Milk metabolites as noninvasive indicators of nutritional status of mid-lactation Holstein and Montbéliarde cows

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W18 Transcriptional comparison between total RNA and mRNA isolated from same fecal samples of neonatal dairy calves. F. Rosa* and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Inflammatory-related genes are commonly expressed at a low abundance. However, these can still be detected in fecal RNA isolated from dairy calves, which contains a negligible amount of RNA from immune cells under non-diarrheic conditions. Additionally, genes related to common functions of the gastrointestinal (GI) tract were observed in total RNA from fecal samples. However, fecal RNA isolation remains a challenge, because of the potential enrichment of bacterial RNA, which can dilute the targeted eukaryotic RNA and consequently dampen the sensitivity of the fecal RNA method. Therefore, our objective in this study was to determine the differential eukaryotic RNA enrichment in total RNA vs mRNA from same fecal samples of healthy neonatal dairy calves. To test this, 200 mg of feces were used from 6 neonatal Holstein calves for total RNA isolation, using a Trizol based method along with the RNeasy Plus Mini Kit (Qiagen). Then, 45 µg of fecal total RNA was used to isolate mRNA through a magnetic selection using Dynabeads Oligo (dT)25 (Invitrogen). The cDNA synthesis was performed using the SuperScript IV reverse transcriptase (Invitrogen). The standard curve was composite from all samples including cDNA from total RNA and mRNA. The internal control genes used in this experiment were *B2M*, *ACTB*, *GAPDH*, *RPS9*, and *PPIA*. Normalized gene expression data were log-transformed before statistical analysis using the Proc Mixed of SAS (SAS 9.4). Expression of genes specific for epithelial cells including cytokeratin 8 (*KRT8)* and aquaporin (*AQP3*) as well as inflammatory-related genes (*TLR4* and *IL1B*) were evaluated. The expression of *KRT8* was greater ($P = 0.03$) in fecal mRNA than in fecal total RNA. A trend ($P = 0.09$) was observed for greater expression of *TLR4* in fecal total RNA than in fecal mRNA. The expression of *AQP3* and *IL1B* was not different. Greater expression of *KRT8* in mRNA than in total RNA suggests that this additional selection of gene transcripts within fecal RNA might improve the sensitivity of this method and consequently the accuracy and robustness. These results further confirms that the fecal RNA method has a potential to be used as a tool to evaluate GI tract molecular adaptations in dairy calves.

Key Words: calf, fecal RNA, gastrointestinal tract

W19 Comparative transcriptomic analysis of epithelial cell markers across gastrointestinal tissues and fecal RNA isolated from dairy calves. F. Rosa*, N. Carpinelli, R. Mohan, and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Fecal RNA method can be used to evaluate biological adaptations of the gastrointestinal (GI) tract of dairy calves through gene expression analysis. A limitation with this method is the current lack of data indicating how the transcriptomic profile observed in fecal RNA mirrors that in specific sections of the GI tract. Therefore, our objective in this study was to compare the transcription of gene markers for GI epithelial cells, fatty acid binding protein 2 (*FABP2*) and cytokeratin 8 (*KRT8*) in fecal RNA against several GI tract sections in dairy calves. To test this, postmortem samples were collected from ruminal epithelium, cecum, large intestine, duodenum, jejunum, ileum, and feces from 6 healthy male Jersey calves (5 wk of age) for total RNA isolation. The standard curve was composite from all samples including cDNA from tissues and fecal. The internal control genes used in this experiment were *B2M*, *ACTB*, *GAPDH*, *RPS9*, *RPS15A*, and *PPIA*. Normalized gene expression data were log-transformed before statistical analysis using Proc Mixed of SAS. The expression of *FABP2* was greater ($P < 0.01$) in the duodenum tissue than in GI section associated with fermentation (i.e., rumen, large intestine, and cecum). Within the small intestine the mRNA expression of *FABP2* was greater ($P = 0.01$) in duodenum than in jejunum, but not different than ileum. In fecal RNA, the *FABP2* expression was greater ($P \leq 0.03$) than in GI section related to fermentation. However, *FABP2* was similar ($P = 0.3$) between fecal RNA and ileum. The expression of *KRT8* was greater ($P \leq 0.02$) in cecum and large intestine than in rumen and jejunum. Among the small intestine sections *KRT8* was greater ($P = 0.03$) expressed in duodenum than in jejunum. The fecal RNA had greater ($P \leq 0.02$) expression of *KRT8* than jejunum and ileum. In contrast, the *KRT8* expression in fecal was not different than the transcripts observed in cecum and large intestine. Since the transcription of the genes specific for GI epithelial cells were significant observed in the RNA isolated from feces, these preliminary data further confirms that fecal RNA has a potential to be used as a tool to evaluate molecular adaptations in the GI tract of dairy calves.

