T361 In vitro methane production from increasing levels of corn- or wheat-based dried distillers grains with solubles. M. Hünerberg*[1], L. Holtshausen[2], T. A. McAllister[2], K. A. Beauchemin[2], and E. Okine[1], [1]*University of Alberta*, *Edmonton, AB, Canada*, [2]*Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*.

Increasing dietary fat levels have shown to depress ruminal methane (CH_4) production, but the reduction may depend upon the dietary forage to concentrate ratio. The objective of this study was to compare in vitro CH_4 production from wheat- or corn-based dried distillers grains with solubles (WDDGS, CDDGS). The WDDGS (4.9% crude fat) and CDDGS (11.5% crude fat) replaced barley silage as substrate at levels of 0, 20, 40, 60 or 100% (DM basis). Each treatment, dried and ground through a 1-mm screen, was incubated (n = 5) in Ankom bags in 125-mL sealed batch culture flasks (0.3 g substrate + 40 mL of anaerobic medium and 10 mL of inoculum). The inoculum was obtained from cows fed a high forage (65% of diet DM) diet. Gas pressures arising from substrate fermentation were measured at 3, 6, 12 and 24 h post inoculation. Pressure values, corrected for gas released from 3 negative controls (no substrate) were used to generate volume estimates. Gas samples collected at the same 4 time points were analyzed for CH_4 concentration. Disappearance of DM and VFA concentration in incubation liquid were measured at 24 h. Production of CH_4 increased linearly (r^2 = 0.98) for CDDGS from 15.2 to 33.3 mL CH_4/g of DM loss at 20 and 100% DDGS, respectively. Production of CH_4 was greater ($P < 0.01$) for WDDGS (averaging 32.6 ± 0.3 mL/g DM loss) than for CDDGS at 20, 40 and 60% of inclusion rates. The percentages of propionate in fluid were greater ($P < 0.01$) for CDDG (22.3, 21.3 and 20.3% of total VFA) than for WDDGS (19.4, 19.4 and 19.5% of total VFA) at 20, 40 and 60% of inclusion. The results suggest: (i) in vitro CH_4 production as per unit of DM loss is lower at lower DDGS inclusion rates (ii) including CDDGS, most likely as response to its higher fat content, produced less CH_4 per unit of DM loss then including WDDG at up to 60% of dietary DM.

Key Words: methane, dried distillers grains with solubles, in vitro

T362 The impact of DDGS on presence of ruminal bacteria, ruminal protozoa and yeast during in vitro fermentation. E. Castillo-Lopez*, J. L. Miner, and P. J. Kononoff, *University of Nebraska-Lincoln*, *Lincoln*.

Changes in ruminal microbial populations is of interest because of their role in feed degradation and metabolizable protein supply to the animal. The objective of this experiment was to evaluate the effect of dried distillers grains and solubles (DDGS) on presence of ruminal bacteria, protozoa and yeast during in vitro fermentation. Treatments were, CONTROL (50% grass hay and 50% rolled-corn), LOW DDGS (33% grass hay, 33% rolled-corn and 33% DDGS) and HIGH DDGS (100% DDGS). Substrates were incubated in rumen inoculum and replicated 3 times. At 0 and 48h fermentation a pellet was isolated from each sample, then bacterial, protozoal and yeast crude protein was estimated by real-time PCR. To do so, microbial markers were designed from the 16S rRNA, 18S rRNA and the second chromosome; for bacteria, protozoa and yeast. Data were analyzed as a 3 × 2 factorial design to test the effects of 3 treatments, 2 time points and interaction between treatment and time. Treatment did not ($P = 0.31$) affect the estimates of bacterial crude protein and averaged 206, 200 and 171 (SEM = 16.08) mg/g DM for the CONTROL, LOW DDGS and HIGH DDGS. However, treatment affected ($P < 0.05$) the estimate of protozoal crude protein and averaged 46, 27 and 6 (SEM = 3.74) mg/g DM for the CONTROL, LOW DDGS and HIGH DDGS. In addition, treatment did not affect ($P = 0.25$) the estimate of yeast crude protein and averaged 0.05, 0.09 and 0.19 (SEM = 0.06) mg/kg DM for the CONTROL, LOW DDGS and HIGH DDGS. Fermentation time did not affect ($P = 0.66$) the estimates of bacterial crude protein and were 197 and 189 (SEM = 12.38) mg/g DM at 0 and 48h. However, time affected ($P < 0.05$) the estimates of protozoal crude protein and were 14 and 39 (SEM = 3.14) mg/g DM at 0 and 48h and the interaction of treatment by time was significant ($P < 0.05$). Furthermore, time did not affect ($P = 0.66$) the estimates of yeast crude protein and were 0.09 and 0.12 (SEM = 0.04) mg/kg DM at 0 and 48h. With real-time PCR, it was possible to estimate the variation of ruminal bacterial, protozoal and yeast crude protein. Level of DDGS may affect the in vitro microbial growth.

Key Words: bacteria, protozoa, real-time PCR, yeast

T363 Effects of low dose of *Saccharomyces cerevisiae* on metabolism by ruminal microbes in dual flow continuous culture fermenters. M. Ruiz-Moreno*[1], M. D. Stern[1], and J. Sullivan[2], [1]*University of Minnesota*, *St Paul,* [2]*Lallemand Animal Nutrition - North America, Milwaukee, WI.*

Effects of *Saccharomyces cerevisiae* (SC) on rumen fermentation were evaluated using a dual flow continuous culture system. Eight fermenters were inoculated with ruminal fluid from a dairy cow in early lactation on d 1 of a 10-d experimental period. Fermenters were provided with 75 g of DM/d of a pelleted diet formulated for a high lactating dairy cow (40 kg milk/d, 3.8% fat, 3.7% protein). Two levels of SC (Levucell, SC20, Lallemand) at 0 or 2 mg/fermenter/day (SC0 and SC2, respectively) were infused twice a day at 0900 and 2100 h to the fermenters in a completely randomized arrangement of treatments. The latter concentration would be equivalent to supplementing 0.5 g/d of SC to a dairy cow. Apparent and true organic matter degradability were not affected ($P > 0.05$) by SC averaging 55.6 vs. 56.0 and 65.5 vs. 64.7% for SC0 and SC2, respectively. Similarly, no differences were obtained ($P > 0.05$) in NDF and ADF digestibility (51.1 vs. 49.4% and 50.3 vs. 48.1% for SC0 and SC2, respectively). Total VFA concentrations were not affected ($P > 0.05$) by treatments (140.2 and 140.8 m*M* for SC0 and SC2, respectively). There was a trend ($P < 0.1$) for a higher branched chain VFA (isobutyrate, isovalerate and 2-methylbutyrate) concentration in SC0 compared with SC2 (2.34 vs. 1.82 m*M*, respectively). The addition of SC resulted in a lower ($P < 0.05$) NH_3-N concentration and NH_3-N flow (6.28 vs. 3.85 mg/100 mL and 0.19 vs. 0.12 g/d for SC0 and SC2, respectively), without affecting ($P > 0.05$) CP degradation and efficiency of microbial protein synthesis (35.7 vs. 29% and 29.1 vs. 25.8 g of N/kg OM truly digested for SC0 and SC2, respectively). Average and minimum pH of fermenters did not differ between treatments ($P > 0.05$) but a trend ($P < 0.1$) for a lower maximum pH was obtained at 5.78 vs. 5.71 for SC0 and SC2, respectively. A low dose of SC may benefit NH_3-N metabolism, without having any negative effects on in vitro rumen fermentation.

Key Words: *Saccharomyces cerevisiae*, rumen, continuous fermenters

T364 Effects of copper and zinc on in vitro ruminal fermentation of total mixed ration using goat inoculum. J. F. Vázquez-Armijo[1], R. Rojo*[1], D. López[1], A. Z. M. Salem[1], and J. M. González-Alvarado[2],

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One in vitro experiment was conducted to examine the effects of supplemental copper (Cu) and zinc (Zn) on ruminal parameters, in vitro dry matter degradability (IVDMD), gas production (GP) and metabolizable energy (ME) (MJ kg^{-1} DM). Total mixed ration was incubated in vitro for 96 h with 4 different supplemental treatments (Control, Cu (860 ppm), Zn (224 ppm), Cu-Zn (860–224 ppm)) provided as mineral premixed. Added Zn increased fraction B (ml g^{-1} DM), but added Zn-Cu treatment decreased fraction B. Supplemental treatments did not alter the initial delay before gas production begins (L) and IVDMD (g^{-1} DM). Added Cu tended to increase the amount of GP (ml g^{-1} DM) at 24, 48 and 96 h (GP_{24}, GP_{48}, and GP_{96}, respectively) of incubation. Cu treatment was the highest value for the fraction the rate of gas production (K) and ME, while Zn was the lowest values. In conclusion, the addition of Cu to in vitro ruminal fermentation was found to increase gas production volume and efficient use of energy.

Table 1. In vitro ruminal fermentation parameters of total mixed ration with different supplemental treatments

Parameters	Control	Zn	Cu	Zn-Cu	SEM	P-value
B	273.57^{bc}	334.90^{a}	288.80^{b}	241.63^{c}	10.78	0.004
K	0.014^{c}	0.008^{d}	0.037^{a}	0.019^{b}	0.003	<0.001
L	0.87	1.32	1.78	0.68	0.19	0.153
GP_{24}	69.35^{c}	58.51^{c}	169.39^{a}	90.72^{b}	13.22	<0.001
GP_{48}	140.68^{bc}	114.03^{c}	237.07^{a}	153.22^{b}	14.11	<0.001
GP_{96}	202.61^{b}	182.96^{b}	291.02^{a}	210.02^{b}	12.84	<0.001
IVDMD	724.30	714.16	707.07	703.33	4.09	0.309
ME	15.14^{c}	14.40^{c}	21.94^{a}	16.59^{b}	0.90	<0.001

Different superscripts in the same row differ (P<0.05).

Key Words: gas production, minerals, goats

T365 Effects of high rates of extruded flaxseed fed to dairy cows on n-3 fatty acids enrichment in milk-fat and the interaction with milk fat content and yield. U. Moallem*[1], M. Zachut[1,2], H. Lehrer[1], L. Livshitz[1], and A. Arieli[2], [1]*Agriculture Research Organization*, *Bet Dagan, Israel*, [2]*Faculty of Agriculture, Hebrew University*, *Rehovot, Israel*.

The objectives were to examine the effects of high rates of dietary extruded flaxseed (EF) containing high proportion of C18:3n-3 on fatty acids (FAs) composition in milk fat, and the interaction with milk fat content and yield. Multiparous Israeli-Holstein dry cows (256 d pregnant) were assigned to 2 treatments: (i) control (n = 22) were fed a dry cow diet and postpartum (PP) lactating cow diet, and (ii) EF (n = 22) supplemented prepartum with 1 kg/d per cow of EF providing 141 g/d of C18:3n-3, and PP to 100 d in milk a diet consisted of 9.2% EF providing on average 382 g/d of C18:3n-3. Milk solids content was determined from 3 consecutive milkings every 2 weeks. Composition of FAs in milk fat was determined in 50 milk samples (24 controls and 26 EF). Milk production was 6.4% higher and fat percentage was 0.4% units lower in the EF group than in the control, with no differences in fat yields. Content and yield of C18:3n-3 in milk fat was 5.1 and 4.6 times higher in the EF group than in controls, respectively. However, the content of C18:3n-3 in milk fat reached a maximum of ≈2% and increasing the dietary supply of C18:3n-3 did not benefit to enrich milk fat. Within group test revealed that the content of C18:3n-3 in milk fat in the EF group was negatively correlated with milk fat percentage (r = –0.91) and yield (r = –0.89). However, no decrease in de novo synthesis of less than 16 carbons FAs was found in the EF group, whereas C16:0 yield were markedly decreased. Moreover, C16:0 yield in the EF cows was negatively correlated with 18:3n-3 content (r = –0.91) and yield (r = –0.65) in milk fat. It appears that the enrichment of 18:3n-3 in milk fat is limited to ≈2%, and it is negatively correlated with milk fat content and yield. It might also be speculated that C18:3n-3 itself suppresses de novo synthesis of C16:0, but not lower chain FAs, which reduced the overall milk fat content in milk.

Key Words: omega-3, milk fat

T366 Effect of grain source and milling process in ethanol production on nutrient contents and in vitro digestibility of ethanol by-product. W. Z. Yang*[1], T. A. McAllister[1], J. J. Mckinnon[2], K. A. Beauchemin[1], and D. Gibb[1], [1]*Agriculture and Agri-Food Canada, Research Centre*, *Lethbridge, AB, Canada*, [2]*Department of Animal and Poultry Science, University of Saskatchewan*, *Saskatoon, SK, Canada*.

