, 95%CI: -4.4, 4.7%) and Algorithm (90.4%, RD = +0.6%, 95%CI: -3.9, 5.2%) quarters. New IMI risk was similar in Blanket (15.1%), Culture (15.3%, RD = +0.2%, 95%CI: -2.5, 2.9%) and Algorithm (14.9%, RD = -0.2%, 95%CI: -2.9, 2.5%) quarters. CM incidence was similar for Blanket (14.5%), Culture (12.2%, HR = 0.82, 95%CI: 0.6–1.2), and Algorithm (12.2%, HR = 0.82, 95%CI: 0.6, 1.1) cows. Risk of culling was similar for Blanket (10.8%), Culture (9.8%, HR = 0.89, 95%CI: 0.6, 1.3) and Algorithm (10.6%, HR = 0.98, 95%CI: 0.7, 1.4) cows. Adjusted geometric mean SCC was similar for Blanket (55, 95%CI: 47, 65), Culture (57, 95%CI: 49, 68), and Algorithm (59, 95%CI: 50, 69) cows. Adjusted average daily milk yield (kg/day) was: Blanket (48.6, 95%CI: 46.2, 51.1), Culture (48.6, 95%CI: 46.2, 51.1), and Algorithm (47.8, 95% CI: 45.3, 50.2). SDCT successfully reduced ABX use by 55%, without causing negative effects on health and productivity.

**Key Words:** selective dry cow therapy, on-farm culture, algorithm

**LB4** Ileal digestibility of an enzyme-treated soybean meal for milk replacer in preweaned dairy calves. I. Ansini,* H. H. Steinn, C. Brokner, D. A. Vermeire1, and J. K. Drackley1, University of Illinois, Urbana, IL, 1Hamlet Protein A/S, Horsens, Denmark, 2Nouriche Nutrition, Lake St. Louis, MO.

Our objective was to measure and compare apparent (AID), standard (SID), and true ileal digestibility (TID) of protein and AA in milk replacers (MR) containing all milk proteins (CON) or an enzyme-treated soybean meal-based (ESBM) protein. A T-cannula was placed in the ileum of 9 Holstein calves at 15 d of age. After 2 wk post-surgery, calves were randomly assigned to a 3 × 3 replicated Latin square with 5-d periods. Calves were fed 2× daily at a rate of 2% (DM) of BW, adjusted weekly. No starter was offered to minimize rumen development. Digesta samples were collected continuously during 12 h on d 4 and 5 of each period. Basal endogenous losses of AA (AA$_{endo}$) and CP (CP$_{endo}$) were estimated by feeding a nitrogen-free MR to each calf during 1 period. Total (basal + specific) AA$_{endo}$ and CP$_{endo}$ were estimated by multivariate regression of the χ distances between digesta and reference protein AA profiles. Ileal digesta pH with the ESBM (7.27) diet was lower (P < 0.01) than with CON (7.51). According to the piecewise nonlinear model of pH fluctuation, digesta pH during ESBM decreased slower after feeding and reached its nadir later than with the CON diet. Diet did not affect (P = 0.45) ADG, but calves on the ESBM diet showed a bigger increment of withers height (0.89 vs 1.61 cm) and lower mean fecal scores (2.81 vs 1.71). Basal ΣAA$_{endo}$ and CP$_{endo}$ were 13.9 and 22.4 g/kg of DM, respectively. Total ΣAA$_{endo}$ (24.6 vs 32.1 g/kg DM) and CP$_{endo}$ (28.2 vs 37.1 g/kg DM) were higher (P < 0.05) with ESBM than with CON. Accordingly, AID and SID of most AA, CP, and ΣAA were lower or tended to be lower with ESBM. However, TID did not differ between diets for CP and all AA except Ala and Ile; TID for Arg tended to be lower with ESBM.
(P = 0.07) to be greater with ESBM. According to the estimation model, the differences of protein endogenous losses were caused by an increase of gut bacterial protein rather than by host protein. In fact, we found that flows of digesta DNA were greater (P < 0.01) with ESBM (3.2 vs 4.7 g/kg DMI), but the mucin flow (estimated using glucosamine concentration as marker) did not differ (2.1 vs 1.8 g/kg DMI). Adjusting digestibilities of AA in MR by endogenous losses is crucial when comparing alternative proteins to milk proteins.