Key Words: calf, fecal RNA, gastrointestinal tract

W20 Milk metabolites as noninvasive indicators of nutritional status of mid-lactation Holstein and Montbéliarde cows. P.-A. Billa*[1], Y. Faulconnier[1], T. Larsen[2], C. Leroux[1,3], and J. Pires[1], [1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France,* [2]*Department of Animal Science, Aarhus University, Tjele, Denmark,* [3]*Department of Food Science and Technology, University of California Davis, Davis, CA.*

The objective was to investigate the effects of feed restriction on concentrations of selected milk metabolites in mid-lactation Holstein and Montbéliarde cows, and explore their correlations with energy balance. Nine Holstein and 10 Montbéliarde cows (165 ± 21 DIM) underwent 6 d of feed restriction during which feed allowance was reduced to meet 50% of NE_L requirements calculated before initiation of the challenge. The experiment was divided in 4 periods: Control (CONT; d −3 to −1), restriction (REST; d 1 to 6), WK1 (d 7 to 13) and WK2 (d 18). Milk concentrations of β-hydroxybutyrate (BHB), glucose, glucose-6-phosphate (Glu6P), isocitrate and glutamate were measured and statistical analyses performed using mixed models of SAS with fixed effects of period and breed, and the random effect of cow. Relationships among variables were explored by Spearman correlations. Feed restriction induced a negative EB, increased Glu6P and isocitrate (+38% and +39%, respectively) and decreased BHB, glucose and glutamate concentrations in milk (−20%, −57% and −65%, respectively) compared with pre-challenge values (Table 1). All milk metabolites were significantly correlated with EB (0.46, 0.62, −0.25, −0.41, 0.59 for BHB, glucose, Glu6P, isocitrate and glutamate, respectively). Results suggest that milk metabolites may be used as noninvasive indicators of nutritional status of mid-lactation cows.

Key Words: milk metabolite, dairy cow, energy balance

W21 Use of circulating metabolites and milk production variables to generate linear regression models for prediction of postpartum liver triglycerides. C. R. Seely*, H. T. Holdorf, R. S. Pralle, R. C. Oliveira, J. L. Woolf, M. R. Moede, S. J. Erb, and H. M. White, *University of Wisconsin-Madison, Madison, WI.*

Given the potential impacts of liver triglyceride (lvTG) accumulation on hepatic metabolism, the ability to diagnose fatty liver without a liver biopsy could be advantageous in both the research and applied settings as accumulation of lvTG can only be diagnosed by liver biopsy. Since fatty liver is related to the overall metabolic status of the cow, the

Table 1 (Abstr. W20). Effects of feed restriction on energy balance and milk metabolite concentrations in mid-lactation cows

		Period[1]					*P*-value		
		CONT	REST	WK1	WK2	SEM	Breed	Period	Breed × Period
	HOLS	46[a,y]	−40[b]	41[a,y]	41[a]	4.0			
EB (MJ/d)	MONT	31[x]	−40	29[x]	42	3.6	0.06	0.001	0.04
	HOLS	0.05[b]	0.04[c]	0.06[a]	0.05[b]	0.01			
BHB (mM)	MONT	0.05	0.04	0.06	0.05	0.01	0.93	0.001	0.55
	HOLS	0.54[a]	0.22[c]	0.47[b]	0.51[ab]	0.03			
Glucose (m*M*)	MONT	0.47	0.21	0.45	0.45	0.02	0.15	0.001	0.17
	HOLS	0.02[b]	0.03[a]	0.01[c]	0.03[a]	0.01			
Glu6P (m*M*)	MONT	0.04	0.05	0.02	0.05	0.01	0.24	0.001	0.12
	HOLS	0.12[c]	0.17[a]	0.13[c]	0.13[b]	0.01			
Isocitrate (m*M*)	MONT	0.14	0.19	0.13	0.16	0.01	0.05	0.001	0.29
	HOLS	0.39[a]	0.14[c]	0.39[a]	0.33[b]	0.06			
Glutamate (m*M*)	MONT	0.38	0.13	0.38	0.31	0.06	0.77	0.001	0.99

[a–d]Period LSMEANS not sharing a common superscript differ ($P \leq 0.05$).