Dried distiller grains with solubles (DDGS) can be derived from ethanol fermentation of varying with type of grains (e.g., corn, wheat, or blend of the 2) or milling process (traditional and fractional). Traditional DDGS contain the residual components (bran, protein, germ, and minerals) of the grain after the majority of the starch has been fermented, whereas, fractional DDGS contain no residual bran and germ which are removed before fermentation. The objective of this study was to compare the nutrient content and in vitro digestibility of different DDGS. Sixty DDGS samples varying grain source and milling process were collected from different ethanol plants in Canada and in US. The DDGS samples were determined for the contents of CP, NDF and fat, and were incubated in a batch culture for 0, 4, 8, 14, 24 and 48 h to measure gas production and DM digestion (DMD). The CP content (% DM) was different ($P < 0.01$) with the highest (51.8) for fractional corn DDGS, medium for wheat (37.9) and blend DDGS (35.9), and the lowest for corn DDGS (30.6). The NDF content (% DM) was lower ($P < 0.01$) for fractional DDGS (24.5), but the fat content (% DM) was higher ($P < 0.01$) for corn DDGS (10.1) than for other DDGS (mean ± SD; NDF, 32.0 ± 1.4; fat, 4.3 ± 0.4). DMD linearly ($P < 0.01$) increased with increasing time of batch culture, and no plateau was obtained after 48 h of fermentation. The DMD were lower ($P < 0.01$) for fractional corn DDGS after 24 h (33%) and 48 h (42%) of fermentation than for other DDGS (mean ± SD; 24h, 44 ± 2.1%; 48h, 54 ± 2.8%). The gas production followed the same variation pattern of the DMD. The results indicate that the nutrient contents of DDGS and extent of digestion varied with DDGS source. The information on the type of grain used and milling process before ethanol fermentation is needed to choose DDGS for accurately formulating ruminant diet.

Key Words: distillers grain, nutrient content and digestion, batch culture

T367 In vitro digestion and gas production of two varieties of barley grain sown with different seeding and N fertilization rates in seven sites across Canada. W. Z. Yang*[1], T. A. McAllister[1], M. Oba[2], and D. Gibb[1], [1]*Agriculture and Agri-Food Canada, Research Centre*, *Lethbridge, AB, Canada*, [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*.

Seeding rate (SR) and N fertilizer application rate (NR) have the potential to alter the starch and protein content of the grain as well as the rate and extent of its digestion. The objective of this study was to determine

whether SR and NR change DM digestion (DMD) and gas production (GP) of barley grain in batch culture. Two malting barley varieties, Copeland and Metcalfe, were seeded at rates of 200 or 400 plants/m^2 with N fertilizer at rates of 0, 30, 60, 90 and 120 kg/ha, respectively, from 7 sites across Canada. Total 560 samples (i.e., 7 sites × 2 varieties × 2 SR × 5 NR × 4 replications) were collected and ground through a 6-mm screen. Fermentability was assessed by measuring in vitro GP and DMD at 0, 4, 8, 12, 18 and 24 h of incubation. The CP content (% DM) of malt barley linearly ($P < 0.01$) increased from 10.3 to 12.2, whereas the starch content (% DM) linearly ($P < 0.01$) decreased from 61.6 to 59.8 with increasing NR. The SR had marginal effect on the contents of CP and starch of barley grains. The DMD after 24 h of batch fermentation ranged on average from 36 to 42% for both varieties and were overall not affected by the SR or NR. There were only one of 7 sites where the SR decreased ($P < 0.01$) DMD of Copeland by 12%, but increased ($P < 0.01$) the DMD of Metcalfe by 17%. The NR linearly ($P < 0.01$) reduced DMD of Copeland from 2 sites and reduced DMD of Metcalfe from one site. There was interaction ($P < 0.01$) between SR and NR on GP: the GP was higher ($P < 0.01$) for 60NR when SR was 200, whereas GP was lower ($P < 0.01$) for 120NR when SR was 400 compared with other NR, respectively. This work demonstrates that the NR changed nutrient content of barley grains and would affect its ruminal degradation rate as shown by the variation of GP.

Key Words: malt barley grain, DM digestion, batch culture

T368 Impact of monensin on rumen microbiota and its stochastic succession. P. Kongmun*[1,2], M. Wanapat[1], and Z. Yu[2], [1]*Department of Animal Science, Khon Kaen University*, *Khon Kaen, Thailand, 40002*, [2]*Department of Animal Science, The Ohio State University*.

This study examined the long-term effects of monensin on rumen microbiota and the effect of monensin on the stochasticity of rumen microbiota using an in vitro model. Rumen fluid samples were collected from 2 Holstein Friesian cows at 6 h post-feeding and constituted into a composite sample as the inoculum. Two sets of cultures (n = 5 each) were incubated at 39°C under anaerobic condition: the monensin cultures contained 5 ppm monensin, while the control cultures contained no monensin. The cultures were transferred every 2 d for 15 d. Samples were collected over the course of the incubation and subjected to analysis for microbiota using both DGGE and sequencing analysis of 16S rRNA genes. The monensin cultures had fewer bands than the control cultures, especially in the high-denaturant area of the DGGE gel that corresponds to bacteria with low GC contact. Principal component analysis (PCA) of the DGGE profiles also showed clear differences between the monensin and the control cultures, with samples collected at d 9 exhibiting the greatest difference. Considerable temporal successions of the microbiota were also evident in both sets of cultures, especially during the initial 9 d of the incubation. Both the DGGE banding patterns and the PCA analysis of the DGGE profiles showed variations among the 5 replicates within the same set of cultures. Because all the cultures were grown in identical test tubes under identical conditions, we attributed these variations among replicates to stochastic succession. It is interesting to note that the early monensin cultures had little stochastic succession among the 5 replicates. A total of 233 random clones were sequenced from individual 16S rRNA gene libraries. At 97% sequence identity level, 46 unique phylotypes were identified that were assigned to genera of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and uncultured bacteria. PCA analysis of these phylotypes is concordant with that of the DGGE profiles. The observations in this study may help explain, at least partially, the variations often observed among individual animals fed the same diet.

Key Words: monensin, rumen microbiota, stochasticity

T369 The effect of body condition at calving and supplementation with *Saccharomyces cerevisiae* on energy status and some reproductive parameters in early lactation dairy cows. R. M. Al Ibrahim*, M. A. Crowe, P. Duffy, L. O`Grady, M. E. Beltman, and F. J. Mulligan, *University College Dublin*, *Dublin 4, Ireland*.

The objective was to examine potential benefits of live yeast culture (YS) supplementation on postpartum (PP) energy status and fertility indices of dairy cows managed to have low or high body condition score (BCS, 1 – 5 scale) at calving. Forty Holstein dairy cows were randomly allocated to a 2 × 2 factorial arrangement. Treatments were: BCS at calving (low, L ≤3.5 or high, H ≥3.75; n = 20) and YS supplementation (2.5 g/cow/d for pre-calving and 10 g/cow/d for post-calving × 10^9 CFU of *S. cerevisiae*/g) (supplemented, Y or control, C; n = 20). Daily milk yield was recorded and weekly milk composition, BCS and BW were assessed from calving to wk 10 PP. Estimated energy balance PP was calculated on a weekly basis individually as the difference between the net energy (NE) intake and the sum of NE for maintenance and milk production. Insulin and IGF-I concentrations were determined on d 14 and 7 pre-calving and 1, 5, 15, 25 and 35 PP. Daily ovarian ultrasonography was performed from d 10 PP to monitor the size and development of the first dominant follicle, first ovulatory follicle and days to first ovulation PP. Pre-ovulatory peak of serum estradiol concentration was determined. Data were analyzed using the Mixed procedure in SAS v 9.1, 2004. Cows in H group (over-conditioned) at calving ingested less NE, produced more milk NE output, and consequently had a significantly ($P < 0.05$) exacerbated negative energy balance in comparison with L group (moderately conditioned) during early lactation. Higher ($P < 0.05$) insulin concentrations and a tendency for higher ($P = 0.06$) preovulatory peak estradiol concentrations in L group were detected in the early PP period. Feeding YS had no effect on energy status of lactating dairy cows with high or low BCS at calving, while it improved serum insulin concentration, preovulatory peak of estradiol and the size of first ovulatory follicle in the early PP period. These observed effects of YS supplementation require to be substantiated with further research.

Key Words: dairy cows, yeast culture, energy balance, reproduction

T370 Effect of supplemented diets with sucrose and/or starch on ruminal peptide-N concentration of Holstein steers. M. Danesh Mesgaran*, F. Rezaii, A. R. Heravi Moussavi, and A. Vakili, *Dept. Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran*.

The objective of this study was to determine the effect of diets containing different types of non-fiber carbohydrates [sucrose (Su), starch (St) or equal mixture of Su and St (Su+St)] on ruminal peptide-N concentration of Holstein steers. Four ruminally fistulated steers (body weight = 280 ± 15 kg) were assigned to a 4 × 4 Latin square with 28 d periods. The basal diet contained alfalfa hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/kg respectively). The non-fiber carbohydrates were added to the basal diet at the rate of 70 g/kg DM. Diets were offered at 2.5 times of the maintenance requirements (7 kg DM/day). Animals were fed twice daily at 08:30 and 16:30. Samples of rumen contents were taken, by suction, at 0.0, 2, 4 and 6 h after the morning feed. Ruminal fluid was prepared for peptide-N analysis using sulfate-tungstate precipitation method. Tungstate acid-precipitate nitrogen was assayed by a standard macro-Kjeldahl procedure. Data were analyzed using mixed procedure of SAS (2003) for repeated measures. Results of the present experiment indicate that peptide-N concentration tended to be lower when steers were fed Su, St and Su+St than BD ($P = 0.09$). Mean Peptide-N concentrations of the sampling times were BD = 2.1, Su = 1.7, St = 1.4 and Su+St = 1.5 mg/dL. Therefore, it might be concluded that the nitrogen metabolism in the rumen is affected by the

type of non-fiber carbohydrates used in the present diets. The effect of sampling time on Peptide-N concentrations was significant ($P < 0.05$). Peptide-N concentrations showed a quadratic significant response to the sampling time ($P < 0.05$). Peptide-N concentrations increased after the morning feeding and declined at 6 h after that. The concentrations of peptide-N at 6 h after the morning feeding (BD = 1.8, Su = 1.4, St = 1.2 and Su+St = 1.3 mg/dL) was less than those of before feeding (BD = 1.9, Su = 1.6, St = 1.3 and Su+St = 1.4 mg/dL).

Key Words: rumen, peptide-N, carbohydrates

T371 Effect of diets supplemented by sucrose and/or starch on in vivo ruminal *Ruminococcus flavefaciens* populations of Holstein steers determined by real time-PCR. M. Danesh Mesgaran*, F. Rezaii, A. R. Moussavi Heravi, M. Nassiri, and A. Vakili, *Dept. Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran*.

The objective of the present work was to investigate the effect of diets containing different types of non-fiber carbohydrates on *Ruminococcus flavefaciens* populations in the rumen fluid of Holstein steers determined by real-time polymerase chain reaction (RT-PCR). Four ruminally fistulated Holstein steers (body weight = 280 ± 15 kg) were assigned to a 4x4 Latin square with 28 d of each period. Basal diet (BD) was formulated to contain of alfalfa hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/ kg DM, respectively). Sucrose (Su), starch (St) or a 1:1 mixture of Su and St (Su+St) was added to the basal (70 g/ kg DM). Diets were offered as 2.5 times of maintenance requirements (7 kg DM/d) at 0830 and 1630 h. Rumen fluid samples were collected before and 4 h after the morning feeding at the last day of each period. Samples were analyzed for *Ruminococcus flavefaciens* quantitation using RT-PCR. The DNA extraction was performed from the samples using the QIAamp DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK). *Ruminococcus flavefaciens* rDNA concentrations were measured by RT-PCR relative to the total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: CGAACGGAGATAATTTGAGTTTACTTAGG and reverse: CGGTCTCTGTATGTTATGAGGTATTACC. Cycling conditions were 95°C for 5 min, 40 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 30 s. Data were expressed relative to the quantification of the total bacterial population, and analyzed using mixed procedure of SAS (2003) and means were compared by the Tukey test at $P < 0.05$. Present results indicate that the supplementation of the basal diet with Su+St tended to decrease ($P = 0.07$) the relative population of *Ruminococcus flavefaciens* in the rumen samples taken before the morning feeding (95×10^{-5} vs. 279×10^{-5}). The adding of Su to the basal diet increased the population of *Ruminococcus flavefaciens* (8689×10^{-5}) compared with BD ($P = 0.06$; 279×10^{-5}) and Su+St ($P = 0.05$; 307×10^{-5}) in the rumen samples of 4 h after the morning feeding.

Key Words: rumen, carbohydrates, *Ruminococcus*

T372 Exogenous proteolytic enzyme increases degradation of dried distillers grains with solubles during in vitro ruminal fermentation. J. M. Vera, J.-S. Eun*, D. R. ZoBell, and A. J. Young, *Utah State University*, *Logan*.

