

Ruminant Nutrition: Dairy: Ruminal Fermentation and Health

454 Design and validation of primers to access rumen *Treponema saccharophilum* population in both in vitro and in vivo systems. J. Liu^{*1,2}, W. Zhu^{1,2}, Y. Y. Pu¹, J. K. Wang^{1,2}, and J. X. Liu^{1,2}, ¹*Institute of Dairy Science, College of Animal Sciences, ²MoE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, Zhejiang, China.*

Treponema saccharophilum was isolated in the early decades and described as highly preferred to utilize pectin for growth. Typically, alfalfa hay contains 10–15% pectin as a component of the cell wall matrix, whereas grass or corn stover has pectin content less than 5%. The objective of this study was to develop a set of PCR primers targeting the 16S rRNA gene of *T. saccharophilum* and to assess the relative abundance of *T. saccharophilum* associated with different forages that vary in pectin content using real-time PCR method. Fifteen 16S rRNA gene sequences having higher than 97% similarity with *T. saccharophilum*, retrieved from our pyrosequencing data, were used as target templates along with type strain *T. saccharophilum* DSM2985 for primer designing. To confirm the specificity of our designed primers, 23 clones of the PCR amplicons amplifying from total rumen DNA were randomly picked and sequenced to build phylogenetic tree. Phylogenetic analysis revealed 2 closely-related clusters: 3 clones grouping with the type strain and the rest of clones grouping together with no cultivable representatives, suggesting the existence of a subspecies of *T. saccharophilum*. Growth of *T. saccharophilum* under in vitro system was compared among alfalfa hay (AH), Chinese wild rye hay (CW) or corn stover (CS) alone and in combination with pectin or corn starch as substrate, to investigate the efficiency of this method. After incubation for 24 h, *T. saccharophilum* grew better in AH or CW with pectin than CW or CS as sole fiber source ($P < 0.05$). Supplementation of corn starch did not enhance the growth of *T. saccharophilum*. The validation of this method was also investigated in vivo. Samples of rumen fluid were collected from 12 primiparous Chinese Holstein cows that were fed on diets with AH, CW or CS as main forage source. The results of real time-PCR showed that the relative abundance of *T. saccharophilum* in rumen of cows fed on AH as forage source was significantly higher than those on CW and CS ($P < 0.05$).

Key Words: *Treponema saccharophilum*, pectin, forage

455 Effect of yeast-derived microbial protein in low and high forage diets on lactation performance of dairy cows. A. K. Manthey^{*1}, K. F. Kalscheur¹, A. D. Garcia¹, and K. Mjoun², ¹*South Dakota State University, Brookings, ²Alltech, Brookings, SD.*

The objective of this study was to investigate the effect of substituting soybean meal with yeast-derived microbial protein [(YMP) DEMP; Alltech Inc., Nicholasville, KY] in diets containing 2 forage concentrations. Sixteen Holstein cows (4 primiparous and 12 multiparous) were randomly assigned to a 4 × 4 Latin square in a 2 × 2 factorial arrangement of treatments. Diets contained low (LF; 45% of diet DM) or high forage (HF; 65% of diet DM) and YMP at 0 (NYMP) or 2.25% (WYMP) of the diet. Forage consisted of 67% corn silage and 33% alfalfa hay (DM basis). Cows fed LF consumed more DMI (28.3 vs. 26.8 kg; $P < 0.007$) and produced more milk (39.9 vs. 39.7 kg; $P < 0.005$) than cows fed HF regardless of the addition of YMP (Table 1). Milk fat percentage was lower in cows fed LF compared with HF (3.76 vs. 3.94; $P < 0.04$), whereas fat yield tended to be lower ($P < 0.07$) in cows fed YMP than in those that were not. Although milk protein percentage did not differ

between forage concentrations with or without the addition of YMP, protein yield and total solids were greater in cows fed LF. Cows fed LF produced more energy-corrected milk (ECM) than those fed HF (41.9 vs. 40.2; $P < 0.04$). Feed efficiency (ECM/DMI) was greater for cows fed NYMP compared with WYMP (1.52 vs. 1.45; $P < 0.02$). There were no interactions of forage and YMP for any of the parameters measured. Results suggested that the forage level as well as YMP affected cow performance. Cows fed LF diets produced more milk and ECM, yet resulted in a lower fat percentage.