Key Words: amino acid, ileal digestibility, calf


Neutrophils are cells of the innate immune system that have the ability to respond to stimuli. Galectin-8, a part of the family of galectins, has been shown to modulate the innate and adaptive immune system. The objective of this study was to analyze the effect of exogenous galectin-8 on global transcription in bovine neutrophils. Whole blood was collected from the jugular vein of clinically healthy Holstein-Friesian cows from the North Carolina A&T State University Dairy Unit (n = 5). Neutrophils were isolated by differential centrifugation and hypotonic lysis, and TC20 was used to measure viability. Neutrophils were then treated (1 × 10^6 cells/mL viable) with rGal-8 (2 μg), LPS (10 μg), rGal-8 + LPS, or maintained in PBS at 37°C for 1 h, 5% CO2. Total RNA was extracted using Trizol, RNA integrity (RIN) was determined using Agilent Bioanalyzer, and RNA with RIN of >7 was used. The RNA sequences were generated using an Illumina HiSeq 4000 sequencer. Short RNA fragment reads were in FASTQ format and aligned to the cow reference genome (bosTau8), using Spliced Transcripts Alignment to a Reference (STAR) software. Mapped reads were counted with HTSeq. Genes were normalized against the control, PBS. Differentially expressed genes were analyzed using DESeq2. Pathway analysis was conducted using Ingenuity Pathway Analysis (IPA) software and Database for Annotation, Visualization and Integrated Discovery (DAVID). Our results show that 14,023 genes were expressed. Transcriptome profiling identified differentially expressed transcripts with the treatment of galectin-8 (2037), LPS (477), and Gal-8+LPS (1065) (P < 0.05). Galectin-8 targeted 78 pathways, including MAPK signaling pathway; Gal8+LPS targeted 63 pathways including TNF signaling pathway; and LPS targeted 41 pathways including toll-like receptor signaling pathway using DAVID. Integrin Signaling pathway was one of the top canonical pathways targeted by galectin-8 using IPA. These results suggest galectin-8 has broad interactions with several molecules modulating cytokines, chemokines, and innate and adaptive immune genes.

Key Words: cow, galectin, RNA sequencing

**LB6** Synergistic associations of bacteria and archaea in DNA and cDNA components of rumen samples collected using stomach tube and cannula methods in dairy cows. D. W. Pitta1, C. F. A. Lage2, J. S. Bender1, N. Indugu1, M. L. Hennessy1, V. K. Shabtai1, B. Vecchiarelli1, A. Fernandez1, A. Spitzer1, S. E. Raisanen1, A. Melgar2, K. Nedelkov1, X. Cher1, J. Oh1, and A. N. Hristov1, University of Pennsylvania, Pennsylvania State University, University Park, PA, USA. 2University of Pennsylvania, Pennsylvania State University, University Park, PA, USA. 3University of Minas Gerais, Belo Horizonte, Brazil. 4College of Pastorial Agriculture Science and Technology, Nanzhou University, China. 5Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria. 6Ehryer College of Arts and Sciences, West Virginia University, Morgantown, WV, USA. 7School of Veterinary Medicine, Cornell University, Ithaca, NY, USA.

The rumen microbial ecosystem is comprised of bacteria, protozoa, fungi and archaea that work synergistically to facilitate feed digestion. However, the synergistic interactions between different microbes is seldom investigated. Here we investigated rumen bacteria and archaea associations of 6-cannulated Holstein cows that were adapted to a basal total mixed ration. Rumen samples were collected via stomach tube (ST) and rumen cannula (RC) at 0, 2, 4, 6, 8, and 12 h post feeding and filtered to collect solid rumen contents. These samples were extracted for genomic DNA (total) and RNA (cDNA; metabolically active), PCR amplified, sequenced and analyzed using QIIME pipeline for DNA and cDNA-based bacteria and archaea diversity. Based on PERMANOVA analysis for bacteria and archaea communities differed (P ≤ 0.05) between DNA and cDNA but showed no differences (P ≥ 0.05) between ST and RC. At the individual taxa level, ANCOM analysis showed that lineages of *Actinobacteria* and *Bacteroidetes* were lower (P ≤ 0.05) and those of *Proteobacteria* and *Fibrobacter* bacterial phyla were higher in the cDNA compared with DNA bacteria across ST and RC samples. Particularly, the abundance of *Ruminococcus* and *Succinivibrionaceae* were more than doubled in the cDNA compared with DNA bacteria. Similarly, for archaea, *Methanobrevibacter* was lower (P ≤ 0.05) and *VadinCA11* (P ≤ 0.05) was higher in cDNA compared with DNA archaea in ST and RC samples. These results indicate that the cDNA approach is more discriminatory than the DNA approach for microbial diversity analysis in ST and RC methods. Correlation analysis of cDNA bacteria with molar proportions of volatile fatty acids in both ST and RC revealed strong positive correlations (P ≤ 0.05) between *Ruminococcus* and acetate, *Succinivibrionaceae*, *Prevotella* and *Bulledia* and propionate and *Clostridium* and butyrate. Co-occurrence analysis revealed that co-associations existed between *Ruminococcus* and *Methanobrevibacter*, and *Succinivibrionaceae* and *Methanospirae*. This is the first study demonstrating the synergistic associations of bacteria-archaea cohorts in the rumen that are individual specific but differed between individual cows.

Key Words: bacteria-archaea cohorts, proxy, ANCOM

**LB7** Effects of increasing propionate concentration on short-term metabolism in liver explants from dairy cows in the postpartum period. K. M. Kennedy* and M. S. Allen, Michigan State University, East Lansing, MI, USA.

