659 Forage concentration and dried distillers grains with solubles in diets for lactating dairy cows. S. D. Ranathunga*, K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe, *South Dakota State University*, *Brookings*.

The objective of this study was to investigate the effects of concentrations of forages and dried distillers grains with solubles (DDGS) on production of lactating dairy cows. Twelve Holstein cows were assigned randomly to replicated 4 × 4 Latin squares in a 2 × 2 factorial arrangement of treatments. Diets were formulated containing low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. Forage consisted of 80% corn silage and 20% alfalfa hay (DM basis). Ground corn and soybean feeds were partially replaced by DDGS from 0% DDGS diets to formulate 18% DDGS diets. Average DMI was not affected by diets (Table 1). Milk yield was greater when cows were fed LF compared with HF regardless of the addition of DDGS (43.3 vs. 41.5 kg/d). Milk fat concentration (3.03 vs. 3.38%) were lesser for cows fed LF compared with HF, whereas protein concentration (3.11 vs. 2.98%) and yield (1.34 vs. 1.24 kg/d) were greater for cows fed LF compared with HF. Yields of fat, total solids (TS), and 4% fat-corrected milk (FCM) were not affected by diets. Cows fed HF had greater feed efficiency FCM/DMI) compared with cows fed LF (1.50 vs. 1.43). Overall, there were no interactions of forage and DDGS for any of the measures. Results suggest that the concentration of forage in diets influences the performance of cows, but not the addition of DDGS. Consequently, partially replacing starch from ground corn and protein from soybean feeds with DDGS at either 41 or 60% of forage in the diet did not affect the production of lactating dairy cows.

Table 1.

	Low forage		High forage			
Item	0DDGS	18DDGS	0DDGS	18DDGS	SE	Pa
DMI, kg/d	25.6	26.1	25.1	25.1	0.56	NS
Milk, kg/d	42.8	43.7	41.7	41.2	1.31	F
Fat, %	3.07	2.99	3.42	3.34	0.25	F
Protein, %	3.09	3.13	3.00	2.96	0.05	F
Fat, kg/d	1.30	1.30	1.43	1.37	0.08	NS
Protein, kg/d	1.32	1.36	1.25	1.22	0.04	F
TS, kg/d	5.17	5.26	5.12	4.96	0.15	NS
4% FCM, kg/d	36.6	37.0	38.1	37.0	1.32	NS
FCM/DMI	1.43	1.42	1.52	1.48	0.04	F

F = Forage effect ($P < 0.05$); NS = No significant effect of forage, DDGS levels, and forage*DDGS interaction.

Key Words: distillers grains, starch, forage

660 In vitro effects of *Escherichia coli* lipopolysaccharide on the function and gene expression of neutrophils isolated from the blood of dairy cows. X. S. Revelo* and M. R. Waldron, *University of Missouri*, *Columbia*.