Table 1.

Item	Low forage		High forage		SEM	P-value ¹
	NYMP	WYMP	NYMP	WYMP		
DMI, kg/d	28.3	28.2	26.0	27.2	0.97	F
Milk, kg/d	39.9	40.4	38.4	37.3	1.09	F
Fat, %	3.84	3.68	3.98	3.89	0.15	F
Fat, kg/d	1.52	1.47	1.52	1.43	0.05	NS
Protein, %	3.26	3.25	3.26	3.19	0.08	NS
Protein, kg/d	1.30	1.31	1.25	1.18	0.03	F
TS, kg/d	5.15	5.12	5.03	4.73	0.13	F
ECM, kg/d	42.1	41.7	41.2	39.2	0.98	F
ECM/DMI	1.50	1.47	1.54	1.44	0.04	D

¹F = Forage effect ($P < 0.05$); D = YMP effect ($P < 0.05$); NS = no significant effect of forage, YMP, and forage × YMP interaction.

Key Words: dairy cow, forage concentration, yeast-derived microbial protein

456 Effects of subacute ruminal acidosis on fecal pH and starch digestibility of Holstein cows. C. S. Fox^{*}, S. Luan, M. R. Murphy, and F. C. Cardoso, *University of Illinois, Urbana-Champaign.*

To investigate the association of subacute ruminal acidosis (SARA), fecal pH (FPH), and starch digestibility (SD), 6 rumen-cannulated Holstein cows (CAN, BW = 762 kg; 287 ± 45 d in milk) were used in a replicated 3 × 3 Latin square design balanced for carry-over effects. Periods (10 d) were divided into 4 stages (S): S1, baseline, d 1–3, ad libitum TMR; S2, restricted feeding, d 4, cows fed for 50% of S1 DMI; S3, challenge, d 5, treatments applied; S4, recovery, d 6–10, all cows were fed ad libitum TMR. Treatments were: CON, no top dress; MOD, 10% of S1 DMI as top dress (pelleted mixture of 50:50-wheat:barley); and HIG, 20% of S1 DMI as top dress. TMR samples were analyzed for starch and lignin contents weekly. Fecal samples were collected in 7 time points (TP): TP1, d 1-afternoon; TP2, d 5-morning; TP3, d 5-afternoon; TP4, d 5-night; TP5, d 6-morning; TP6, d 6-afternoon; and TP7, d 7-afternoon. Fecal pH was measured at sample collection. Fecal samples from TP1 to TP5 were analyzed for starch and lignin contents. Rumen pH was lower ($P < 0.0001$) for HIG (6.25 ± 0.09) than for CON (6.44 ± 0.09). Nadir time (hour relative to feeding) at rumen pH below 6 were 3, 5, and 7 h for CON, MOD and HIG, respectively. Fecal pH was similar ($P = 0.233$) for CON (6.48 ± 0.06), MOD (6.54 ± 0.07), and HIG (6.37 ± 0.07). At TP4 FPH was at its nadir (6.04 ± 0.09, $P < 0.001$). At S3 FPH had a tendency ($P = 0.102$) for a linear effect among CON, MOD, and HIG (6.54 ± 0.08, 6.50 ± 0.08, and 6.38 ± 0.08, respectively). Starch digestibility tended ($P = 0.102$) to be greater for CON than MOD and HIG (96.71% ± 0.8, 96.36% ± 0.8, and 94.15% ± 0.8, respectively).