Our objective was to determine the temporal effects of increasing concentrations of propionate on hepatic metabolism of dairy cows in the postpartum (PP) period. Liver of 6 dairy cows (3 primiparous: 9.00 ± 1.00 (mean ± SD) d PP and 3 multiparous: 4.67 ± 1.15 d PP) was biopsied and used in a block-design liver explant experiment. Treatments consisted of 3 concentrations of ^13^C_sodium propionate at 1, 2 or 4 mM. Explants were incubated in 2 mL of supplemented medium 199 at 38°C and sampled at 0.5, 15 and 60 min. Explants were analyzed for [M+n] citrate, isocitrate, succinate, fumarate, malate, pyruvate, lactate, glutamate, and glucose by GC-MS and for acetyl CoA, propionyl CoA, succinyl CoA, and methylmalonyl CoA by LC-MS/MS. Data were analyzed with mixed models and repeated measures. Increased concentrations of ^13^C-propionate increased total ^13^C% enrichment of propionyl CoA, succinyl CoA, fumarate, malate, and citrate over time (P ≤ 0.01). Treatment did not affect total ^13^C% enrichment of hepatic glucose (0.89 vs. 0.89 vs. 1.00%; P = 0.79) or acetyl CoA (4.23 vs. 4.48 vs. 4.87%; P = 0.79) but total ^13^C% enrichment increased over time for both (P < 0.001). Total ^13^C% enrichment of pyruvate (2.18 vs. 3.36 vs. 4.18%; P < 0.001) and [M+2] pyruvate (1.39 vs. 2.15 vs. 2.54%; P < 0.001) increased over time with increased concentrations of propionate. Increases in ^13^C% enrichment of [M+4] citrate (2.36 vs. 3.30 vs. 4.00%; P < 0.001) and [M+5] citrate (1.28 vs. 1.81 vs. 2.30%; P < 0.001) indicate propionate conversion to acetyl CoA and subsequent entry of acetyl CoA into the TCA cycle. Because these cows were in a lipolytic state, the conversion of pyruvate to acetyl CoA is inhibited. However, the relative partitioning of pyruvate to oxaloacetate or acetyl CoA is dependent upon enzyme activities. This research indicates that at least some propionate can be converted to acetyl CoA and oxidized in the TCA cycle. Metabolic
reactions occur very rapidly and understanding short-term metabolism may improve feeding strategies for dairy cows in the PP period.

**Key Words:** propionate metabolism, liver, dairy cows

LB8 *Moringa oleifera* polyphenols modulate galectin expression in LPS-induced bovine peripheral blood mononuclear cells. S. Adjei-Fremah1, K. Ekwemalor1, E. Asiamah2, and M. Worku*, 1North Carolina A&T State University, Greensboro, NC, USA, 2University of Arkansas at Pine Bluff, Pine Bluff, AR, USA.

Galectins are carbohydrate-binding proteins that function to regulate immune and inflammatory response. The expression of galectin genes LGALS1, LGALS3, and LGALS9 has been associated with innate and adaptive immunity. Bioactive plant-derived polyphenols enhance immunological health in animals. Studies have shown that bioactive plant compounds are able to bind and regulate galectins in inflammatory diseases. Polyphenols derived from *Moringa oleifera* have antioxidant and anti-inflammatory properties but their effect on galectin expression in the bovine peripheral blood mononuclear cells (PBMC) has not been studied. The objective of this study was to investigate the effects of *Moringa oleifera* polyphenol extract (MOPE) on galectin gene transcription and translation in LPS-challenged bovine PBMC ex vivo. Bovine PBMC was isolated from blood collected from lactating Holstein cows (n = 10; age = 3.73 ± 0.35 yr; milk yield = 25.71 ± 3.11 kg/cow per day) using Ficoll technique. LPS-challenged PBMC (1.0 μg/mL *Escherichia coli* O111:B4), was incubated with MOPE (10 μg/mL) for 3 h. Quantitative real-time PCR (qRT-PCR) was used to evaluate the mRNA levels of bovine galectin 1 (LGALS1), galectin 3 (LGALS3), and galectin 9 (LGALS9), using TBP, ACTB, and RPLP0 as internal controls. Secretion of GAL-1, GAL-3, and GAL-9 in culture supernatant was measured using ELISA. Data were analyzed by GLM procedure (SAS 9.4). Results from the qRT-PCR showed decreased mRNA of LGALS1 (fold change, FC = −2.01), LGALS3 (FC = −3.84) and LGALS9 (FC = −3.98) after treatment with MOPE. Treatment with MOPE decreased mean concentration of GAL-1 (41.71 vs. 172.76 ng/mL; P < 0.01), GAL-3 (3.30 vs. 9.95 ng/mL; P < 0.01) and GAL-9 (854.05 vs. 2336.76 pg/mL; P < 0.01). Overall, results from this study showed that polyphenol from *Moringa* decreased GAL-1, GAL-3 and GAL-9 mRNA and protein expression in bovine PBMC. Our finding suggest the use of polyphenols extract from *Moringa* enriched feed supplements may have immunomodulatory properties for bovine health, and aid in the design of galectin-based strategy to counteract LPS-induced inflammation and morbidities.

**Key Words:** bovine PBMC, galectin, *Moringa*