We performed a series of in vitro batch culture experiments to assess if an exogenous proteolytic enzyme (EPE) would improve degradation of dried distillers grains with solubles (DDGS) and beef growing and finishing TMR diets containing DDGS. A commercial enzyme product (Protex 6L, Genencor Division of Danisco, Rochester, NY) having only a protease activity was investigated in this study. In all experiments, strained ruminal fluid was obtained from 2 cannulated beef cows. In experiment 1, the EPE was added to the DDGS at 0, 0.7, 1.4, and 2.1 mg/g DM in a filter bag, and they were incubated for 24 h in gas-tight culture vials (125-mL capacity) with ruminal fluid. The EPE addition resulted in quadratic responses on degradability of DM, NDF, and ADF, and its optimum dose rate was found at 1.4 mg/g DM. In experiment 2, efficacy of the EPE added at 1.4 mg/g DM to DDGS was assessed for 96 h using the Daisy II in vitro fermentation system (Ankom Corp., Macedon, NY). Degradability of NDF and ADF increased starting at 18 h of incubation. In experiment 3, efficacy of the EPE was further investigated using beef growing and finishing TMR diets containing 20% DDGS on a DM basis. Experimental procedures were the same as those used in experiment 2. Addition of the EPE tended to increase ($P = 0.07$) NDF degradability of growing and finishing diets at 12 h of incubation, but the effect of EPE on fiber degradation of beef diets was minor at the later hours of incubation. Total VFA production did not differ due to EPE addition in beef diets. Adding EPE in DDGS as a single substrate resulted in a sizable increase in DM and fiber degradability, but its effects were reduced when added in beef growing and finishing diets containing approximately 20% DDGS. It is recommended that the EPE be further evaluated in a beef steer growth study using diets containing relatively high DDGS inclusion rates.

Key Words: exogenous proteolytic enzyme, dried distillers grains with solubles, in vitro fermentation

T373 Effects of eugenol addition on milk fatty acid composition of dairy cows fed high- or low-concentrate diets. C. Benchaar*[1], W. Z. Yang[2], H. V. Petit[1], and P. Y. Chouinard[3], [1]*Agriculture and Agri-Food Canada*, *Dairy and Swine R&D Centre, Sherbrooke, QC, Canada*, [2]*Agriculture and Agri-Food Canada*, *Lethridge Research Centre, AB, Canada*, [3]*Université*, *Département des Sciences Animales, Québec, QC, Canada*.

Four primiparous lactating cows (BW = 568 kg; DIM = 67) were used in a 4 × 4 Latin square design (28-d periods) with a 2 × 2 factorial arrangement of treatments to determine the effects of eugenol (EUG) addition (0 vs. 50 mg/kg of DMI) and concentrate proportion of the diet (high-concentrate: HC vs. low-concentrate: LC; 65 vs. 35%, DM basis) on milk fatty acid (FA) composition. Diets contained 17.2% CP and were formulated to be isocaloric ($NE_L = 1.65$ Mcal/kg DM) using a commercial source of calcium salts of long-chain FA (Megalac) in LC diets. Analyses of FA were performed on pooled samples collected from 4 consecutive milkings (d 22 to 23). Data were analyzed as a 2 × 2 factorial using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Significance was declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$. No interaction concentrate level × EUG was observed for any of the FA measured. Milk FA profile was not changed by EUG supplementation. Proportions (g/100 g of total FA) of C16:0 (28.2 vs. 24.5%) and *cis*-9 C18:1 (19.4 vs. 16.1%) were higher while the proportion of *cis*-9, *cis*-12 C18:2 (2.04 vs. 2.69%) was lower in milk fat of cows fed LC diets than in that of cows fed HC diets. Milk fat concentrations of *trans*-10 C18:1 (0.34 vs. 0.30%), *trans*-11 C18:1 (1.06 vs. 0.96%; $P = 0.08$), and *cis*-9, *trans*-11 C18:2 (CLA; 0.53 vs. 0.44%) increased in cows fed LC diets as compared with cows fed HC diets, but the ratio *trans*-11 C18:1 to *trans*-10 C18:1 was not significantly affected by concentrate proportion. These results suggest that under the experimental conditions of this study, neither the addition of EUG (50 mg/kg of DMI) nor the increase in dietary concentrate proportion of the diet modified the pathway of biohydrogenation of FA in the rumen.

Key Words: essential oil/eugenol, concentrate proportion, milk fatty acid

T374 Effects of sugar beet pulp substituted for ground corn on the performance and health of Chinese Holstein dairy cows. M. Wang, J. Y. Zhang, J. Q. Wang*, D. P. Bu, L. Y. Zhou, and P. Sun, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

Sixty multiparous Holstein cows (60 ± 22 DIM, 31.2 ± 6.2 kg of milk/d) were used to study the effect of sugar beet pulp (SBP) substituted for ground corn on performance, nutritional status as measured using blood metabolites and health. Cows were randomly divided into 2 pens and within each pen where 3 groups (control, 20SBP or 40SBP) and each group comprised of 10 cows (n = 10). The cows were fed a diet containing either ground corn (control) or SBP (20% or 40% of the ground corn replaced with SBP on the basis of corn DM, 20SBP and 40SBP) as the main energy source. Cows were fed 3 times daily in a tie-stall barn. Feed intake was recorded daily. Cows were milked 3 times daily. Blood samples were collected monthly via venous puncture from coccygeal vein 2 h after morning feeding. Data were analyzed statistically by using PROC MIXED of SAS. The results showed that dry matter intake (21.45, 21.58 and 21.56 kg/d), milk production (28.52, 28.45 and 27.89 kg/d), energy corrected milk (ECM, 28.50, 28.76, 28.33 kg/d) were not effected by increasing SBP substitution ($P > 0.05$). The milk protein (2.95, 2.98, 3.04%), milk fat (3.55, 3.65, 3.66%), milk lactose (4.73, 4.78, 4.79%), total solids (TS,12.18,12.33,12.43%), and solid non-fat (SNF, 8.53, 8.58, 8.66%) showed no significant differences among 3 treatments. In blood metabolite, the concentrations of blood urea nitrogen (BUN, 23.39, 19.76, 20.94 mg/dl) and glucose (52.34, 44.2, 44.66 mg/dl) decreased when cows fed SBP in the diets ($P < 0.01$). Nonesterified fatty acid (NEFA) and β-hydroxybutyrate (BHBA) concentrations were not significantly different among treatments. Manure score was lower (2.97, 2.85, 2.89, respectively) for 20SBP than for the other treatments ($P < 0.05$). However, no significant differences existed among 3 treatments on somatic cell counts (SCC), body condition score (BCS), locomotion score, and average body weight ($P > 0.05$). The results indicate that supplemented dried sugar beet pulp is equal to corn as an energy source for lactating dairy cows when fed at the replacing 20 and 40% of corn the DM.

Key Words: sugar beet pulp, ground corn, performance

T375 Garlic botanical reduces methane production in rumen fluid determined in vitro. S. Cavini[1], D. Bravo[2], S. Calsamiglia[1], G. F. Schroeder*[3], M. Rodriguez[1], and A. Ferret[1], [1]*Universitat Autonoma de Barcelona*, *Spain*, [2]*Pancosma*, *Geneva, Switzerland*, [3]*Cargill Innovation Campus*, *Elk River, MN*.

The intent of this study was to evaluate the effect of increasing doses of a particular garlic extract standardized in propyl propyl thiosulfonate (PPT) on rumen fermentation pattern and methane production in vitro. The effect of PPT on in vitro microbial fermentation using ruminal fluid from a dairy cow was determined using the gas production technique. Thirty milliliters of a 1:4 ruminal fluid-to-buffer solution were introduced into glass polypropylene tubes supplied with 0.5 g of DM of a 60:40 forage:concentrate diet and incubated for 72 h at 39°C. Gas production was measured and samples were collected for VFA, ammonia, and methane (CH_4) concentrations. Treatments were control (CON), 20, 40, 80, 120 and 160 mg/L of PPT (abbreviated PPT20 to PPT160) and 500 mg/L of Monensin (MON) as positive control. Each treatment was tested in duplicate and in 2 replicated periods. Results were analyzed with PROC MIXED and PROC REG of SAS. The PPT linearly decreased total VFA ($Y = -0.084X + 86.20$, R^2=0.76) with PPT160 producing more VFA (69.7 m*M*) than MON (57.9 m*M*). The PPT quadratically depressed the molar proportion of acetate ($Y = -0.0002X^2 + 0.012X + 77.2$, $R^2 = 0.96$). Inversely, PPT quadratically increased the molar proportion of propionate ($Y = 0.0001X^2 - 0.006X + 13.1$, $R^2 = 0.97$) with PPT160 producing still less propionate than MON (14.6 vs. 18.4 mol/100 mol). Increasing doses of PPT quadratically decreased CH_4 production ($Y=-0.0006X^2 + 0.042X + 14.61$, $R^2 = 0.97$) with PPT160 producing 6.01 μL/L (compared with CON = 14.3 and MON = 1.8 μL/L). Furthermore, in order to take into consideration the parallel reduction in CH_4 and VFA, the ratio CH_4/VFA was also analyzed, resulting in decrease by PPT dose ($Y = -7.10^{-6}X^2 = 5.10^{-4}X + 0.174$, $R^2 = 0.94$) with PPT160 being associated with a 50.3% decrease. The present results indicated that a garlic extract standardized in thiosulfonates exhibited promising effect on reduction of CH_4 production in vitro. An in vitro dose between 120 and 160 mg/L may be optimal.

Key Words: methane, garlic, in vitro

T376 In vitro methane production by ruminal microorganisms is affected by the diet of donor animals. M. L. Tejido[1,2], M. J. Ranilla*[1,2], C. Saro[1,2], and M. D. Carro[1,2], [1]*Dpto. Producción Animal, Universidad de León*, *24071, León, Spain*, [2]*Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n*, *24346 Grulleros, León, Spain*.

Six rumen-fistulated sheep were fed 4 diets in a partially replicated 4 × 4 Latin square design to investigate the effects of forage to concentrate ratio (F:C) and type of forage (FOR) in the diet on in vitro methane production. The diets consisted on either 70:30 (HF) or 30:70 (HC) F:C ratio, and either alfalfa hay (A) or grass hay (G) as FOR. In each period, ruminal fluid from each sheep was used to inoculate batch cultures containing the same 4 diets as substrate. Cultures were incubated at 39°C for 24 h. There were no F:C x FOR interactions ($P > 0.05$) for any measured variable. Methane and total volatile fatty acids (VFA) production was 17.5 and 10.0% times greater ($P < 0.05$) with HC-inoculum compared with HF-inoculum. Changing the F:C in the diet of sheep did not affect ($P > 0.05$) propionate production for any substrate, but production of butyrate was augmented ($P < 0.01$) as F:C increased. Methane:VFA ratio and apparent dry matter digestibility were not affected ($P > 0.05$) by F:C in the diet of sheep. For all substrates, inoculum from sheep fed A diets promoted greater ($P < 0.05$) production of methane and total and individual VFA, as well as greater ($P < 0.01$) acetate:propionate ratios and apparent dry matter digestibility compared with inoculum from sheep fed G diets. Methane:VFA ratio was greater ($P < 0.05$) with A-inoculum compared with G-inoculum for HC substrates, but no effect of FOR was observed for HF substrates.
There were clear differences in methane production among inocula from different sheep, which persisted across diets and substrates. Methane emission estimated from VFA production was about 21% greater ($P < 0.001$) than that directly measured, but both values were significantly related ($r = 0.612$; $P < 0.001$; $n = 128$). The results indicate that methane production in vitro is affected by both F:C ratio and type of forage in the diet of donors, and these variables should be taken into account when conducting in vitro experiments.

Key Words: methane, forage:concentrate ratio, forage

T377 Hydrogen sulfide release by ruminal microbes maintained in batch culture. M. Ruiz-Moreno*[1], E. Seitz[1], J. Garrett[2], and M. D. Stern[1], [1]*University of Minnesota*, *St. Paul,* [2]*Quali Tech Inc., Chaska, MN.*

Hydrogen sulfide (H_2S) release in the rumen depends upon ruminal pH, sulfur availability and its interaction with other minerals. An in vitro rumen fluid incubation was conducted using 2 sources of sulfur and 2 sources of Zn, Cu and Mn in a 2 × 2 factorial arrangement of treatments during 2 consecutive 24-h periods. A synthetic diet consisting of 36% cellulose, 32% starch, 19% CP, 5% fat and 2.4% sugar provided substrate for microbial metabolism. Sulfur was added as $NaSO_4$ or sulfur-bound lignosulfonate to a final concentration of 0.75% of DM. Copper, Zn and Mn were added as $CuSO_4$, $ZnSO_4$ and $MnSO_4$ or as protected Cu, Zn and Mn (SQM protected minerals, Quali Tech Inc.) to a final concentration of 16, 56 and 71 ppm of DM, respectively. Rumen fluid was obtained from a ruminal cannulated lactating dairy cow and mixed with McDougall's artificial saliva to a 1:4 ratio. Treatments were assigned in 6 replicates to 120-mL serum bottles containing 40 mL of the inoculum mix and 0.5 g dietary DM. Serum bottles were flushed with N_2, crimp sealed and incubated during 24 h at 39.1°C. At the end of incubations, gas volume was measured, H_2S in the headspace of bottles was analyzed and final pH of incubations was recorded. Results were analyzed as a 2 × 2 factorial design. An interaction between lignosulfonate and mineral source was detected. Addition of SQM minerals and lignosulfonate resulted in lower pH ($P < 0.05$) than that without lignosulfonate (5.87 vs. 5.95, respectively), while absence of SQM minerals resulted in intermediate pH of incubations despite lignosulfonate (5.90 ± 0.04). Addition of lignosulfonate without SQM minerals decreased total gas production ($P < 0.001$) compared with the other treatments (173.1 vs. 175.9 mL/g OM). Lignosulfonate resulted in a lower ($P < 0.001$) production of H_2S (416.2 vs. 475 µg/g OM). In contrast, addition of SQM minerals increased ($P < 0.001$) production of H_2S (469.5 vs. 421.5 µg/g OM). Results indicate that source of trace mineral can influence the dynamics of rumen fermentation.