The objective of this study was to investigate the effects of *Escherichia coli* lipopolysaccharide (LPS) on the function and gene expression of bovine neutrophils (PMNL). PMNL from midlactation cows (161 ± 15 d postpartum; n = 7) were incubated with 0, 1, 25 and 50 μg/mL of LPS for 120 min and the generation of reactive oxygen species (ROS), PMNL extracellular traps (NETs), chemotaxis and killing of *Staphylococcus aureus* were determined. Incubation with 25 μg/mL of LPS increased intracellular ROS by 79% in non-mitogen-stimulated PMNL whereas 50 μg/mL of LPS enhanced intracellular ROS in non-stimulated and stimulated PMNL by 184 and 145%, respectively. Non-stimulated PMNL incubated with 25 and 50 μg/mL of LPS both had a 105% increase in NETs. LPS had no effect on subsequent PMNL chemotaxis or killing of *S. aureus*. To examine the effect of LPS on the expression of genes involved in PMNL function, mRNA was purified from PMNL isolated from midlactation (143 ± 5.6 d postpartum; n = 5) and early lactation cows (7 ± 0 d postpartum; n = 5), after a 120-min incubation with 0 or 50 μg/mL of LPS. Amounts of interleukin-8 (IL-8), tumor necrosis-α (TNF-α), bactericidal/permeability-increasing protein (BPI), myeloperoxidase (MPO), superoxide dismutase (SOD), cytosolic NADPH oxidase (p67-phox), flavocytochrome *b* (p22-phox), histone H2A.1 (H2A.1) and histone H2B-like (H2B) mRNA were determined by real-time quantitative reverse transcription PCR. LPS increased IL-8, TNF-α and SOD mRNA expression in PMNL isolated from all cows (7.9, 21.5 and 2.1 fold change relative to β-actin, respectively) whereas only PMNL collected from midlactation cows had higher p67-phox and flavocytochrome mRNA expression when incubated with LPS (2.10 and 2.06 fold change relative to β-actin, respectively). LPS had no effect on MPO, H2A.1 and H2B mRNA levels. These results suggest that LPS primes the neutrophils toward enhanced immunity by increasing the generation of ROS and expression of NETs along with elevated expression of genes encoding inflammatory mediators and enzymes involved in the production of ROS.

Key Words: neutrophils, lipopolysaccharide, reactive oxygen species

661 Expression analysis of genes of sialic acid metabolism in transition and late lactation Holstein cows using microarrays and RNA sequencing. S. Wickramasinghe*, S. Hua, G. Rincon, A. Islas-Trejo, C. B. Lebrilla, and J. F. Medrano, *University of California, Davis*.

Recent studies on sialylated milk oligosaccharides demonstrated beneficial effects to the suckling neonate. However, the concentrations of sialylated oligosaccharides are low in cow milk and it is important to identify a genetic strategy to optimize the content of sialylated oligosaccharides because cow milk based formula is the first choice as a substitute for human breast milk. The objective of this project was to identify and characterize the genes involved in sialic acid (Sia) metabolic pathways in milk in transition and late lactation cows. Expression analysis of genes in Sia metabolism was conducted by microarrays and RNA-sequencing (RNaseq) in milk cell samples collected from Holstein cows. Gene expression results from microarrays and RNaseq were in good agreement. However, RNaseq offered a larger dynamic range and provided a detailed characterization of genes by identifying alternative splice forms and abundant single nucleotide polymorphisms. Twenty genes in Sia metabolic pathway had low or medium levels of expression in milk and were categorized as genes involved in Sia synthesis, conjugation, transport and breakdown. Eighteen genes showed increased levels of expression in late lactation. ST8SIA1 which regulates synthesis of GD3, the most abundant ganglioside in early lactation showed a higher expression in transition milk, and ST35Gal5 that regulates synthesis of GM3, a prominent ganglioside in late lactation, had high expression in late lactation milk. Among the genes in conjugation, α-2,3-sialyltransferases showed higher levels of expression at the 2 stages of lactation than α-2,6-sialyltransferases, that is characteristically more active in human milk. Sialidases and Sia transporters showed a higher activity in late lactation milk indicating an increase in synthesis and breakdown of sialylconjugates in late lactation. These findings agree with published research on bovine milk oligosaccharide profiles and

variation in type and amount of sialylconjugates, and provide a detailed characterization of the expression of genes determining oligosaccharide content in cow′ milk.

Key Words: milk, sialic acid, gene expression

662 Incidence and risk factors of bovine respiratory disease in dairy heifer calves in Ontario and Minnesota. C. Windeyer*[1], S. J. LeBlanc[1], K. D. Lissemore[1], D. C. Hodgins[1], S. M. Godden[2], and K. E. Leslie[1], [1]*University of Guelph*, *Guelph, ON, Canada*, [2]*University of Minnesota*, *St Paul*.

