W319 In situ ruminal degradability of soybean meal (SBM), canola meal (CM), and corn or wheat dried distillers grains (DDG). G. Maxin,* D. R. Ouellet, and H. Lapierre, *Dairy and Swine Research and Development Center, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Different protein sources, as CM or by-products of ethanol production, are currently used in dairy rations to replace SBM. There is little data available on rumen degradation of these protein sources in a single study. Therefore, the objective of this study was to compare the dry matter (DM) and crude protein (CP) ruminal degradability of SBM, CM, high protein corn DDG with solubles (HPDDG) and wheat DDG with solubles (WDDG). In situ studies were conducted with 4 rumen-fistulated lactating Holstein cows fed a diet containing 38% grass hay and 62% corn-based concentrate. Each protein source was incubated in the rumen in nylon bags for 0, 2, 4, 8, 16, 24 and 48h according to NRC (2001) guidelines. DM and CP ruminal degradabilities were calculated from rumen-undegraded residues corrected or not for small particle loss according to Hvelplung and Weisbjerg (2000). Data were fitted to an exponential model to estimate degradation parameters and effective degradability (ED) was calculated with a passage rate of 7%/h. DM and CP contents for SBM, CM, HPDDG and WDDG were 88.7, 89.7, 92.2, 89.7% and 53.6, 40.0, 40.3, 37.2%. WDDG and SBM had higher uncorrected ED ($P < 0.05$, DM: 75.0 and 72.6%, CP: 84.8 and 66.0%) than CM and HPDDG (DM: 57.2 and 55.5%, CP: 59.3 and 48.2%). This was attributed to a higher soluble fraction in WDDG and a higher potentially degradable fraction and rate of degradation in SBM (9.0 vs. 5.8%/h for the other feeds). Small particle loss from the bags contributed to the DM and CP disappearance, being higher for WDDG (31.6% of DM, 45.7% of CP feed) than for the other feeds (11.2, 14.7, 17.3% of DM and 16.3, 19.7, 19% of CP for SBM, CM and HPDDG). Therefore, the corrected ED was lower than the uncorrected ED for all feeds, especially for WDDG where ED decreased to 53.2% (DM) and 60.8% (CP). However, this correction did not alter feed ranking, with SBM and WDDG being more degradable than CM and HPDDG. The results suggest that small particle loss correction is relevant for this type of feed ingredients and that higher RUP supply would result with CM and HPDDG substituting SBM, assuming similar intestinal digestibility.

Key Words: rumen degradation, protein

W320 Effect of carbohydrate source on performance and ruminal responses of dairy cows fed low-starch diets. H. M. Dann*[1], K. W. Cotanch[1], C. Kokko[1], K. Fujita[2], and R. J. Grant[1], *[1]William H. Miner Agricultural Research Institute, Chazy, NY, [2]ZEN-NOH National Federation of Agricultural Cooperative, Tokyo, Japan.*

Fifteen multiparous Holstein cows (6 ruminally cannulated) were used in a replicated 3 × 3 Latin square design with 21-d periods (7-d collection periods) to measure the effect of forage or nonforage sources of fiber on lactational and ruminal responses when fed to cows in lower-starch diets compared with a higher-starch control diet. Treatments were: 1) control diet (CON) containing 50% forage [20% conventional corn silage (CS), 20% brown midrib CS, and 10% haycrop silage (HCS)], 2) high-forage diet (FOR) containing 63% forage (53% brown midrib CS and 10% HCS), and 3) nonforage fiber source diet (NFFS) containing 50% forage similar to CON and partial replacement of corn meal and soybean meal with beet pulp, wheat middlings, and distillers grains. CON contained 35% neutral detergent fiber (NDF) and 26% starch, FOR contained 38% NDF and 21% starch, and NFFS contained 38% NDF and 21% starch. Data were analyzed as a Latin square by ANOVA with the MIXED procedure of SAS using cow as the experimental unit, treatment, period within square, and square as fixed effects, and cow within square as a random effect. Dry matter intake (DMI), NDF intake, milk yield, and milk composition differed among treatments, which reflected the dietary NDF content. There was no treatment effect ($P > 0.10$) on solids-corrected milk (SCM), efficiency of milk production, ruminal total volatile fatty acid concentration (126 ± 4 m*M*), ammonia concentration (6.7 ± 0.7 mg/dL), or microbial N yield (594 ± 28 g/d). CON was associated with compromised ruminal pH compared with FOR and NFFS. This study demonstrates the value of both a higher forage and a NFFS strategy compared with a higher starch feeding approach.