Starch digestibility displayed a linear effect ($P = 0.048$) among CON, MOD, and HIG. At S3 there was a linear effect ($P = 0.014$) for SD among CON, MOD, and HIG ($97.62\% \pm 1.5$, $97.47\% \pm 1.5$, and $91.84\% \pm 1.6$, respectively). At TP4 SD was at its nadir ($93.74\% \pm 1.2$, $P < 0.053$). In conclusion, cows receiving HIG had lower SD than cows receiving MOD or CON. Linear effects for both SD and FPH with increasing challenge suggest a relationship that might be useful as a diagnostic tool for SARA.

Key Words: subacute ruminal acidosis, fecal pH, starch digestibility

457 Induction of subclinical ruminal acidosis leads to marked alterations in blood immunometabolic markers and minerals in lactating Jersey than Holstein cows in response to. J. S. Osorio^{*1}, F. T. da Rosa¹, E. Trevisi², M. R. Murphy¹, F. Cardoso¹, and J. J. Loo¹, ¹University of Illinois, Urbana, ²Università Cattolica del Sacro Cuore, Piacenza, Italy.

Subacute ruminal acidosis (SARA) has long been regarded as a major reason for economic losses in the dairy industry in the United States. SARA has been associated with decreased milk production and decreased efficiency of milk production, while it increases culling rate and death loss. Although Holstein and Jersey cows have inherent differences in terms of lactation potential their response to SARA in terms of immunometabolic alterations and their resilience to normal levels after induction of SARA are yet to be defined. Twelve lactating cows (>100 DIM; $n = 12/\text{breed}$) were used in a replicated 2×2 Latin square design of represented period and dietary treatment (control and challenge). Each period comprised 10 d divided into 4 stages: baseline (d 1–3), feed restriction (d 4), challenge (d 5), and recovery (d 6–10). In each period a control (CO) diet was offered during baseline and recovery stages. On d 4 cows were restricted to 50% of average baseline feed intake until d 5. The challenge on d 5 consisted of either a CO diet or the CO + 4.6 kg of a 50:50 wheat/barley pellet. Blood samples were collected on d 5 at 0, 3, 6, and 12 h relative to the challenge. Data from cows subjected to challenge were analyzed using the PROC MIXED procedure of SAS, where square, period, breed (b), and h were fixed effects, while cow nested within period was the random effect. An interaction of $b \times h$ ($P < 0.04$) was observed for Mg, Na, K, aspartate transaminase (AST), NEFA, and BHBA with Jerseys having greater concentrations of these biomarkers at either 3 or 6 h after challenge. Besides NEFA and Mg, Jerseys also had greater concentrations of other biomarkers at 0 h. Concentrations of Mg, ceruloplasmin, albumin, alkaline phosphate, haptoglobin, NEFA, and reactive oxygen metabolites (ROMt) were greater ($P < 0.04$) overall in Jersey cows regardless of h after challenge. Similarly, creatinine and myeloperoxidase were greater ($P < 0.03$) in Holstein cows. Overall, our results suggest that the profile of immunometabolic biomarkers and minerals in blood were more affected by SARA in Jerseys.

Key Words: breed, immunometabolite, SARA

458 Evaluation of OmniGen AF in heat-stressed Holstein cows in lactation. L. W. Hall^{*1}, S. D. Anderson¹, F. A. Rivera¹, F. Villar¹, J. D. Chapman², N. M. Long³, and R. J. Collier¹, ¹University of Arizona, Tucson, ²Prince Agri, Quincy, IL, ³Clemson University, Clemson, SC.