Bovine respiratory disease (BRD) during calfhood has substantial impacts on the growth, survival and profitability of replacement heifers. One objective of this study was to evaluate risk factors for BRD in dairy heifers until 4 mo of age. A total of 2882 heifer calves from 15 herds in Ontario and 4 herds in Minnesota were examined at 1–7, 15–21, 35–42, and 84–112 d of age. Height, weight and body temperature were measured and health scores assigned using a standardized system. Serum total protein was assessed to evaluate passive transfer, with values less 5.2 g/dL considered failure of passive transfer (FPT). Farm personnel maintained birth, treatment and death records. Mortality was 2.9% overall (4.4% in Ontario and 1.5% in Minnesota) with herd mortality risk ranging from 0 to 9.5%. FPT occurred in 11.3% of calves, but varied by farm (0 to 53.5%). In Ontario, 19.1% of calves had FPT compared with 4.1% of calves from the herds in Minnesota. Overall, 19.4% of calves were treated for BRD (range: 0 to 36.7%). Mean age at first diagnosis was 33 d. Similar proportions of calves were treated for BRD in Ontario (21.9%) and Minnesota (17.1%). Significant risk factors as determined by a generalized linear mixed model for BRD were FPT, season of birth and assistance at calving, controlling for clustering by farm. Calves with FPT had 1.9 times (CI: 1.4–2.7) the odds of BRD compared with calves with successful passive transfer. Odds ratios of BRD for calves born in winter versus fall and summer, and spring versus summer were 2.1 (CI: 1.2–3.5), 2.7 (CI: 1.7–4.2), 2.1 (CI: 1.5–3.0), respectively. Assistance at calving increased a calf's odds of BRD 1.6 times (CI: 1.0–2.7). FPT, season of birth and assistance at calving significantly affect the odds of BRD. The age of onset of BRD may be earlier than traditionally expected. The variation by farm in FPT, incidence of BRD and mortality warrants further investigation.

Key Words: calf, respiratory disease, passive transfer

663 Effect of antibiotic treatment at post-weaning movement and BRD on growth at multiple time points in commercial dairy calves. A. L. Stanton*[1], S. J. LeBlanc[1], D. Kelton[1], S. T. Millman[2], J. Wormuth[3], and K. E. Leslie[1], [1]*University of Guelph*, *Guelph, Ontario, Canada*, [2]*Iowa State University*, *Ames,* [3]*CY Heifer Farm*, *Elba, NY*.

Bovine respiratory disease (BRD) is common following weaning and movement of calves from individual to group housing. The objective was to evaluate the effects of single injection of tulathromycin administered at post-weaning grouping of calves, and the effect of BRD in the 60 d following grouping on the growth of dairy calves at multiple time points. The study was conducted at a custom heifer raising facility in New York State. 1,367 weaned dairy calves were randomly assigned to receive either tulathromycin (TUL) or oxytetracycline (TET), once at the time of first movement to group housing. The incidence of BRD was 8% and 13% in the TUL and TET groups. A total of 248 heifers were identified and treated for BRD in the 60 d following movement. Post-weaning BRD events were recorded by trained barn staff. Weights and heights were measured at strategic points throughout the growing period based on movement through the facility. On average, calves were 56 d of age at enrollment and were re-weighed at 98, 180, 271 and 381 d of age. The effect of BRD and experimental treatment on the ADG of calves that were retained in the herd (n = 1,271) was evaluated between each time period using a mixed model, controlling for source farm and enrollment cohort. Between 56 and 98 days of age, TUL calves had an ADG of 0.90 ± 0.02 kg/day compared to TET calves with an ADG of 0.82 ± 0.02 kg/day ($P < 0.001$). After 98 days of age, there was no difference in ADG. However, the initial advantage in ADG resulted in TUL calves weighing 3.7 kg more than TET calves at 180 days ($P < 0.05$). Calves with BRD in the 60 days following enrollment gained 0.15 ± 0.02, 0.06 ± 0.01 and 0.03 ± 0.01 kg/day less than non-BRD calves between 56 and 98, 98 and 180, and 180 and 270 days of age, respectively. BRD did not have a significant effect on ADG after 270 days of age. At 381days of age BRD calves weighed 18kg less than non-BRD calves ($P < 0.001$).
In this population of calves, who remained in the herd for over a year, BRD in the 60 days following first movement to group housing continued to affect the ADG until 9 months of age.