Key Words: rumen, hydrogen sulfide, in vitro

T378 Comparison of bacterial diversity in the rumen of sheep and in Rusitec fermenters as assessed by ARISA–PCR. M. J. Ranilla*[1,2], M. L. Tejido[1,2], C. Saro[1,2], and M. D. Carro[1,2], [1]*Dpto. Producción Animal, Universidad de León*, *24071, León, Spain*, [2]*Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n*, *24346 Grulleros, León, Spain*.

This study was designed to compare the effects of 4 diets on bacterial communities in bacterial pellets (BP) isolated from the solid (SAB) and liquid phase (LAB) of the rumen of sheep with those observed in Rusitec fermenters. The 4 experimental diets had forage:concentrate ratios (F:C) of 70:30 (HF) or 30:70 (HC) and alfalfa hay or grass hay as forage (FOR). SAB and LAB were isolated from each sheep (4 per diet) and fermenter (n = 4) immediately before feeding, and bacterial diversity was analyzed by ARISA-PCR of the 16S ribosomal DNA. A total of 170 peaks were detected in the ARISA electropherograms across the full set of 64 BP. The number of peaks (NP) in BP from sheep ranged from 42 to 82 for LAB, and from 31 to 81 for SAB (168 peaks in total). In fermenters, NP ranged from 53 to 79 for LAB, and from 21 to 69 for SAB (162 peaks in total). No effect of F:C ($P > 0.05$) on NP or Shannon index (SI) was observed on LAB in any system. F:C did not affect SAB profile in fermenters, but NP and SI were greater ($P < 0.05$) in SAB from sheep fed HF diets compared with those from HC-sheep. Feeding grass hay diets promoted greater ($P < 0.01$) SAB diversity in both systems compared with alfalfa hay diets. FOR did not ($P > 0.05$) affect LAB profile in sheep, but grass hay-fed fermenters had greater ($P < 0.01$) LAB diversity compared with fermenters fed alfalfa hay diets. The results indicate that bacterial diversity was more markedly affected by FOR than by F:C. There was a positive relationship (P = 0.001) between the NP in LAB and that in SAB in Rusitec, but no relationship ($P = 0.72$) was found in sheep; this would indicate that dietary effects on bacterial diversity were similar in LAB and SAB in fermenters, but contrasting in sheep. When all samples were analyzed together by clustering analysis, 2 distinct clusters were observed for in vivo and in vitro BP, which suggests a different structure of the bacterial communities in sheep and fermenters.

Key Words: rumen, fermenters, bacterial diversity

T379 Effect of supplemented diet by sucrose or starch on fungi populations in rumen fluid as determined by real-time polymerase chain reaction in Holstein steers. A. Vakili*, M. Danesh Mesgaran, H. Jahani Aziz-abadi, F. Rezaii, and S. Ghovvati, *Dept. of Animal Science, Ferdowsi University of Mashhad*, *Mashhad, Iran*.

The objective of this work was to investigate the effect of diets containing different type of non-fiber carbohydrates (sucrose or starch) on fungi populations in rumen fluid as determined by real-time polymerase chain reaction. Four Holstein steers (BW = 280; SD = 15 kg) were assigned to a 4 × 4 Latin square with 21-d periods. A basal diet was formulated to be contained of alfalfa hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/ kg, respectively). Starch (St) or sucrose (Su) or a 1:1 mixture of starch and sucrose (St+Su) was added to the basal diet at the rate of 70g/kg DM. Diets were offered as 2–2.5 times of maintenance requirements (7 kg DM/d). Rumen fluid samples were collected before and 4 h after the morning feeding. DNA was extracted from the samples using the QIAamp DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer′s instructions. Fungi rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: GAGGAAGTAAAAGTCGTAACAAGGTTTC and reverse: CAAATTCACAAAGGGTAGGATGATT. Cycling conditions were 95°C for 5 min, 40 cycles of 95°C for 15s, 60°C for 15s and 72°C for 30s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 60 to 95°C, with fluorescence collection at 0.2°C at intervals. Data are expressed relative to quantification of the total bacterial population. Data were analyzed using mixed procedure of SAS (2003). Statistical model was: $Y_{ijk} = \mu + T_i + C_j + P_k + \varepsilon_{ijk}$, where Y_{ijk} is dependent variable, μ is the overall mean, T_i is treatment effect, C_j is cow effect, P_k is period effect, and ε_{ijk} is error. The results of this experiment showed that different type of non-fiber carbohydrates didn't have any effect on fungi populations before or 4 h after the morning feeding[St = 52 and 44,Su = 48 and 45, St+Su = 47 and 42, SEM = 5 and 3 ($10 \times ^{-7}$) fungi relative to total bacteria, respectively].

Key Words: fungi, real-time PCR, rumen

T380 Sodium acetate/acetic acid as a buffer solution to simulate an acidic in vitro rumen environment. R. C. Araujo*[1], A. V. Pires[1], and A. L. Abdalla[2], [1]*ESALQ, Universidade de São Paulo*, *Piracicaba, SP, Brazil*, [2]*CENA, Universidade de São Paulo*, *Piracicaba, SP, Brazil*.

Incubation media based on $NaHCO_3$ and NH_4HCO_3 as buffers have a pH close to 6.8. A low-pH medium would provide a more realistic in vitro rumen simulation of animals fed feedlot diets. Treatments were: CTL6.8 – Theodorou's medium with a pH of 6.8 based on $NaHCO_3$ (7.28 g/L of medium) and NH_4HCO_3 (0.83 g/L of medium) as buffers; CTL5.8 – control acidified with 72% sulfuric acid to achieve a pH of 5.8; NaAc – Theodorou′s medium with Na acetate (56.6 g/L of medium) and glacial acetic acid (2.08 mL/L of medium) as buffers to achieve a pH of 5.8. In each flask (160 mL), 0.5 g of an 80:20 concentrate:forage diet

(91.4% DM) was incubated with 50 mL of medium and 25 mL of rumen fluid at 39°C for 16h. A randomized complete block design was used with n = 8 for gas production and n = 4 for all other variables. Two inocula (3 animals each; mean pH = 5.61 ± 0.11) from lambs adapted to the above diet were used as a source of variation. Data were analyzed by PROC Mixed of SAS with differences declared when $P < 0.05$. The pH variation after 0, 4, 8, 12, and 16h of incubation was: CTL6.8 – 6.77, 6.57, 6.45, 6.39, 6.31; CTL5.8 – 5.82, 5.51, 5.38, 5.34, 5.31; NaAc – 5.68, 5.65, 5.61, 5.59, 5.57, respectively. Mean pH was lowest for CTL5.8 (5.47), followed by NaAc (5.62) and CTL6.8 (6.50). CTL6.8 showed the greatest values for gas production (151.1 mL), CH_4 production (13.3 mL), and truly degraded DM (TDDM; 70.7%). Gas production (86.9 vs. 80.2 mL), CH_4 production (4.2 vs. 2.1 mL), and TDDM (51.4 vs. 49.6%) were similar between CTL5.8 and NaAc, respectively. It was not possible to determine C_2 concentration for NaAc. Total SCFA (94.0 vs. 61.4 m*M*) and C_2 (50.5 vs. 21.4 m*M*) concentrations as well as C_2 to C_3 ratio (2.20 vs. 0.95) were greater for CTL6.8 than CTL5.8. Concentration of C_3 was the greatest for CTL6.8 (23.8 m*M*), intermediary for CTL5.8 (22.7 m*M*), and the lowest for NaAc (19.4 m*M*). In spite of less pH variation, Na acetate/acetic acid solution as buffer interfered in C_2 determination and showed similar results in comparison with an acidified $NaHCO_3/NH_4HCO_3$-based medium.

Key Words: buffer, medium, pH

T381 Milk selenium content and performance of cows supplemented with selenized yeast. L. Q. Melo[1], L. L. Bitencourt[1], S. Siécola Júnior[1], G. S. Dias Júnior[1], N. M. Lopes[1], V. A. Silveira[1], I. R. Rios[1], R. A. N. Pereira[2], and M. N. Pereira*[1], [1]*Universidade Federal de Lavras*, *Lavras, Brazil*, [2]*Empresa de Pesquisa Agropecuária de Minas Gerais*, *Lavras, Brazil*.

This study evaluated the effect of replacing sodium selenite (45.1% of Se) with selenized yeast (Selemax, Biorigin, Brazil. 2245 ppm of Se). Twenty-eight Holsteins were fed a Se supplement free diet for a 60-d standardization period (0.08 ppm of Se in the diet), before being paired blocked and assigned to a treatment for 105 d: Orally given gelatin capsules containing 2.1 g of Selemax (4.64 mg of Se) or 0.011 g of Selenite (4.88 mg of Se) daily. Milk yield and DMI were measured daily, and milk composition on days −1 and 0, 15 to 17, 36 to 38, 57 to 59, and 99 to 101. Milk Se content was determined on d 0, 4, 8, 16, 37 and 57. Total collection of feces and urine was performed on d 39 to 41 for Se balance. Blood samples were obtained on d 0, 43 and 106 to determine Se content and glutathione peroxidase activity. Data were analyzed as repeated measures over time with Mixed of SAS. The model contained the effects of covariate (measure of the same variable at the end of the standardization period), block, treatment, time, and time by treatment interaction. Cow within treatment tested the treatment effect. Daily milk yield was 26.7 kg for Selemax and 26.9 kg for Selenite ($P = 0.84$), milk SCC (× 1,000 cells) was 354 and 352, respectively ($P = 0.99$), and no difference in milk solids or DMI were detected ($P > 0.22$). Selemax increased milk Se content from 8 to 32.3 μg/kg ($P < 0.01$), observed after 4 d of supplementation and throughout data sampling. Plasma Se content was 91.4 and 77.3 μg/L ($P = 0.14$) for Selemax and Selenite, respectively. Glutathione peroxidase activity was greater for Selenite on d 43 and for Selemax on d 106 ($P = 0.05$ for the interaction). There was no difference in Se excreted in urine and feces or retained ($P > 0.46$). Selenized yeast increased milk Se content shortly after starting the supplementation.

Key Words: selenium, yeast, glutathione peroxidase

T382 Effect of direct-fed microbial (DFM) products on rumen bacterial communities in Holstein cows at 2 and 6 weeks postcalving. E. A. Galbraith*[1], A. H. Smith[1], K. J. Mertz[1], Z. Wu[2], and J. D. Ferguson[2], [1]*Danisco*, *Waukesha, WI*, [2]*University of Pennsylvania School of Veterinary Medicine*, *Kennett Square*.

Increase in performance was measured in a study to determine the efficacy of 3 DFM treatments in dairy production. Treatment with a *Propionibacterium* DFM 2 weeks prepartum followed by a *Lactobacillus* DFM through 22 weeks postpartum or *Bacillus pumilus* 8G-134 at either 5×10^9 or 1×10^{10} CFU/head/day both pre- and postpartum resulted in improved milk volume (*B. pumilus* 8G-134) and milk fat (all DFM treatments). This study investigated if these DFM treatments were also associated with changes in ruminal microbial populations at 2 and 6 weeks postcalving. At 8 h postfeeding, cows were restrained and rumen fluid collected via stomach tube. The microbial community of rumen fluid samples was monitored by terminal restriction fragment length polymorphism (T-RFLP) analysis of amplified 16S rDNA genes. Peak profiles from all samples using 3 restriction enzymes were between 70% and 93% similar. All samples tended to have several major peaks and the taxonomic identities of species responsible for these peaks were determined by searching 16S databases. Major peaks present in most samples included common rumen species of *Prevotella*, *Bacteroides*, *Butyrvibrio fibrisolvens*, *Selenomonas ruminantium*, and *Clostridium clostridioforme* and *coccoides*. Analysis of dendrograms indicated no overall clustering by treatment, sampling date, or lactation, but MANOVA analysis did pinpoint several minor TRF peaks which were significantly associated ($P < 0.1$) with treatments. On a CFU per gram basis, the level of DFM treatments fed in this trial would constitute less than 0.1% of the total rumen bacterial population, therefore below the threshold of detection of microbial ecology techniques such as T-RFLP. However, even at low concentrations, the DFM treatments affected performance and bacterial populations, suggesting an impact on bacteria that may be part of a rarer biosphere in the rumen. Examination of rumen bacterial communities may help elucidate the mode of action of these direct-fed microbials.

Key Words: dairy cows, rumen bacteria, direct-fed microbial

T383 Effects of a rumen protected B vitamin complex supplemented to multiparous Holstein cows on milk production and reproductive performance. S. O. Juchem*[1,2], P. H. Robinson[1], and E. Evans[3], [1]*University of California*, *Davis*, [2]*California State University*, *Fresno*, [3]*Technical Advisory Services*, *Bowmanville, ON, Canada*.