OmniGen AF is a feed supplement containing yeast and B vitamins. A total of 60 cows on a commercial dairy in Arizona were balanced for DIM, parity and milk production and assigned to 1 of 2 treatment groups fed OmniGen AF (OG, 30 cows) or control (CON, 30 cows) diets for 52 d postcalving. At 52 d of lactation cows were randomly

selected ($n = 12$) from both groups (6 OG and 6 CON) and housed in environmentally controlled modules for 21 d at the University of Arizona. The OG was top-dressed $2 \times/d$ with molasses as the carrier and the CON cows received the molasses carrier $2 \times/d$. Both were mixed into the top one-third of the TMR. During the environmental room phase of the study cows fed OG had higher feed intake than CON during heat stress (HS) (46.8 kg vs. 42.9 kg, $P < 0.0001$) and no difference during thermoneutral (TN). A temperature-humidity index (THI) threshold of 68 or greater was used to achieve HS. Feeding OG maintained a numerical 1 kg milk yield advantage compared with CON (30.3 kg vs. 31.4 kg, $P = 0.26$) during HS but not during TN. Cows fed OG had lower milk fat (%) (4.2% vs. 3.8%, $P = 0.02$) and milk protein (%) ($P = 0.04$). There was no difference in 3.5% FCM between treatments. Water consumption was lower (12.4 l/d in OG treated cows, $P < 0.01$) than control cows. Respiration rates were lower in treated cows at 1400 h and 1700 h (4.7 and 8.4 less respirations/min, $P = 0.05$, < 0.001) and rectal temperatures were also lower (0.15°C and 0.25°C lower than CON, $P = 0.05$, < 0.001) in treated cows. Feeding OG reduced physiological responses to heat stress in lactating dairy cows.

Key Words: heat stress, nutrition

459 The effects of feeding time on the circadian pattern of feed intake, milk production, and plasma hormones and metabolites in dairy cows. M. Niu^{*}, Y. Ying, P. A. Bartell, and K. J. Harvatine, Penn State University, University Park.

The timing of feed intake entrains circadian rhythms (24 h repeating cycles) in other mammals. The object of this study was to determine if the timing of feeding can entrain circadian rhythms in dairy cows. Nine Holstein cows were arranged in a replicated 3×3 Latin Square design with 14-d periods in an automated feed observation system that recorded feed weight every 10 s. Treatments were feeding $1 \times/d$ at 0830 h (AM) or 2030 h (PM) and feeding $2 \times/d$ in equal amounts at 0830 and 2030 h (AMPM). All treatments were fed at 110% of daily intake. Cows were milked $2 \times/d$ at 0500 and 1700 h. Daily measurements were analyzed by a mixed model that included the random effect of cow and period and the preplanned contrasts were AM vs. PM and AM vs. AMPM. Time course data were analyzed with mixed model procedures using repeated measures and the planned contrast were tested at each time point. Second, a cosine function was fit to time course data by nonlinear regression. There was no effect of treatment on DMI, digestible DMI, or total tract digestibility of DM, OM, and NDF. Over 16 and 24% of DMI was consumed in the first hour after feeding for the AM and PM, and 12 and 19% for AMPM in the morning and evening, respectively. Milk yield and composition did not differ among treatments. Milk yield at the 0500 h milking was 1.3 kg higher than the 1700 h milking ($P < 0.01$). Plasma BUN was increased 0.52 and 0.89 mg/dL by PM and AMPM compared with AM, respectively ($P < 0.05$). Treatment did not affect fecal NDF or plasma insulin, glucose, and NEFA, but there was an interaction of treatment and time ($P < 0.05$). Treatment modified the circadian pattern of fecal NDF ($P < 0.01$), plasma glucose ($P < 0.05$), and insulin ($P < 0.01$), but no effect on plasma BUN or NEFA was observed. Phase (time of peak) of fecal NDF was advanced 4.4 h by PM compared with AM ($P < 0.05$), and amplitude tended to be reduced by AMPM compared with AM ($P = 0.08$). In conclusion, feeding time entrains the circadian rhythms of fecal NDF, plasma glucose, and insulin, but has little effect on daily DMI and milk yield.

Key Words: feeding time, circadian rhythm, dairy cow

460 Effects of subacute ruminal acidosis on milk yield and milk composition of Holstein and Jersey dairy cows. K. J. Haerr*, S. Luan, M. R. Murphy, and F. C. Cardoso, *University of Illinois, Champaign-Urbana*.