[y,x]Breed LSMEANS not sharing a common superscript differ within the period ($P \leq 0.05$).

objective of this study was to determine if the concentration of lvTG could be predicted from milk production variables and circulating blood metabolites related to energy balance and liver health. Blood and liver samples were taken at −14, +1, and +14 d relative to calving (DRTC) from multiparous Holstein cows (n = 37) enrolled in 2 previously reported studies. Daily milk production and weekly milk composition were collected. Liver TG (% dry matter) was quantified and serum was analyzed for aspartate amino transferase (AST), alanine amino transferase (ALT), albumin (alb), BHB, BUN, and triglyceride (TG). Plasma was analyzed for glucose (glc) and nonesterified fatty acids (NEFA). Through the PROC REG procedures of SAS (9.4), forward stepwise linear regression models utilizing a $P < 0.1$ and minimum AIC inclusion criterion were fit to predict either +1 or +14 DRTC, or maximum lvTG% from the analyzed blood metabolites and milk variables. Two types of models were explored; 1) a predictive model that used prepartum metabolites to predict maximum lvTG% and 2) a diagnostic model that utilized +1 and +14 DRTC metabolites and milk variables to predict respective lvTG%. Maximum lvTG% was 18.9 ± 1.4% and occurred at +14 ± 1 DRTC. Diagnostic models at +1 DRTC included ALT and AST ($R^2 = 0.23$) and +14 DRTC included blood TG, NEFA, glc, and cumulative milk yield to date ($R^2 = 0.66$). The predictive regression model for maximum lvTG% based on −14 DRTC metabolites included ALT, alb, BUN, and TG ($R^2 = 0.41$). Overall, postpartum diagnostic models were stronger than predictive models; however, additional metabolites should be explored to improve the ability to diagnose or predict lvTG%.

Key Words: transition cow, biomarker, fatty liver

W22 Antimicrobial usage for the treatment on respiratory diseases in calves: A systematic review. E. Gürdal* and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA.*

Our objective was to conduct a systematic review of the quality of previous publications that evaluated the efficacy of antimicrobials for the treatment of bovine respiratory disease (BRD) in calves. The literature search strategy, based on population, intervention, and outcome of studies written in English from CabDirect, PubMed, Web of Science and Scopus, was conducted on December 2018; a total of 2,058 publications were retrieved. Publications of interest were clinical trials and experimental challenges that used antimicrobials to treat BRD in calves <6 mo. Thirty-four manuscripts containing 37 trials were retained after screening the titles (n = 901), the abstracts (n = 308) and the full papers. The selected trials included clinical trials (n = 22) and challenge trials (n = 15) that dated back from 1979. The median number of animals enrolled was 49 and ranged from 11 to 696 calves. Seventeen manuscripts were either funded or had authors affiliated with pharmaceutical companies. A total of 29 trials were randomized but only 14 of those were blinded. Fifteen trials included a negative control treatment; but only 6 were randomized and blinded. Trials with negative control evaluated the efficacy of: one (n = 3) or more (n = 2) antimicrobials, anti-inflammatories combined with antimicrobials (n = 2), various dosages or timing of treatments (n = 7), or combination of antimicrobial treatments (n = 1). Macrolides were the most common antimicrobial class evaluated (n = 14). The length of the observational period for health outcomes ranged from 3 d to 8 wks. Fever was the most frequent clinical sign of BRD evaluated (n = 26). Only 8 trials evaluated clinical signs of respiratory disease using a scoring tool. In addition to clinical signs, 13 trials performed pathological examination of euthanized calves. Although considerable numbers of studies have been conducted on antimicrobial use for BRD in calves, very few studies were controlled and randomized. Future research on BRD should follow standardized methods for the evaluation of clinical outcomes. Funding provided by CDFA–AUS project.

Key Words: antimicrobial, calf, respiratory disease

W23 Effect of acupuncture therapy in dairy cows affected by pyometra: A randomized controlled clinical trial. P. Pinedo*[1], L. Caixeta[2,3], E. Barrell[2,3], J. Herman[2], J. Velez[4], D. Manriquez[1], and T. Holt[2], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Clinical Sciences, Colorado State University, Fort Collins, CO,* [3]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN,* [4]*Aurora Organic Dairy, Platteville, CO.*

Pyometra (PYO) is a uterine disease characterized by the accumulation of purulent or mucopurulent material within the uterine lumen in the presence of an active corpus luteum (CL). Due to prohibited use of artificial hormones in US certified organic dairies, conventional therapies for treatment of PYI are not applicable. The objective of this study was