Increase in milk yield of dairy cows through supplementation with B vitamins was reported, but the impact on reproductive performance of dairy cows in unknown. Objectives were to evaluate the effect of supplementation with a complex of rumen protected B vitamins (RPBV) that contained biotin, pantothenic acid, folic acid, cyanocobalamin, and pyridoxine to early lactating multiparous cows on milk yield, milk composition and reproductive performance during the first 170 d of lactation. Multiparous Holstein cows (n = 1243) that calved between November of 2007 and April of 2008 were assigned to 2 treatments as cows moved from fresh to one of the 4 early lactation pens: control diet (CT); and B vitamin diet (BV), supplementation with 3.6 g/cow/d of RPBV. Early lactation diets were identical for CT and BV treatments, except for the RPBV supplement fed in the first TMR load to the 2 treatment pens. Cows were artificially inseminated upon estrous detection every morning, and pregnancy diagnosis was performed by per rectum palpation at 42 ± 3 d after breeding. Yields of milk and milk composition were measured monthly. Body condition (BC) was scored from a subgroup of 170 cows at 40 and 100 DIM. A total of 949 cows provided data for statistical analysis, 448 CT and 501 BV cows. Cows were moved to treatment pens at 22.3 DIM. Loss of BC was similar ($P = 0.11$) for CT

(−0.055) and BV (−0.034) cows during early lactation, as well as group DMI, 25.4 and 25.0 kg/d, respectively. Milk yield was similar ($P = 0.18$) for BV and CT during the first 170 d of lactation, 44.9 vs. 44.4 kg/d, respectively. Milk fat content was reduced by feeding RPBV (3.29 vs. 3.38%; $P < 0.01$), but milk fat yield was not affected (1464 vs. 1485 g/d; $P > 0.15$). Day at first service was not different ($P = 0.44$) for BV and CT cows (67.9 vs. 67.2 d), but BV cows had higher ($P < 0.05$) first service conception rate than CT cows (40.8 vs. 35.8%). In summary, supplementation with a RPBV complex improved first service conception rate, whereas milk yield was not affected.

Key Words: biotin, folic acid, cyanocobalamin

T384 Effect of feeding live yeast on performance of Holstein cows during summer. R. S. Marsola*, M. Favoreto, F. T. Silvestre, J. H. Shin, A. T. Adesogan, C. R. Staples, and J. E. P. Santos, *University of Florida*, *Gainesville*.

Objectives were to evaluate the effect of amount of dietary live yeast (LY) intake on performance of cows under heat stress. Holstein cows, 27 multiparous and 33 primiparous, were blocked by parity and milk yield in the first 20 DIM and randomly assigned to receive 0 g/d LY, 0.5 g/d LY (20 billion cells/g, *Saccharomyces cerevisiae* strain CNCM I-1077, Levucell SC20, Lallemand Animal Nutrition, Milwaukee, WI), or 1 g/d LY in the diet from 30 to 107 DIM. Cows were milked twice daily and DM intake and milk yield were measured daily. Milk components were measured once weekly. Cows were weighed weekly. Blood was sampled weekly and analyzed for concentrations of NEFA. Cows were fed chromic oxide in the last 2 wk of the study for calculation of total tract digestibility of nutrients. Ruminal fluid was collected once by rumenocentisis 6 h after feeding for measurement of pH. During the study period, the mean daily temperature was 26.8 C and humidity was 83.2%, and the temperature and humidity index ranged from 73 to 81. Data were analyzed by the GLIMMIX procedure of SAS and linear and quadratic orthogonal contracsts were used. Rectal temperature was not affected by LY and averaged 38.9 ± 0.04°C. Feeding LY did not influence DMI, yields of milk, 3.5% FCM, energy-corrected milk (ECM), and milk fat. Feeding LY caused a linear increase in feed efficiency (ECM/DMI) and milk true protein yield, and had a quadratic effect on OM digestibility, but tended to decrease calculated energy balance. Mean rumen pH increased, and proportion of cows with low pH (<5.8) decreased linearly with LY. Feeding 1 g/d of LY increased efficiency of feed conversion into ECM, yield of true protein, and rumen pH, and reduced the risk of sub-acute rumen acidosis.

Table 1.

	g/d				P	
	0	0.5	1	SEM	Linear	Quadratic
DM intake, kg/d	20.9	20.3	20.3	0.4	0.25	0.61
Milk, kg/d	37.1	36.9	38.1	0.8	0.36	0.46
3.5% FCM, kg/d	36.0	35.0	37.4	0.9	0.33	0.12
ECM, kg/d	34.8	34.1	36.4	0.9	0.23	0.12
ECM/DMI	1.66	1.69	1.78	0.04	0.03	0.52
Milk fat, kg/d	1.23	1.18	1.27	0.04	0.42	0.12
Milk protein, kg/d	0.99	0.99	1.05	0.02	0.03	0.15
Energy balance, Mcal/d	3.33	2.99	1.68	0.61	0.06	0.51
NEFA, mEq/L	84	107	118	12	0.04	0.68
BW, kg	612	608	613	4	0.91	0.35
Rumen pH	5.99	6.03	6.30	0.11	0.04	0.40
pH < 5.8, % cows	45.0	36.8	10.5	—	0.02	0.27
OM digestibility, %	70.9	72.3	69.5	0.9	0.22	0.06

Key Words: dairy cow, heat stress, live yeast

T385 Population dynamics of protozoa in dairy cows fed with Rumensin200 and tallow during dry and lactating stages. H. Castillo, A. Castillo*, D. Dominguez, G. Villalobos, M. Arana, and J. A. Ortega, *Universidad Autonoma de Chihuahua*, *Chihuahua, Chihuahua, Mexico*.

Addition of Rumensin200 and tallow in TMR for dairy cows on protozoan populations was explored in dry and early-lactating cows. Ionophores have been used in ruminants to decrease acidosis and to mitigate gaseous emissions in dairy operations, by inhibiting growth of microorganisms such as protozoa. Also, tallow as an energy alternative in TMR has shown changes in fiber digestibility and gases production, mainly due to the interaction of its unsaturated component with rumen microorganisms. For this experiment, 4 ruminally fistulated Holstein cows were fed rations based on a 90:10 (dry) and 40:60 (lactating) forage to concentrate ratios. Four treatments were randomly assigned in a 4 × 4 Latin Square experimental design as follows: TMR (T1), TMR + 2/3.3 g Rumensin 200(dry/lactating), (T2), TMR + 3,2% DM tallow (T3) and TMR + 2/3,3 g Rumensin 200+ 3,2% DM tallow (T4). Samples of ruminal content were taken at 0, 1, 2, 4, 8, 12, 18 and 24 h after feeding, filtered, preserved with an equal volume of 5% formalin and frozen. Thawed samples were treated with brilliant green and glycerol for direct protozoa count on a Neubauer chamber under a microscope at 40X. Oxidation-reduction potential (ORP) and pH were recorded *in rumen* during the same sampling times. Total number of protozoa at 24 h after feeding did not differ ($P > 0.001$) among treatments in lactating cows, whereas the addition of Rumensin200 to TMR for dry cows caused a significant decrease ($P > 0.001$) in population size (5.0^5 vs. 1.06^5, respectively). Also, protozoa were less diverse in lactating compared with dry cows; while the *Diplodiniae* species were dominant (98%) in lactating cows with any treatment, dry cows fed T3 exhibited a more diverse community formed by 68% *Diplodiniae* and 29% *Entodinium*. Monitoring of pH did not show significant differences ($P > 0.001$) among treatments in both dry and lactating stages, while ORP values suggested a more reduced environment (−241 to –310 mV) in lactating than in dry cows (−234 to –294 mV). This experiment showed changes in protozoan community composition led by modification of the rumen environment.

Key Words: Rumensin, tallow, protozoa

T386 Construction and analysis of metagenomic fosmid library from rumen microflora of Chinese Holstein dairy cow. D. Li, J. Q. Wang*, K. L. Liu, D. P. Bu, and W. Feng, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

The vast majority of rumen microbial diversity has been inaccessible by culture dependent methods. Recent progress in molecular microbial ecology has reveled that traditional culturing methods fail to represent the scope of microbial diversity in rumen, since only less than 11% of viable microorganisms are recovered by culturing techniques. To investigate the full extent of microbial diversity and compare genomic studies among ruminant microflora species by metagenomic method, a fosmid library was constructed with genomic DNA isolated directly from rumen content. Preparation of ruminant genomic DNA for library construction was extracted by LMP agarose gel plug. Extracted ruminant genomic DNA was digested with *Hin*dIII. Purified DNA of 36–48 kb length was recovered by pulsed field electrophoresis and ligated with pcc2FOS vector and transformed into *E. coli* EPI300. The fosmid genomic library of rumen microbe was successfully constructed with the capacity of 1050 Mb in which the insert fragment size was about 35 kb, and about 30000 clones. Excluding the 2% of empty clones, the

coverage of this library is 93% genome equivalents. This fosmid library offers a new tool for gene screening and cloning, and for comparative genomic studies among ruminant microflora species.

Key Words: metagenomics, fosmid library, rumen microflora

T387 Effects of *Saccharomyces cerevisiae* and *Aspergillus niger* (fermentation soluble meal extracted) on productivity of Holstein cows in early lactation. R. Heydari, M. Dehghan-Banadaky*, K. Rezayazdi, and A. Zali, *Department of Animal Science, Campus of Agriculture and Natural Resources, University of Tehran*, *Karaj, Tehran, Iran*.

The objective of this study was to investigate the effects of feeding *Saccharomyces cerevisiae* (SC) and *Aspergillus niger* fermentation solubles meal extracted (AN) to early lactation cows. Twenty-four Holstein lactating cows (8 primiparous and 16 multiparous; 24 ± 7 DIM) were assigned to 1 of 4 dietary treatments as follows: 1) SC (Biosaf SC47)10 g/d/cow; 2) AN (Bospro) 30 g/d/cow; 3) SC 10 g/d/cow and AN 30 g/d/cow; 4) control (no additive). Cows were fed the same total mixed ration (19.5% alfalfa hay, 19.5% corn silage, 7.1% beet pulp and 53.9% concentrate on dry matter basis) and additives were top-dressed during experimental period (75 d). Milk production and DM intake were recorded daily and milk samples were collected weekly from all cows for measurement of somatic cell count (SCC) and milk composition. Blood samples were taken from each cow on the last day of experiment, 3 h after morning meal for metabolic profiling. Data (except blood data) were statistically analyzed using the repeated measures option in Proc Mixed of SAS. DMI was similar between treatments (21.44, 20.41, 21.83 and 21.54 kg/d, respectively). Cows fed SC (treatment 1 and 3) produced more milk than other groups, but fat corrected milk (FCM4%) was not significantly affected by treatments ($P > 0.05$). Milk protein percent significantly increased in cows fed SC as well as milk protein yield. However, other milk composition percentage and SCC were similar for all treatments. Changes in body weight and body condition score (BCS) were not influenced by treatments. Blood metabolites includes: glucose, nonesterified fatty acids, urea nitrogen, triglycerides and phosphorous were unaffected by treatments. These results indicate that supplementation of SC can improve milk production and milk composition, but AN did not affect productivity.

Key Words: *Saccharomyces cerevisiae*, *Aspergillus niger*, milk production

T388 Diversity of nitrogen-fixing bacteria in Holstein dairy cow rumen. S. Zhao, J. Wang*, D. Bu, L. Zhou, and C. Zhang, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

The rumen has a suitable environment for N_2-fixation, because of N_2 presence and the presents of some nitrogen fixing bacteria. The gene *nif*H encodes the dinitrogenase reductase, an enzyme for N_2 fixation, is conserved and used to analyze the phylogenetic diversity of the nitrogen-fixing microorganisms. To reveal the diversity of nitrogen-fixing microorganisms in dairy cow rumen, the *nif*H clone library was constructed. Rumen total DNA was extracted based on the method of freezing/thawing and N-lauroyl sarcosine/proteinase K lysis. Polymerase chain reaction was used to amplify the *nif*H genes from rumen total DNA. PCR products were purified and ligated to pMD19-T vector. *Nif*H gene clones got by transforming ligation product into *E.coli* JM109 were sequenced. The sequences were analyzed by Blast on GenBank and phylogenetic tree was built by Mega 4.0. In results, a total of 64 *nif*H gene clones were obtained. Most of the *nif*H genes belonged to *Fermicute* with the percent of 85.93%, and some belonged to *Archaea*, *α-Proteobacteria*, *Chlorobi* with the percent of 7.81%, 4.70%, 0.56%, respectively. The *nif*H phylogenetic tree also revealed that there was a remarkable diversity of nitrogenase genes in the rumen. The study provided first evidence for the diversity of nitrogen-fixing bacteria from dairy rumen.

Key Words: nitrogen-fixing bacteria, *nif*H, rumen

T389 Dietary cation-anion difference: Effects on fluid metabolites and health status of transition cows in Karst area. W. X. Wu*, *College of Animal Science, Guizhou University*, *Guiyang, China*.