To investigate the association between Subacute ruminal acidosis (SARA) on milk yield and milk composition, 6 Holstein cows (HOL, BW = 717 kg; 258 ± 16 d in milk), 6 rumen-cannulated Holstein cows (CAN, BW = 762 kg; 287 ± 45 d in milk), and 6 Jersey cows (JER, BW = 470 kg; 190 ± 86 d in milk) were used in a replicated 3 × 3 Latin square design to measure carry-over effects. Periods consisted of 10 d and were divided in 4 stages. Stage 1 (S1), baseline, d 1–3, ad libitum TMR; S2 restricted feeding, d 4, all cows fed for 50% of their S1 DMI; S3 challenge, d 5, treatments applied; S4, recovery, d 6–10, cows were fed ad libitum TMR. Cows were fed according to NRC (2001) recommendations. Treatments were: CON, no top dress (TD, pelleted mixture of 50:50 of wheat:barley); MOD, 10% of DMI in S1 as TD; and HIG, 20% of DMI in S1 as TD. Milk yield was recorded daily and milk composition was obtained for d 2, 4, 5, and 8. Dry matter intake was recorded daily. Body weight and BCS were measured at each period. Statistical analysis was performed using the MIXED procedure of SAS 9.2 (SAS Institute, Inc.). No treatments carry-over effects were observed for any outcome variable ($P > 0.30$). Milk yield was similar ($P = 0.141$) for CON (21.2 ± 35 kg/d), MOD (22.22 ± 35 kg/d), and HIG (21.85 ± 36 kg/d). Holstein milk yield was higher ($P < 0.001$) than JER (24.9 ± 49 and 19.2 ± 54 kg/d, respectively). There was no difference ($P = 0.486$) in milk fat (MF) among treatments. Holstein MF was lower ($P < 0.001$) than JER MF (3.64% ± 0.1 and 4.74% ± 0.1, respectively). There was no difference ($P = 0.615$) in milk protein (MP) among treatments. Holstein MP was lower ($P < 0.001$) than JER MP (3.08% ± 0.06 and 3.65% ± 0.02, respectively). Feed efficiency (FE = energy-corrected milk/DMI) was greater ($P < 0.001$) for S2 (3.34 ± 0.2) than for S1 (1.66 ± 0.2), S3 (1.16 ± 0.2), and S4 (1.65 ± 0.2). In conclusion, MOD and HIG did not result in greater milk fat depression or lower milk yield compared with cows receiving CON.

Key Words: milk, ruminal acidosis, SARA

461 Effects of *Saccharomyces cerevisiae* fermentation product on bacteria in the rumen and hindgut of lactating dairy cows during subacute ruminal acidosis (SARA). S. C. Li*, E. Khafipour¹, I. Yoon², M. Scott², and J. C. Plaizier¹, ¹*University of Manitoba, Winnipeg, MB, Canada*, ²*Diamond V, Cedar Rapids, IA*.

The effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on bacteria in the rumen and hindgut during normal feeding (first 4 wk) and subacute ruminal acidosis (SARA_ challenge (following 1 wk grain-based feeding) were studied using 8 rumen- and cecal-fistulated lactating dairy cows in a crossover experiment. A 3-wk washout period separated the experimental periods. During the SARA challenge, 22% of the corn silage in the diet was replaced with wheat/barley pellets. Cows were either supplemented with 14 g/d of Diamond V Original XPC mixed with 140 g/d ground corn (SCFP) or with 140 g/d of ground corn only (Control). During wk 4 and 5 of each period, ruminal, cecal, and rectal contents were sampled at 0 and 6 h after feeding. Genomic DNA was tested using a relative qPCR SYBR Green assay with the primers targeting the 16s rRNA gene of tested bacteria groups except *E. coli*, for which FimH gene was targeted. The challenge successfully induced SARA, and the time below pH 5.6 increased ($P < 0.01$) from 11 to 311 min/d. In the rumen, SARA increased ($P < 0.05$) *Lactobacillus* spp., *Megasphaera elsdenii*, and *Prevotella albensis*, and decreased ($P < 0.05$) *Prevotella brevis* and *Treponema bryantii*. Supplemental SCFP increased ($P < 0.05$) *P. brevis* and *Bifidobacterium* and showed the potential to