Table 1.

Item	CON	FOR	NFFS	SE
DMI, kg/d	28.2^{x}	27.2^{y}	27.7^{xy}	0.8
NDF intake, kg/d	9.1^{b}	10.0^{a}	9.9^{a}	0.3
Milk, kg/d	51.6^{ax}	48.4^{by}	50.5^{abx}	2.3
SCM, kg/d	49.0	47.3	48.5	1.9
Fat, %	3.66^{y}	3.98^{x}	3.76^{xy}	0.17
Fat, kg/d	1.86	1.88	1.86	0.08
True protein, %	3.10	3.07	3.08	0.06
True protein, kg/d	1.58^{ax}	1.45^{by}	1.54^{abx}	0.05
SCM/DMI	1.73	1.74	1.75	0.04
Rumen pH	6.09	6.16	6.11	0.04
Rumen pH range	1.17^{x}	0.99^{xy}	0.92^{y}	0.08
Rumen pH < 5.5, min/d	85^{x}	11^{y}	30^{xy}	22
Ruminating, min/d	530	554	541	12
Ruminating, min/kg NDF	58.3^{a}	56.1^{ab}	54.8^{b}	1.7

$^{ab}P \leq 0.05$, $^{xy}P \leq 0.10$.

Key Words: nonforage fiber source, low-starch, NDF

W321 Duodenal bioavailability of quercetin and rutin in German Holstein cows. A. Gohlke[1], C. J. Ingelmann[1], S. Wolffram[2], and C. C. Metges*[1], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Institute of Animal Nutrition & Physiology, Christian-Albrechts-University of Kiel, Germany.*

Flavonoids were shown to have health promoting effects in vitro but evidence for in vivo efficacy is scarce. Whether they are effective depends on their bioavailability and metabolism. Since flavonoids are thought to be degraded by the rumenal microbiota we studied the bioavailability of the flavonol quercetin after duodenal administration of the aglycone and its glucorhamnosid rutin. Six German Holstein cows (>10,000 kg milk 305 d; 2nd lactation; 87 DIM) were fitted with duodenal cannulas. Bioavailability was tested at 4 different doses of each flavonoid (0 (NaCl only), 30, 60 and 90 µmol/kg BW). Before and after duodenal short-term infusion (10 min) at 0700 h, timed blood samples were taken until 24 h post-administration. Levels of plasma quercetin (Q), and its methylated (isorhamnetin, tamarixetin; I, T) and dehydroxylated (kaempferol; K) derivatives were measured after treatment with β-glucuronidase and sulfatase by HPLC with fluorescence detection. Data evaluation was performed using PROC MIXED of SAS. After rutin administration levels of plasma Q, I, T, and K did not increase above baseline. Administration of quercetin aglycone resulted in increased plasma concentrations of Q, I, T and K within 1 h ($P \leq 0.05$). Although maximal plasma

Q concentrations showed high interindividual variability (331.9–842.8 nmol/L at 90 μmol/kg) peak time (115 min) did not differ ($P > 0.9$) among cows. With quercetin aglycone, Q was the main metabolite in plasma followed by I, K and T (81.5, 8.8, 5.8, 3.8% of total flavonol plasma area under curve). Flavonoids did not change DMI and plasma glucose levels but increased daily milk yield by 8% as compared with NaCl administration ($P \leq 0.05$). In contrast to findings in monogastric animals quercetin from rutin seems not to be available in cows when administered duodenally. Different intestinal microbiota composition in ruminants and monogastrics as well as differences in passage time could explain these findings. The increased milk yield upon flavonoid administration needs verification. Supported by BMBF, Germany.

Key Words: bioavailability, dairy cows, flavonoids

W322 Differences in rate of ruminal hydrogenation of C18 fatty acids in clover and ryegrass. J. Lejonklev*[1], A. C. Storm[2], M. K. Larsen[1], G. Mortensen[1], and M. R. Weisbjerg[2], *[1]Aarhus University, Department of Food Science, Tjele, Denmark, [2]Aarhus University, Department of Animal Science, Tjele, Denmark.*