There has little information of dietary cation-anion difference (DCAD, mmol/kg DM) on the performance of dairy cows in Karst area, especially in southwest, China. This study is conducted to evaluate the effects of DCAD on the fluid metabolites, health status, and subsequent lactation performance of transition cows in Karst area. Thirty pregnant, nonlactating Holstein multiparous cows were randomly assigned to 3 blocks of 10 cows based on their age (4 yr), body weight (600 kg), and expected calving date (21 d). Animals were fed 1 of 3 DCAD diets: control (+81), treatment 1 (+20), and treatment 2 (−32), respectively. Anionic salts were included to reduce DCAD. Feeding of reduced DCAD resulted in lower urinary pH than control ($P < 0.05$). Plasma Ca and Cl levels in treatment 2 was higher over those in control ($P < 0.05$). There were no significant difference in plasma glucose and urea nitrogen; Na, K, and P concentrations for 3 dietary treatments ($P > 0.05$). Anionic salts supplementation reduced the cases of hypocalcemia (3:1:1) and retained placenta (4:2:1). Dry matter intake, milk yield and contents of protein, fat, and lactose were unaffected by DCAD modulation ($P > 0.05$). These results suggested that negative DCAD is beneficial for transition cows in Karst area. Further study is necessary to investigate the effect of DCAD on the reproductive performance.

Key Words: dietary cation-anion difference, Karst area, transition cows

T390 Effects of subacute ruminal acidosis challenges on lipopolysaccharide endotoxin (LPS) in the rumen, cecum, and feces of dairy cows. S. Li, A. Kroeker, E. Khafipour, J. C. Rodriguez, D. O. Krause, and J. C. Plaizier*, *Department of Animal Science, University of Manitoba*, *Winnipeg, MB, Canada*.

Feeding high grain diets to cows can cause subacute ruminal acidosis (SARA) and increase the amount of dietary starch that is digested in the large intestine. Grain-induced SARA can increase free lipopolysaccharide endotoxins (LPS) in the rumen, due to increased lysis of gram-negative bacteria. Increased starch in the large intestine may also reduce the pH of the digesta, and increase LPS in the large intestine and in the feces. This could explain symptoms of SARA, as it may be easier for LPS to translocate into the blood in the large intestine than in the rumen. SARA can also be induced by feeding forage pellets. This does not increase the content of starch in the diet. Hence, the effects of this form of SARA on the large intestine may differ from that of grain-induced SARA. To test this, a study was conducted with non-lactating dairy cows with cannulas in the rumen and in the cecum. A Latin square with 3 4-wk periods was used. In wk 1–3 cows received a control diet containing 70% of forage (DM basis) and 30% mixed concentrates. In wk 4 cows received either the control diet, a high grain diet for a grain pellet-induced SARA challenge (GPI_SARA, 38% wheat-barley pellets, 32% mixed concentrates, and 30% of forages), or a diet that contained alfalfa pellets for an alfalfa-pellet induced SARA challenge (API_SARA, 45% of mixed concentrates, 32% of alfalfa pellets, and 23% of other forages). During this week, rumen pH was monitored continuously in all cows.

Rumen fluid, digesta from the cecum, and feces were sampled immediately before feed delivery and at 6 h after feed delivery. All samples were analyzed for LPS. The pH of cecum samples were determined. Both SARA challenges resulted in depressions of rumen pH that were representative of SARA, and decreased the pH of digesta in the cecum, but not as much as the rumen pH. GPI_SARA greatly increased LPS in the rumen, cecum, and feces. API_SARA increased LPS in the rumen, but not in the cecum and in the feces. Results confirm our hypothesis that grain-induced SARA, but not SARA induced by feeding pelleted forages, increases LPS in the large intestine and in the feces.

Table 1. Rumen and cecum pH and LPS in the rumen, cecum and feces

	Control	API_SARA	GPI_SARA	SEM	P-value
Average rumen pH	6.30[a]	5.99[b]	5.98[b]	0.04	<0.01
Time < rumen pH 6, min/d	332[b]	770[a]	744[a]	57	<0.01
Time < rumen pH 5.6, min/d	56[b]	255[a]	299[a]	30.7	<0.01
Cecum pH	7.07[a]	6.86[b]	6.79[b]	0.06	<0.01
Rumen LPS, EU/mL	8,333[b]	18,425[b]	124,566[a]	8,738	<0.01
Cecum LPS, EU/mL	18,289[b]	15,631[b]	128,410[a]	20,379	<0.01
Feces LPS, EU/mL	13,909[b]	18,998[b]	101.555[a]	16,355	<0.01

[a, b]Means with different superscripts in a row differ (P < 0.05).

Key Words: SARA, LPS, grain

T391 Supplementing *Megasphaera elsdenii* modulates diurnal rumen fermentation profile in dairy cows. Q. Zebeli[1], S. Iqbal[1], A. Mazzolari[1], S. M. Dunn[1], W. Z. Yang[2], and B. N. Ametaj*[1], [1]*University of Alberta*, *Edmonton, AB, Canada*, [2]*Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*.

Megasphaera elsdenii is a direct-fed microbial possessing lactate-utilizing properties. We hypothesized that its supplementation might modulate the fermentation profile in the rumen of dairy cows. This study sought to evaluate the effects of supplementing *M. elsdenii* on diurnal volatile fatty acids (VFA) concentration and profile in the rumen fluid of mid-lactation dairy cows. Eight rumen-cannulated Holstein cows were used in a paired 2 × 2 crossover design with 2 21-d periods. All cows were offered a total mixed ration containing (dry matter basis) 32% rolled barley grain, 15% alfalfa hay, 40% barley silage, and 13% protein-, and vitamin-mineral supplement. A culture of 35 mL of *M. elsdenii* ATCC 25940TM, containing 10^7 - 10^9 CFU/mL, was inoculated daily via rumen fistula to each cow pertaining to the treatment group (TRT), whereas control cows (CTR) were inoculated with 35 mL of carrier only. Rumen samples were collected on d 21, shortly before the morning feeding at 0800 and every 2 h up to 2000, and VFA were analyzed by GC. ANOVA was conducted with MIXED procedure of SAS accounting for repeated measures. Data showed that treatment did not affect the concentration of total VFA (128 vs. 125 m*M*; $P > 0.05$), but lowered the molar proportion of acetate (61.3 vs. 59.9% of total VFA; $P < 0.01$) and isobutyrate (1.9 vs. 1.7%; $P < 0.01$). On the other hand, the TRT cows had greater proportions of butyrate (12.7 vs. 14.4%; $P < 0.01$) and valerate (1.9 vs. 2.3%; $P < 0.01$) than the CTR cows. There was an hour by treatment interaction for acetate to propionate ratio ($P = 0.03$) and the proportion of propionate in the rumen fluid ($P = 0.05$). Rumen caproate and isovalerate were not affected by treatment ($P > 0.05$). In conclusion, data of this study indicated that supplementation of *M. elsdenii* modulated the fermentation in the rumen of dairy cows shifting its profile from acetate to the production of butyrate and valerate.

Key Words: dairy cow, fermentation profile, *Megasphaera elsdenii*

T392 Effects of supplementing *Megasphaera elsdenii* on preprandial rumen fermentation profile in dairy cows. Q. Zebeli[1], S. Iqbal[1], A. Mazzolari[1], S. M. Dunn[1], W. Z. Yang[2], and B. N. Ametaj*[1], [1]*University of Alberta*, *Edmonton, AB, Canada*, [2]*Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*.

Ingestion of large amounts of cereal grains leads to rapid accumulation of volatile fatty acids (VFA) and lactate in the rumen fluid and major changes in their profile. *Megasphaera elsdenii*, a rumen obligate anaerobe, has lactate-utilizing properties and the potential to modulate rumen fermentation profile by converting lactate into VFA. This study sought to evaluate the effects of supplementing *M. elsdenii* on VFA concentration and profile in the rumen fluid in mid-lactation dairy cows. Eight rumen-cannulated Holstein cows were used in a paired 2 × 2 crossover design with 2 21-d periods (first 11 d used for adaptation). All cows were offered a total mixed ration containing (dry matter basis) 32% rolled barley grain, 15% alfalfa hay, 40% barley silage, and 13% protein-, and vitamin-mineral supplement. A culture of 35 mL of *M. elsdenii* ATCC 25940TM, containing 10^7 - 10^9 cfu/mL, was inoculated daily via rumen fistula to each cow pertaining to the treatment group (TRT), whereas control cows (CTR) were inoculated with 35 mL of carrier only. Preprandial rumen samples were collected shortly before morning feeding on d 12, 14, 16, 18, and 21, and VFA were analyzed by GC. ANOVA was conducted with MIXED procedure of SAS accounting for repeated measures. Data showed that treatment did not affect the concentration of total VFA in the rumen fluid ($P > 0.05$), but tended to increase the molar proportion of propionate (19.9 vs. 21.0% of total VFA; $P = 0.09$), and lower the acetate to propionate ratio (3.32 vs. 3.09; $P = 0.07$). The TRT cows also had lower concentration of valerate (2.4 vs. 1.8 m*M*; $P = 0.04$) and isovalerate (2.7 vs. 2.2 m*M*; $P < 0.01$) than their CTR counterparts. Other VFA such as acetate, butyrate, isobutyrate, and caproate were not affected by the treatment ($P > 0.05$). In conclusion, results of this study indicated that supplementation of *M. elsdenii* slightly modulated the preprandial fermentation profile in dairy cows.

Key Words: dairy cow, fermentation profile, *Megasphaera elsdenii*

T393 Diagnosis of subacute ruminal acidosis (SARA) using the Optium Xceed Diabetes Monitoring System. S. Li[1], A. Kroeker[1], D. O'Gorman[2], D. O. Krause[1], J. C. Rodriguez[1], and J. C. Plaizier*[1], [1]*Department of Animal Science, University of Manitoba*, *Winnipeg, MB, Canada*, [2]*Marigot Ltd.*, *Carrigaline, Co. Cork, Ireland*.

The current diagnosis of subacute ruminal acidosis (SARA) relies on measuring the pH of rumen fluid samples, which are difficult to collect and may not be representative. Studies have reported that monitoring of blood glucose may aid in the diagnosis of SARA. However, SARA caused by feeding high grain diets and SARA caused by feeding diets with insufficient physically effective fiber, e.g., a diet containing alfalfa pellets, can affect blood glucose differently. This may affect the accuracy of this measurement for the diagnosis of SARA. The Optium Xceed Diabetes Monitoring System for glucose measurement was tested for the diagnosis of SARA. The study included 6 rumen cannulated nonlactating dairy cows in a 3 × 3 Latin square with 4 wk periods. In wk 1–3 cows received a control diet (70% forage and 30% mixed concentrates (DM basis)) . In wk 4, cows received either the control diet, a high grain diet for a grain pellet-induced SARA challenge (GPI_SARA, 38% wheat-barley pellets, 32% other mixed concentrate, and 30% forages), or a diet that contained alfalfa pellets for an alfalfa-pellet induced SARA challenge (API_SARA, 45% mixed concentrate, 32% alfalfa pellets, and 23% other forages). During wk 4, rumen pH was monitored in all cows, and blood was sampled immediately before feed delivery and at 6 h after feed delivery. Glucose in whole blood was measured using

the Optium Xceed Diabetes Monitoring System, which can be used on farm. The average daily rumen pHs were 6.30, 5.99, and 5.98, for control, API_SARA, and GPI_SARA, respectively. The durations of the rumen pH below 5.6 were 56.4, 225.2 and 298.7 min/d for control, API_SARA, and GPI_SARA, respectively. This shows that both forms of SARA resulted in similar depressions of rumen pH and that SARA was induced. Blood glucose was higher during GPI_SARA than during control (4.47 vs. 4.25 mmol/L,), but blood glucose did not differ between the API_SARA and control. This shows that the Optium Xceed Diabetes Monitoring System can aid in the diagnosis of grain induced SARA, but that additional tests may be needed for the diagnosis of SARA caused by diets with insufficient physical effective fiber.

Key Words: SARA, diagnosis, blood

T394 Simplified procedure for quantifying ruminal microbe populations using real-time PCR. C. R. Mullins*, L. K. Mamedova, and B. J. Bradford, *Kansas State University*, *Manhattan*.

A variety of molecular techniques exist to quantify ruminal microbiota; however, sample processing requirements for most techniques are complex and time consuming. The objective of this work was to use real-time PCR to quantify relative abundance of 10 microbial populations while simplifying the sample preparation process. Our trial utilized ruminal contents from 8 ruminally cannulated Holstein cows used in a 4 × 4 Latin square experiment that examined the effect of varying wet corn gluten feed inclusion rate (0–36% DM). Rumen samples were collected every 9 h over a 3-d period so that 8 samples were taken from each cow each period, representing every 3 h of a 24-h period, thus accounting for diurnal variation. Digesta and rumen fluid were collected as one sample to capture the free-floating and particle adherent microbes in similar proportions as found in the rumen. Samples were collected from 5 locations throughout the rumen, mixed, and a representative subsample (200 g) was collected and frozen at −20°C. Prior to processing, samples were thawed at room temperature until they became pliant, then composited by cow period. Each composited sample was diluted with distilled, deionized water at a 1:1 ratio and homogenized. A subsample was then obtained from the homogenized mixture and used for microbial DNA isolation using a commercial kit (Zymo Research Fecal DNA kit). Quantitative real-time PCR was used to determine relative abundance of bacterial populations using previously validated primers specific for genes encoding 16S ribosomal RNA. Efficiencies were calculated to determine population abundance relative to the total bacterial population. Dietary treatments had few effects, but diets that decreased ruminal pH tended to decrease the *Butyrovibrio fibrosolvens* population ($P = 0.09$).The relative population densities for most species quantified were within the range reported previously; for example, the *Prevotella* genera and *Fibrobacter succinogenes* accounted for 39.9% and 1.0% of the ruminal bacteria, respectively. This procedure offers a simpler and quicker means to quantify relative abundance of rumen microbial populations.