alleviate changes caused by SARA. In the cecum, SARA reduced ($P < 0.05$) *P. brevis*, *Clostridium perfringens* while increasing ($P < 0.05$) *M. elsdenii* and *E. coli*. Supplemental SCFP tended to increase ($P = 0.11$) *Bifidobacterium* in the cecum under normal and SARA challenge conditions. In the rectum, SARA increased ($P < 0.05$) *Lactobacillus* spp., *M. elsdenii*, *T. bryantii*, and *E. coli*, and reduced ($P < 0.05$) *Bifidobacterium*. Supplemental SCFP tended ($P = 0.06$) to increase *T. bryantii*, *Bifidobacterium* and *Butyrivibrio fibrisolvens* in the rectum. Neither SARA nor SCFP affected *Streptococcus bovis* and *C. perfringens* in the rumen and hindgut. In conclusion, SCFP stabilized rumen conditions during SARA by alleviating the microbial population changes caused by SARA and increased bacterial populations with beneficial health effects, such as *Bifidobacterium*.

Key Words: bacteria, Original XPC, SARA

462 Factors associated with the type and levels of mastitis pathogens isolated from milk samples collected from milk of organically and conventionally managed dairy cattle. Z. Ullah*, J. K. Margerison¹, D. Simcock², and N. Lopez Villolobos³, ¹*Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand*, ²*Institute of Food Nutrition and Human Health, Massey University, Palmerston North, New Zealand*, ³*Institute of Veterinary and Biological Sciences, Massey University, Palmerston North, New Zealand*.

This study assessed the factors associated with mastitis related pathogens isolated from individual quarter milk samples collected from 46 organically (Org) and 51 conventionally (Conv) managed dairy cows. The Rapid Mastitis Test and pathogen isolation were completed on milk samples collected at 1, 14, 130, 260 d in milk (DIM). Presence of mastitis pathogens was treated as a binomial trait (0 = absence, 1 = presence), and modeled, following a logit-transformation, using PROC GLM MIX (SAS version 9.3) including herd, lactation number, DIM and interaction between management type and lactation number as fixed effects and individual cow as a random effect. Monthly somatic cell count (Org: 145,000; Conv: 139,000/ml) did not differ between organic and conventional management. *Bacillus* spp. were isolated more frequently from organic than conventional quarters (Org: 8.6; Conv: 5.3 isolates) whereas coagulase-negative staphylococci (CNS) tended ($P = 0.055$) to be isolated more frequently in organically (Org: 24.3) than conventionally (Conv: 17.3) managed quarters. The CNS cases were greater in all cows at 1 DIM (CNS: Org: 35.6%; Conv 29.7%) compared with 130 DIM (CNS: Org: 24.9%; Conv: 11.8%). Conventional quarters had a higher incidence of *Bacillus* spp. and *Streptococcus uberis* at 1 DIM than organically managed quarters. *Bacillus* spp. was isolated more frequent in older (>5 lactations) conventionally managed quarters, while cases of *Streptococcus uberis* were greater in 3+4 lactation organically managed animals. Clinical mastitis (CM) and CNS were greater in organic cows in 1 and > 5 lactations, while conventional cows showed no significant differences between lactations. In conclusion, organically managed cows had a greater number of cases of *Streptococcus uberis* in 3+4 lactation and cases of animal culling were higher. Overall, mastitis pathogens were more frequent at 1 DIM and first lactation animals, indicating the opportunity to improve prevention in dry cows and prepartum heifers.

Key Words: mastitis, organic, dairy

463 Associations of subclinical endometritis with energy metabolism and inflammation during the periparturient period and early lactation in dairy cows. T. Yasui*, K. McCann, R. O. Gilbert, D. V. Nydam, and T. R. Overton, *Cornell University, Ithaca, NY*.