Key Words: respiratory disease, dairy heifers, tulathromycin

664 Effects of glucose and essential amino acids on phosphorylation of signaling proteins for protein synthesis in bovine mammary epithelial cells. J. A. D. R. N. Appuhamy*[1], J. Escobar[2], and M. D. Hanigan[1], [1]*Department of Dairy Science, Virginia Polytechnic Institute and State University*, *Blacksburg,* [2]*Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University*, *Blacksburg*.

Protein synthesis responds to nutrient signals such as amino acids and energy supply which involve AMP-activated protein kinase (AMPK), tuberous sclerosis complex 2 (TSC2), mammalian target of rapamycine (mTOR), ribosomal protein S6 (rpS6), and eukaryotic elongation factor 2 (eEF2). Increasing phosphorylation (PhS) of AMPK, TSC2, and eEF2 impair protein synthesis while increasing PhS of mTOR and rpS6 stimulate it. Our objective was to investigate the individual and interactive effects of essential amino acids (EAA) and glucose on PhS of AMPK, TSC2, eEF2, mTOR and rpS6 in MAC-T cells. Cells were deprived of serum, EAA, and glucose overnight and then incubated in complete or EAA-deprived DMEM/F12 with and without glucose (3.51 g/L) in a 2 × 2 factorial design for 1 h. Cell lysates were subjected to Western immunoblotting with antibodies against total and phosphorylated mTOR (Ser2448), rpS6 (Ser235/236), eEF2 (Thr56), AMPK (Thr172), and TSC2 (Thr1462). The PhS of each signaling protein was determined as a ratio of the phosphorylated to total forms. Glucose and EAA deprivations increased ($P < 0.10$) PhS of TSC2 (47 and 85%) and AMPK (29 and 28%), and reduced ($P < 0.01$) PhS of rpS6 (31 and 58%). Deprivation of EAA alone reduced ($P = 0.02$) PhS of mTOR by 31% and increased ($P = 0.01$) PhS of eEF2 by 20%. Interactive effects between glucose and EAA for PhS of AMPK, TSC2, and eEF2 were significant ($P < 0.06$) but that for mTOR and rpS6 were non-significant ($P > 0.51$). Glucose and EAA availability appear to synergistically modulate PhS of AMPK, TSC2 and eEF2. Regulation of rpS6 by EAA appears to be mediated by mTOR (Ser2448), but regulation by glucose occurred through an alternative mechanism in MAC-T cells. The effects of EAA on mTOR and rpS6 and the effects of glucose on rpS6 are supportive of independent effects of energy and EAA on protein synthesis.

Key Words: energy, amino acid, cellular signal

665 Prevention of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection in Balb/c mice by feeding probiotic *Lactobacillus acidophilus* NP-51. M. A. Osman*[1], J. R. Stabel[2], J. M. Hostetter[3], D. S Nettleton[4], and D. C. Beitz1,5, [1]*Department of Animal Science, Iowa State University*, *Ames,* [2]*US Department of Agriculture, ARS, National Animal Disease Center*, *Ames, IA,* [3]*Department of Veterinary Pathology, Iowa State University*, *Ames,* [4]*Department of Statistics, Iowa State University*, *Ames,* [5]*Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University*, *Ames*.