Key Words: rumen, real-time PCR, DNA extraction

T395 Effects of forage-to-concentrate ratio and rumen fermentation characteristics on apparent ruminal synthesis of niacin and vitamin B6 in lactating dairy cows. M. Seck*[1,3], J. A. Voelker Linton[2], M. S. Allen[2], P. Y. Chouinard[3], and C. L. Girard[1], [1]*Agriculture and Agri-Food Canada*, *Sherbrooke, Quebec, Canada*, [2]*Department of Animal Science, Michigan State University*, *East Lansing,* [3]*Departement de sciences animales, Universite Laval*, *Quebec, Quebec, Canada*.

Effects of forage-to-concentrate ratio and rumen fermentation characteristics on apparent ruminal synthesis and post-ruminal supply of niacin (B3) and vitamin B6 were evaluated in an experiment using 14 ruminally and duodenally cannulated Holstein cows. The experiment was a crossover design with 2 15-d treatment periods and a preliminary period in which dry matter intake (pDMI) of a diet intermediate in composition between the treatments was determined. Treatments were diets containing low-forage (LF; 44.8% forage, 32.8% starch, 24.4% NDF) or high-forage (HF; 64.1% forage, 22.5% starch, 30.7% NDF) concentrations. No interactions between treatment and pDMI were observed ($P \geq 0.2$). LF decreased B3 intake (1035 vs. 1135 ± 16 mg/d; $P \leq 0.01$) but increased apparent ruminal synthesis (2831 vs. 1885 ± 250 mg/d; $P \leq 0.01$) and duodenal flow (3866 vs. 3020 ± 255 mg/d; $P \leq 0.01$) of B3 compared with HF. Although B6 intake was not influenced (91 vs. 89 ± 1; $P > 0.1$) by treatments, LF decreased apparent ruminal degradation (−3 vs. −20 ± 3 mg/d; $P \leq 0.01$) and increased B6 duodenal flow (88 vs. 68 ± 4 mg/d; $P \leq 0.01$) compared with HF. B3 flow tended to be correlated positively to B3 intake (r = 0.36, $P = 0.06$) while B6 flow was correlated positively to B6 intake (r = 0.73, $P < 0.01$). Ruminal synthesis and duodenal flow of B3 and B6 were correlated negatively to mean ruminal pH (r = −0.45, $P < 0.02$ for all) and correlated positively to true ruminally degraded starch (kg/d; r = 0.42, $P < 0.03$ for all). Ruminal synthesis and duodenal flow of B3 and duodenal flow of B6 were correlated positively to microbial N flow (g/d, r = 0.51, $P < 0.01$ for all). Niacin and vitamin B6 supply to dairy cows is increased with greater dietary starch concentration and starch digestion in the rumen.

Key Words: dairy cow, niacin, pyridoxine

T396 The effect of high inclusion of monensin on lactation performance in dairy cows. L. R. Behling*[1], K. Perfield[2], R. Martin[1], R. Greenfield[1], and S. Onetti[1], [1]*Vita Plus Corporation*, *Madison, WI*, [2]*Elanco Animal Health*, *Greenfield, IN*.

Three free-stall herds were used to conduct on-farm trials to determine if a high inclusion of monensin (MON) in the lactating cow TMR would affect milk fat production. Each herd provided 2 pens each of cows at similar DIM, parity and age. Herds were fed typical Midwestern diets, composed of alfalfa haylage and corn silage. Diets were formulated for 17.3% CP, 25.6% starch and 22.2% NDF from forage, DM basis. The trials were conducted during winter (Dec and Jan) and summer (Jul and Aug) in 2009 to evaluate potential interactions between season and MON inclusion. All cows were fed MON at an inclusion of 11.4 g/ton DM before the start of the trial. At the beginning of the trial, MON was removed from the diets in the control pens, and increased to 15.3 g/ton in the treatment pens, based on formulated DMI of 23.7 kg/d. After 2 weeks, treatment pens were increased to 19.1 g MON/ton. After 4 weeks at 19.1 g/ton, milk samples were collected from the milk line for each pen and pen milk weights were recorded. Data were analyzed using MIXED models of SAS. The model included fixed effects of treatment, season and their interaction. Herd was the specified term for the random statement. There were no season x treatment interactions on milk production and components. Inclusion of MON had no effect on milk yield, milk fat % or milk fat and protein yield. Milk protein % was significantly decreased by MON inclusion and MUN was significantly lower in the winter. In summary, inclusion of MON at 19.1 g/ton did not affect milk fat production.

Table 1. Milk production of dairy cows after 4 weeks of supplementation with 19.1 g MON/ton DM

	Winter		Summer	
	g MON/ton DM			
Variable	0	19.1	0	19.1
Milk, kg	36.3	37.1	34.5	36.1
Fat, %	3.71	3.73	3.80	3.74
Fat, kg	1.33	1.37	1.33	1.37
Protein, %[a]	3.21	3.14	3.17	3.09
Protein, kg	1.15	1.14	1.11	1.15
MUN, mg/dL[b]	11.8	11.7	15.4	15.9

[a]Significant treatment effect at $P < 0.05$.

[b]Significant seasonal effect at $P < 0.05$.

Key Words: monensin, milk fat, dairy cows

T397 Effects of a microbial fermentation product on milk production and blood metabolites on commercial dairies in eastern Canada. A. M. Gehman*[1], J. D. Johnston[2], and J. M. Tricarico[1], [1]*Alltech*, *Brookings, SD,* [2]*Ritchie Feed and Seed, Ottawa, Ontario, Canada.*

Three dairy farms located in Ontario and Quebec, Canada, were utilized to determine effects of feeding a microbial fermentation product (MFP; CP = 47% DM; soluble CP = 40% CP) on milk production, components, and blood metabolites of lactating dairy cattle. The study was conducted as a crossover design with 2 21-d periods. Experimental rations were: 1) control, 0 g/d MFP; or 2) MFP, 600 g/d MFP. Diets were isonitrogenous and isoenergetic. The MFP ration was formulated to provide 600 g/head/d MFP (2.1% ration DM) by replacing a portion of plant-based protein. Each farm was assigned to one of 2 treatment sequences: control followed by MFP or MFP followed by control. Milk production and feed intake were recorded for the last 2 d of each period, and blood samples were taken from 15 randomly selected cows on each farm during the last week of each period. Average DIM for cows that were blood sampled was 200 for control and 147 for MFP. Milk was analyzed for fat and protein, and blood was analyzed for non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), and blood urea nitrogen (BUN). Energy-corrected milk tended to be greater ($P = 0.09$) for MFP than control (36.1 vs. 33.3 ± 0.8 kg/d), while dry matter intake was not different, ave: 24.0 ± 0.5 kg/d. Milk fat content was and yield tended to be higher ($P = 0.03$ and 0.09) for MFP than control (3.96 vs. 3.86 ± 0.05% and 1.34 vs. 1.22 ± 0.03 kg/d). Milk protein content was not different between treatments, ave: 3.34 ± 0.06%, but yield was greater ($P = 0.04$) for MFP than control (1.13 vs. 1.05 ± 0.02 kg/d). While BHBA and NEFA were not different between treatments, ave: 0.68 ± 0.03 and 0.17 ± 0.04 mmol/L respectively, BUN was greater ($P = 0.02$) for MFP than control (4.95 vs. 4.53 ± 0.04 mmol/L). Including MFP in a ration at 600 g/d increased energy-corrected milk by 2.8 kg/d and both milk fat and protein yield by 0.12 kg/d, while not affecting dry matter intake. Blood metabolites BHBA and NEFA were not affected by MFP, suggesting the increase in production and components was not due to mobilization of body reserves.

Key Words: dairy cow, microbial fermentation product, milk

T398 Effect of *Megasphaera elsdenii* NCIMB 41125 (Me) on production of lactating dairy cows. P. H. Henning*[1], L. J. Erasmus[2], C. H. Horn[3], and H. H. Meissner[1], [1]*MS Biotech*, *Centurion, South Africa*, [2]*University of Pretoria*, *Pretoria, South Africa*, [3]*Biotherapeutics*, *Centurion, South Africa*.

High concentrate intake in fresh cows pose an acidosis risk. *M. elsdenii* (Me) is a key ruminal lactic acid utilizer, but its numbers may be low in early lactation. Me was isolated from rumens of cattle adapted to high concentrate diets. The objective of this study was to evaluate use of Me as direct fed microbial (DFM) for dairy cows. Sixty multiparous Holstein cows were blocked according to previous milk production and BW, and randomly assigned to 4 treatments in a 2x2 factorial design. Treatments were + or - Me and low (L) or high (H) concentrate diet. The +Me cows were orally dosed with Me (10^{11} cfu once on each of d 1, 10 and 20 postpartum). The -Me cows received a placebo. Diets L and H, respectively, contained (g/kg DM) ground corn (354, 482), alfalfa hay (319, 196), *Eragrostis curvula* hay (79, 79), non-fiber carbohydrate (NFC) (448, 504) and neutral detergent fiber) NDF (282, 238). DM intake and milk yield (daily), milk composition (weekly) and BW and BCS (monthly) were measured for the first 80 d of lactation. Data were analyzed as a completely randomized block design (Genstat 5). Contrasts (+ vs. –Me for all cows, L cows and H cows, respectively) were used to determine significance of treatment effects. Since higher-producing cows may be more prone to acidosis results were also analyzed using only the 10 highest-producing cows in each treatment group. With all cows included, dosing with Me resulted in greater milk production ($P = 0.10$) (35.1 vs. 33.1 kg/d), higher mean BW ($P = 0.02$) (640 vs. 610 kg) and better BCS ($P = 0.06$) (2.63 vs. 2.38), while milk fat % was increased ($P = 0.03$) (3.14 vs. 3.07) for L cows. With only highest-producing cows included, dosing with Me increased milk production for the H group ($P = 0.06$) (39.3 vs. 35.9), without a significant increase in DM intake, and increased (both $P = 0.02$) BW (644 vs. 597) and BCS (2.71 vs. 2.26). Milk fat was again increased ($P = 0.06$) (3.56 vs. 3.21) for the L cows. Results suggest that dosing with *Megasphaera elsdenii* NCIMB 41125 may improve milk production, milk fat, body weight and body condition score, with greater benefit likely for higher-producing cows on higher concentrate diets.

Key Words: *M. elsdenii*, acidosis, dairy cows

T399 Effect of soluble yeast protein extract and dietary fermentable carbohydrate on fermentation, digestion, and N flow in rumen-simulating fermenters. G. A. Harrison*, M. D. Meyer, and K. A. Dawson, *Alltech*, *Nicholasville, KY*.

Effects of addition of soluble yeast protein extract (SYPE) to diets differing in fermentable carbohydrate (fCHO) content were investigated in single-flow rumen-simulating fermenter cultures. Twelve cultures were used in a 2 × 2 factorial design with 4 dietary treatments and 3 replications per treatment. Daily feed amounts provided to cultures were 24.1, 24.76, 24.34, and 25 g for low fCHO, low fCHO + SYPE, hi fCHO, and hi fCHO + SYPE treatments, respectively, with twice daily feeding for 6 d. SYPE was included at 2.64% of diet to raise dietary CP to 17.5 from 16.6% (DM basis). Fermentation samples were collected from cultures before morning feeding during the last 3 d of experiment. Composite effluent samples from each fermenter were used for DM and NDF disappearance and volatile fatty acid (VFA) analyses. Nitrogen flow measures were estimated by using purine to N ratios for effluent and bacteria. Data were analyzed for effects of treatment using GLM procedure of SAS and fCHO and SYPE effects determined by orthogonal contrasts. Culture pH and ammonia concentration before morning feeding were higher in cultures fed low fCHO diets ($P < 0.05$) and ammonia concentration higher in cultures fed SYPE ($P < 0.05$). Cultures fed lower fCHO diets had greater molar proportion of acetate, lower molar proportion of butyrate ($P < 0.0001$), and lower total VFA concentration ($P < 0.01$). Digestion of true DM was greater when cultures received higher fCHO diets ($P < 0.05$). Bacterial N yield was not affected by fCHO or

SYPE ($P > 0.10$) but an interaction between fCHO and SYPE was noted with an increase in bacterial N yield with SYPE addition to hi fCHO diets of 9.6% ($P < 0.05$). Efficiency of bacterial N production based on fCHO provided was greater in lower fCHO cultures ($P < 0.001$). The effects of soluble yeast protein extract addition were dependent upon dietary fermentable carbohydrate with positive responses in bacterial yield on higher fCHO diets.

Key Words: soluble yeast protein extract, fermentable carbohydrate, ruminal metabolism

T400 Effect of soluble yeast protein extract and culture feed rate on fermentation, digestion, and N flow in rumen-simulating fermenters. G. A. Harrison*, M. D. Meyer, and K. A. Dawson, *Alltech*, *Nicholasville, KY*.