Multiparous Holstein cows (n = 108) were used to determine the associations of subclinical endometritis (SCE) with plasma nonesterified fatty acids (NEFA) and B-hydroxybutyrate (BHBA) as markers of energy metabolism, calculated energy balance (EB), and plasma haptoglobin (Hp) as a marker of inflammation during the periparturient period and early lactation. Evaluation of endometrial cytology by low volume uterine lavage was conducted on one day between 40 and 60 d postcalving. The incidence of SCE among cows sampled was 40%. Area under the curve (AUC) were calculated for both NEFA and BHBA using data collected from -3 to +3 wk relative to parturition. Overall, cows that developed SCE tended to have higher AUC for NEFA (7759 vs. 6602 $\mu\text{Eq/L} \times \text{d}$; $P = 0.08$) and BHBA (126.9 vs. 112.6 $\text{mg/dL} \times \text{d}$; $P = 0.08$). Further stratification into prepartum (wk -3 to parturition) and postpartum (parturition to wk +3) revealed that prepartum AUC for both NEFA and BHBA were not associated with subsequent SCE, whereas postpartum AUC for NEFA (5391 vs. 4427; $P = 0.11$) tended to be increased and BHBA was increased (72.0 vs. 58.9; $P < 0.05$) for cows that developed SCE. Consistent with the results for plasma NEFA and BHBA, calculated EB during the prepartum period was not different in cows that did or did not develop SCE; however, cows with SCE had lower (-3.8 vs. -1.9 Mcal/d ; $P = 0.02$) EB during the 6-wk postpartum period compared with cows without SCE. Analysis of EB by week (wk -3 to -1 before calving and wk 1 to 6 postcalving) indicated that EB in cows with SCE was lower at wk 1 (-8.1 vs. -4.9; $P = 0.01$), wk 2 (-7.9 vs. -5.5; $P = 0.04$), and wk 3 (-4.9 vs. -2.7; $P < 0.05$) and tended to be lower at wk 6 (0.9 vs. 2.5; $P = 0.10$) than cows without SCE. Plasma Hp concentrations were analyzed from wk 1 to 8 of lactation; concentrations of Hp were not different during either wk 1 or the entire postpartum period between cows that did or did not develop SCE. These results suggest that decreased energy status, particularly during the first 3 wk postpartum, predisposes dairy cows to subsequent SCE.

Key Words: subclinical endometritis, energy status, haptoglobin

464 Meta-genomics of rumen bacteria in cows exposed to different feeding strategies. H. M. Golder^{*1,2}, S. E. Denman³, C. McSweeney³, W. J. Wales⁴, M. J. Auld⁴, A. R. Rabiee^{1,2}, P. Celi^{2,5}, and I. J. Lean^{1,2}, ¹*SBS*Scibus, Camden, NSW, Australia, ²*University of Sydney, Faculty of Veterinary Science, Camden, NSW, Australia*, ³*CSIRO Animal, Food and Health Services, Queensland Bioscience Precinct, St. Lucia, QLD, Australia*, ⁴*Future Farming Systems Research Division, Department of Primary Industries, Ellinbank, VIC, Australia*, ⁵*Melbourne School of Land and Environment, The University of Melbourne, Parkville, VIC, Australia*.

We hypothesized that distinct bacterial communities would result from different feeding strategies. Twenty-four rumen fistulated, lactating Holstein cows in a prospective cohort study grazed pasture that provided approximately 8 kg DM/cow per d and were offered 1 of 3 diets: (1) Control (10 cows); (2) partial mixed ration (PMR; 10 cows); or (3) PMR plus protein (PMR+P; 4 cows). The study compared feeding PMR with or without increased protein with controls. The control diet included wheat grain fed twice daily at milking and ryegrass silage fed on the pasture. The PMR comprised corn silage, corn grain, wheat grain and the same silage fed on a feedpad twice daily after milking. The PMR+P cows were managed similarly to the PMR cows, but the ration contained lucerne hay, no silage and 16% DM of the wheat grain was replaced with canola meal. Monensin (220 mg/hd/d), tylosin (220 mg/hd/d), vitamins and minerals were included in all diets. The diets were replicated at 5