The objective of this study was to examine effects of feeding *Lactobacillus acidophilus* strain NP51 to mice challenged with *Mycobacterium avium* ssp. *paratuberculosis* (MAP), the causative agent of Johne's disease. We hypothesized that feeding NP51 would increase Th-1 responses and decrease progression of MAP infection in mice. Thus, Balb/c mice were randomized to treatment groups in a factorial design including mice either fed heat-killed or viable NP51and challenged with either heat-killed or viable MAP. Mice were fed 1×10^6 CFU of either heat-killed or viable NP51·mice^{-1}·day^{-1} along with normal mouse chow until the end of the study. On d 45, mice were challenged with 1×10^8 CFU of heat-killed or viable MAP injected intraperitoneally. Ten mice from each group were killed on d 45, 90, 135, and 180. At each sampling period, tissues were excised from mice and cultured for MAP. Splenocytes were cultured in vitro with either MAP antigen or concanavalin A and examined for proliferation of T cells subpopulations. Overall, feeding NP51 to mice (either heat-killed or viable) significantly increased the frequency of CD8+ cytotoxic T cells in spleens of mice infected with viable MAP. Most importantly, MAP burden was decreased in the mesenteric lymph nodes, livers, and spleens of mice fed the NP51 compared with the MAP-infected controls on d 135. These results suggest that feeding NP51 modifies the immune responses and prevents progression of MAP infection in Balb/c in mice.

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Key Words: MAP, Johne's disease, *Lactobacillus acidophilus*

666 Effects of varying DCAD and Na:K on production, rumen and urine parameters in lactating dairy cows. K. E. Cowles* and M. R. Murphy, *University of Illinois*, *Urbana*.

Six multiparous Holstein cows, fitted with rumen cannulas, averaging 122 ± 31 d in milk were randomly assigned to 6 treatments allocated in an equiradial (pentagonal) second-order response surface design with a center point to examine the effects of dietary cation-anion difference (DCAD) and Na:K on lactating dairy cows. Replication of treatments within a 6 × 6 Latin square minimized the potential effects of outliers and allowed a surface covering a 3 × 3 matrix of DCAD and Na:K combinations to be examined. Ranges in DCAD and Na:K were chosen to be equally spaced on logarithmic scales; tripling each time from 0.25 for the former, and 1.5-fold each time from 25 mEq/100 g DM for the latter. The response surface was centered on a molar Na:K of 0.75 (0.60% Na and 1.37% K in DM) and a DCAD of 37.5 mEq/100 g of DM. The other 5 treatments were: 1.63, 50.0 (Na:K, DCAD); 0.46, 53.8; 0.25, 35.2; 0.63, 25.1; and 2.00, 31.2. Percentages of Na and K in DM of the TMR for vertices of the pentagon were calculated as 1.05, 1.10; 0.56, 2.08; 0.27, 1.84; 0.44, 1.17; and 0.84, 0.72. Diets were based on corn silage and corn-based grain mix. The Na:K ratios were varied with $NaHCO_3$ and K_2CO_3. Periods were 14 d. Daily feed intake of each cow was recorded during each period; samples of feed and orts were collected daily. Milk production was measured daily; samples were collected weekly and analyzed for components. Rumen and urine samples were collected and analyzed for pH on the last 3 d of each period. The MIXED procedure of SAS was used for ANOVA. There were no response surface effects of treatment on milk production and components, and DMI ($P < 0.05$). A linear relationship ($r^2 = 0.15$, $P < 0.022$) between mean rumen pH and mean urine was found. A quadratic effect of Na:K ($P < 0.01$) and interaction of DCAD ($P < 0.003$) indicated that urine pH was maximal (8.24 or above) at high DCAD and low Na:K. Linear ($P < 0.05$) and quadratic effects ($P < 0.05$) of DCAD on rumen pH were indicated. In conclusion, relationships exist among rumen pH and urine pH. Urine pH was maximized when diets had high DCAD and low Na:K. Rumen pH responded quadratically to DCAD.

Key Words: DCAD, rumen pH, urine pH