Effects of soluble yeast protein extract (SYPE) and culture feed rate (FR) were investigated in single-flow rumen-simulating fermenter cultures. Twelve cultures were used in a 2 × 2 factorial design with 4 dietary treatments and 3 replications per treatment. Dietary treatments were low FR SBM, low FR SYPE, hi FR SBM, and hi FR SYPE with twice daily feeding for 6 d. Culture daily feed rates (as fed) were 20 and 30 g for low and high FR, respectively, and SYPE was included at 2.64% (DM basis) and primarily replaced soybean meal. Fermentation samples were collected from cultures before morning feeding during the last 3 d of experiment. Composite effluent samples from each fermenter were used for DM and NDF disappearance and volatile fatty acid (VFA) analyses. Nitrogen flow measures were estimated by using purine to N ratios for effluent and bacteria. Data were analyzed for effects of treatment using GLM procedure of SAS with FR and SYPE effects determined by orthogonal contrasts. Culture pH before morning feeding was lower in hi FR cultures ($P < 0.0001$). Mean ammonia concentration before morning feeding was not affected by treatment ($P > 0.10$). Volatile fatty acid pattern was altered by feed rate with molar proportions of acetate and isoacids being lower ($P < 0.05$) and molar proportion of butyrate higher with increased feed rate ($P < 0.001$). Cultures fed more feed had increased total VFA concentration ($P < 0.0001$). Digestion of true DM and NDF were not affected by treatment ($P > 0.10$). When feed rate was increased, increases were noted in measured g of RDP and bacterial N yield ($P < 0.01$). No differences were detected in fermentation, digestion, and N flow due to SYPE ($P > 0.10$). A numerical increase in bacterial N yield was observed when SYPE replaced SBM in the higher FR cultures (10.2%) but not in the lower FR cultures (interaction; $P > 0.10$). Culture feed rate influences fermentation pattern N flow in rumen-simulating fermenters.

Key Words: soluble yeast protein extract, culture feed rate, ruminal metabolism

T401 Effect of essential oils on rumen fermentation, milk production, and feeding behavior in lactating dairy cows. L. R. Tager* and K. M. Krause, *West Virginia University*, *Morgantown*.

Eight ruminally cannulated lactating Holstein dairy cows were used in a Latin rectangle design to assess the effects of 2 commercial essential oil (EO) products on rumen fermentation, milk production, and feeding behavior. Cows were fed a TMR with a 42:58 forage:concentrate ratio (DM basis). Treatments included addition of: 0.5 g/d XT 6965 (CEL; 85 mg cinnamaldehyde and 140 mg eugenol), 10 g/d XT 6965 (CEH; 1700 mg cinnamaldehyde and 2800 mg eugenol), 0.25 g/d XT 6933 (CAP; capsicum), or no oil (CON). Cows were fed ad-libitum twice daily for 21 d per period. Total VFA, individual VFA, acetate:propionate ratio, and ammonia production were not affected by EO ($P > 0.05$). Mean rumen pH as well as bouts, total h, mean bout length, total area, and mean bout area under pH 5.6 did not differ among treatments ($P > 0.05$). Total tract digestibility of OM, DM, NDF, ADF, CP, and NSC were not affected by EO ($P > 0.05$). In situ DM disappearance was not affected by EO ($P > 0.05$). However, OM disappearance tended to decrease compared with CON ($P = 0.08$; 60.3% vs. 57.6%) with CEH. Compared with CON, NDF disappearance ($P = 0.05$; 41.5% vs. 37.6%) and ADF disappearance ($P = 0.04$; 44.5% vs. 38.8%) decreased with addition of CEH. DMI, number of meals/d, h eating/d, mean meal length, rumination events/d, h ruminating/d and mean rumination length were not affected by EO ($P > 0.05$). However, length of the first meal after feeding decreased with addition of CEH (47.2 min) and CAP (49.4 min) compared with CON (65.4 min; $P = 0.01$). Milk yield and composition did not differ. CEL had no effect on rumen fermentation, milk production, or feeding behavior. CAP shortened length of the first meal without changing rumen fermentation or production, making it a possible additive for altering feeding behavior. CEH negatively affected rumen fermentation and altered feeding behavior, suggesting that a dose of 10 g/d is not beneficial to lactating dairy cows.

Key Words: essential oil, dairy nutrition, rumen fermentation

T402 Rumen-protected choline affects methionine methyl group metabolism in lactating dairy cows. S. L. A. Benoit, B. J. Bequette, and R. A. Erdman*, *University of Maryland*, *College Park*.

Methionine (Met) is a precursor for protein synthesis and the primary donor of labile methyl groups. We hypothesized that milk production responses to rumen protected choline (RPC) relate to choline sparing Met as a methyl donor. The objectives of this study were to determine the bio-availability of RPC and whether Met methyl group flux is reduced when dairy cows are fed RPC. Four multiparous Holstein cows in mid lactation were fed a nutritionally complete basal diet except for Met that was limited to 1.49% of metabolizable protein. Treatments included the basal diet or the basal diet plus 15g/d RPC as choline chloride (Reashure, Balchem Corp., New Hampton, NY) in single reversal design with 2 wk periods. Metabolic fates of Met were measured by continuous i.v. infusion of [1-^{13}C] and [methyl-^{13}C] Met, and [trimethyl-C^2H_3] choline for 12 h on d 14 of each period. Milk was collected at 3 h intervals and blood taken over the last 6 h. Supplementation with RPC did not affect total milk yield or milk fat and protein yields which averaged 39 kg/d, 1634 g/d and 1110 g/d, respectively. Based on plasma [1-^{13}C] Met and [methyl-^{13}C] Met enrichments, total Met flux, irreversible loss, and remethylation were not affected by treatment, averaging 15.2, 11.5, and 4.2 mmol/h, suggesting that 24% of Met was remethylated. In contrast, using plasma [1-^{13}C] homocysteine as the true intracellular precursor, total Met flux, irreversible loss, and remethylation rates (mmol/h) were 80.1, 67.6 ($P = 0.04$); 38.3, 33.5; and 41.8, 34.1 ($P = 0.07$), for control and RPC, respectively. Differences in plasma vs. casein [methyl-$^2H_3$] Met labeling, which arises from [trimethyl-C^2H_3] choline, suggested that ~40% of Met in the mammary gland underwent transmethylation with choline serving as the methyl donor. Finally, based on treatment differences in Met methyl flux, the bio-availability of RPC approximated to 72%. These results illustrate the central role of Met and choline in methyl metabolism and the importance of methyl group transactions in the high producing dairy cow.

Key Words: methionine, methyl metabolism, dairy cows

T403 Cloning and identification of novel hydrolase genes from a metagenomic library of dairy cow rumen microflora and characterization of the expressed cellulases. X. Gong*, M. Qi, R. J. Forster, T.

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A 6,000-clone metagenomic bacterial artificial chromosome (BAC) library was constructed from microbial flora DNA extracted from the rumen contents of a grass hay-fed dairy cow and activity-based screening was employed to explore the functional hydrolase genes. Ninety-four independent clones specifying distinct hydrolytic activities (51 esterases, 18 xylanases and 25 cellulases) were identified. Subcloning and sequence analysis of a subset of these hydrolase-positive clones identified 10 endoglucanase genes. Amino acid sequences of 5 of these genes indicated less than 55% homology among them, while similarity to the cellulases in the National Center for Biotechnology Information (NCBI) databases averaged 70%. Glycoside hydrolase families 5, 8 and 9 were represented by 6, one, and 3 of the 10 endoglucanases, respectively. Subcloning and sequence analysis of a subset of the esterase-positive clones identified 10 esterase genes. These shared less than 33% homology, with an average similarity of 53% to esterases in the databases, as assessed by predicted amino acid sequence. Preliminary characterization of the encoded cellulases was carried out using crude extracts of each of the subclones. Zymogram analysis using carboxymethylcellulose as a substrate showed a single positive band for each sample, confirming that only one functional cellulase gene was present in each subclone. Optimal pH for these cellulases ranged from 6.5 to 7.0 and their optimal temperatures were 40°C to 50°C. All the endoglucanases could hydrolyze a wide range of β-1,3-, and β-1,4-linked polysaccharides with varying activities. The present work revealed an increased diversity of functional cellulases and esterases in the rumen.

Key Words: esterase, ruminal microorganisms, BAC library

T404 Development of a diet inoculate with two substrates by submerged solid fermentation. D. Díaz-Plascencia*[1], C. Rodríguez-Muela[1], F. Salvador[1], J. Jiménez[1], H. Rubio[2], S. Mena[3], and A. Elías[4], [1]*Universidad Autónoma de Chihuahua*, *Chihuahua, México*, [2]*Instituto Nacional de Investigaciones Agrícolas Forestales y Pecuarias*, *Chihuahua, México*, [3]*Universidad de Guadalajara*, *Jalisco, México*, [4]*Instituro de Ciencia Animal*, *La Habana, Cuba*.

To evaluate 2 substrates (apple byproduct (AB) and sugar cane molasses (CM)) in the preparation of an inoculate with yeasts for ruminant rations, 2 yeasts strains (YS) were used by submerged solid fermentation (YS A, commercial yeast of the *Saccharomyces cerevisiae* and YS D, *Kluyveromyces lactis*, obtained by apple pomace fermentation) under aerobic conditions in a liquid medium. Treatments used were Tr1: 200 mL of AB + 1 g of YS. Tr2: 132 mL of AB + 34 g of CM + 1 g of YS. Tr3: 66 mL of AB + 66 g of CM + 1 g of YS. Tr4: 100 g of CM + 1 g of YS. All treatments were employed for growth of both YS. Treatments were added with 1.2% of urea, 0.2% of ammonium sulfate, 0.5% of mineral supplement. Fermentation was carried out in flasks of 1,000 mL with distilled water. Five replicates by Tr and different sampling times (0, 12, 24, 48 and 96 h) were used. Variables evaluated were: pH, temperature, soluble carbohydrates, yeast count, ammonia and lactic acid. Data were analyzed as a randomized 4 × 2 factorial design in a split-plot experiment. Results showed different ($P < 0.01$) pH behavior among the substrates. Temperature had an increase ($P < 0.01$) from 0 h to 12 h in all treatments. Ammonia was different ($P < 0.01$) among treatments and among YS. Lactic acid showed effect ($P < 0.01$) among treatments and among YS. Soluble carbohydrates were different ($P < 0.01$) among treatments, YS and sampling time. Yeast count of YS D was higher ($P < 0.01$) in Tr4 with value of $2.8 \times 10^9 \pm 0.05$ cell.mL^{-1} at 48 h, versus YS A count of $9.6 \times 10^8 \pm 0.05$ cell.mL^{-1} in Tr4 at the 96 h. It can be concluded that the use of sugar cane molasses, increases the growth of yeast, especially *K. lactis*, during the preparation of a diet inoculated by submerged solid fermentation.

Key Words: yeasts, fermentation, apple byproduct

T405 Glycerol can replace corn grain in diets for transition dairy cows. E. R. Carvalho*, N. S. Schmelz, H. White, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Expansion of the biofuels industry has increased the availability of glycerol as an alternative feed for dairy cows. The objective of this study was to determine the effects of glycerol on feed intake, milk production, rumen VFA, and metabolic parameters in transition dairy cows. Twenty-six multiparous Holstein cows were paired by expected calving date and fed diets containing either high moisture corn or glycerol from −28 through +56 d relative to calving. Glycerol was included at 11.5 and 10.8% of the ration DM for the pre- and postpartum diets, respectively. Prepartum feed intake was not changed ($P \geq 0.05$) by glycerol feeding (14.6 vs. 14.9 kg/d, glycerol vs. control) nor did postpartum feed intake differ ($P \geq 0.05$; 20.7 vs. 19.8 kg/d, glycerol vs. control). Overall milk yield did not differ ($P \geq 0.05$; 37 vs. 35.8 kg/d, glycerol vs. control), but there was a tendency ($P \leq 0.15$) for a treatment × week of lactation effect that was greater for glycerol. There were no effects of glycerol on milk composition, milk urea nitrogen, somatic cells, and energy balance ($P \geq 0.05$). During the prepartum period, blood glucose was reduced ($P \leq 0.05$; 53.4 vs. 59.1 mg/dL, glycerol vs. control) and β-hydroxybutyrate was increased ($P \leq 0.05$; 0.82 vs. 0.58 mmol/L, glycerol vs. control) in cows fed glycerol. Concentrations of blood nonesterified fatty acids did not differ between the treatment groups ($P \geq 0.05$), and there was no response ($P \geq 0.05$) to glycerol for blood metabolites during the postpartum period. Total rumen VFA (mmol/L) did not differ ($P \geq 0.05$; 85.9 vs. 82.3, glycerol vs. control), but percentage of rumen propionate (28.6 vs. 22.7%, glycerol vs. control) and butyrate (15.3 vs. 11.5%, glycerol vs. control) were greater ($P \leq 0.05$) for cows fed glycerol at the expense of acetate (51.5 vs. 61.4%, glycerol vs. control). These data indicate that glycerol is a suitable replacement for corn grain in diets for transition dairy cows and suggest that glycerin, a biofuels coproduct, is compatible with transition cow health and productivity.

Key Words: biofuels, glycerol, transition cows