rates: 8, 10, 12, 14 or 16 kg DM/cow per d (27:73 ryegrass silage:wheat DM; 2 cows/rate per diet). The PMR+P was offered at 14 or 16 kg only. Rate within diet comparison was isoenergetic. Three rumen fluid samples were collected over a 10 h period after AM milking. Genomic DNA 454 tag amplicon pyrosequence data was processed using QIIME software. Microbiomes were not different; however, distinct bacterial populations between diets ($P < 0.001$) and rates ($P < 0.001$) were evident on principal components between group analysis. Co-inertia analysis showed feeding rate was a larger influence on bacterial composition than time of sampling, individual rumen VFA, ammonia, lactate or pH. Rate had a large effect on bacterial composition for the Control and PMR groups fed the 8 or 10 kg rate. A strong positive association was present between ammonia and bacteria in 12 and 14 kg PMR groups and 14 kg PMR+P cattle. Bacteria populations of these 3 groups were positively associated with lactate, butyrate, acetate and pH. Bacteria in the control group fed at 12, 14 or 16 kg were strongly influenced by propionate concentrations. Feeding system and rate influenced rumen measures and bacterial populations.

Key Words: acidosis, meta-genomics

465 The influence of immunological stress on the limiting sequence and ratio of lysine, methionine and threonine in preruminant calves. N. Zhang^{*}, H. Li, Y. Tu, C. Jiang, and Q. Diao, *Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China*.

The redistribution of nutrients from the growth process to support of immune system function suggests altered nutritional requirements during immune challenge. However, it is not clear how the amino acid requirements altered following immune stress. This study investigated the effect of immunological stress on the limiting sequence and ratio of Lys, Met, and Thr in preruminant calves. According to the method of amino acid deletion, 25% of Lys, Met or Thr was deleted from the milk replacers, which contained 22.00% CP, 1.60% Lys, 0.50% Met and 1.03% Thr, to form 4 kind of experimental diets. Forty male Chinese Holstein calves (between 6 and 8 d old, 40 ± 1 kg of body weight) were randomly divided into 8 groups with 5 replicates (1 calf per replicate). Per Four groups of calves feed 4 kinds of diets respectively. The first 4 groups injected intraperitoneally with 2.5 μg *E. coli* lipopolysaccharides (LPS) /kg BW at 24, 26 and 28 d of age. The other 4 groups injected with an equivalent volume of sterile saline as control. A total collection of feces and urine was conducted between 25 and 27 d for analysis of nitrogen metabolism. The average daily gain of calves was decreased (438.7 vs. 366.5 g/d, $P < 0.05$, for the Control and LPS group calves, respectively) and the ratio of feed to gain of calves was increased (1.89 vs. 2.21, $P < 0.01$) by the immunological stress. The nitrogen retention was suppressed by the immunological stress (0.54 vs. 0.49 g N /kg $\text{BW}^{0.75}/\text{d}$, $P < 0.05$). The ratio of the change of N retention (g /kg $\text{BW}^{0.75}/\text{d}$) to the change of Lys, Met, or Thr intake (g /kg $\text{BW}^{0.75}/\text{d}$) changed from 1.34, 1.07 and 0.71 to 0.92, 0.49 and 0.67, suggested that the limit sequence of Lys, Met, and Thr were changed from Lys > Met > Thr to Lys > Thr > Met, with the immunological stress. The ratio of Lys / Met / Thr for calves changed from 100/29/58 to 100/27/61 with the immunological stress. The results indicated that the immunological stress suppressed the growth performance and nitrogen balance, and changed the limit sequence and the optimum ratio of Lys, Met, and Thr.

Key Words: amino acid, immunological stress, limiting sequence and ratio