The effect of forage type on ruminal hydrogenation was investigated by in vitro incubation of feed samples in rumen fluid from 2 rumen fistulated Holstein cows. Silages of red clover (RC), white clover (WC) and perennial ryegrass (PR) were produced in laboratory scale using forage harvested in primary growth and in third regrowth, resulting in 6 silages. Fatty acid content was analyzed after 0, 2, 4, 6, 8 and 24 h of incubation in triplicates to study the rate of hydrogenation of unsaturated C18 fatty acids. Initial composition of C18 fatty acids in dry matter varied between the different forages and growths. The amount of polyunsaturated fatty acids decreased with increased incubation time, whereas the amounts of monounsaturated vaccenic acid and saturated stearic acid increased. A dynamic mechanistic model was constructed assuming mass action driven fluxes between the following pools of C18 fatty acids: C18:3 (linolenic acid), C18:2 (linoleic acid), C18:1 (vaccenic acid, oleic acid, petroselidic acid) and C18:0 (stearic acid) as the end point. Longitudinal data from each single in vitro run was fitted to the final model and the rate constants (/h) of the hydrogenation were estimated using a Nelder-Mead optimization algorithm while maximizing the log-likelihood function. The estimated rate constants of hydrogenation were 0.069 (RC), 0.071 (WC) and 0.087 (PR) for $k_{C18:2->C18:1}$. 0.081 (RC), 0.077 (WC) and 0.102 (PR) for $k_{C18:3->C18:1}$. 0.046 (RC), 0.055 (WC) and 0.050 (PR) for $k_{C18:1->C18:0}$. Type of forage had a significant effect on $k_{C18:2->C18:1}$ ($P = 0.005$) and a tendency to influence $k_{C18:3->C18:1}$ ($P = 0.062$). The growth (primary or regrowth) had no effect on $k_{C18:2->C18:1}$ ($P = 0.42$) or $k_{C18:3->C18:1}$ ($P = 0.14$). Neither forage nor growth significantly affected $k_{C18:1->C18:0}$ ($P > 0.5$). Both RC and WC resulted in lower rates of hydrogenation of polyunsaturated fatty acids to monounsaturated than did PR, whereas the hydrogenation of monounsaturated to saturated fatty acids was similar between forages. This effect is present irrespective of the cutting time, i.e., primary growth or regrowth, despite large variations in fatty acid content between the cuts.

Key Words: clover, hydrogenation, rumen

W323 Corn source and dietary protein degradability: effects on ruminal measures and proposed mechanism for degradable protein effects. M. B. Hall,* *U. S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Effects of corn source and ruminal degradability of dietary protein (RDP) on ruminal measures was evaluated in a partially balanced, incomplete Latin square design with 3 21-d periods, 8 ruminally cannulated lactating dairy cows, and a 2 × 2 factorial arrangement of treatments (trt). Trt were corn source (corn; DG: dry ground corn, HM: high moisture corn) and RDP (+RDP: added protein from soybean meal; -RDP: heat-treated expeller soybean product partially substituted for soybean meal). Diets were formulated to be isonitrogenous and similar in starch and NDF. Data were analyzed with mixed models with cow (a random variable), period, corn, RDP, and corn x RDP as factors. Rumen concentrations over time were analyzed as repeated measures. Significance was declared at $P < 0.05$, and tendency $0.05 \leq P < 0.15$. DMI was 1 kg less on -RDP as compared with +RDP diets. Weights of total digesta and liquid tended to be greater with +RDP than with -RDP at 2 h post-feeding, with digesta DM% greater with -RDP than with +RDP (DM%: 14.6, 15.7, 14.9, and 15.3% for DG+RDP, DG-RDP, HM+RDP, and HM-RDP, respectively, SED = 0.44). Differences in the digesta DM:liquid ratio could introduce dilution effects on pH and organic acid concentrations (OA), hence, 2 h post-feeding digesta DM% for each cow in each period were used as covariates for analysis of ruminal pH and OA. *P*-values of the covariate were < 0.04. pH was greater for DG (6.27) than for HM (6.09; SED = 0.12), and tended to be affected by RDP x time with HM+RDP numerically lower than other trt after 1 h post-feeding. Corn x RDP tended to affect mean OA with +RDP 4% lower than -RDP with DG, but 9% greater with HM. Interaction of time and RDP affected OA with +RDP (155 mM) greater than -RDP (141 mM; SED = 5.9) at 2 h post-feeding and with more similar values for trt at all other times. Average lactate concentration was greater and maxima tended to be greater for +RDP than for –RDP. +RDP effects on OA and pH may be due to more immediate fermentation of starch instead of storage as microbial glycogen, but presence of dietary starch precludes measurement of glycogen. The basis for RDP effects needs further exploration.

Key Words: corn, protein degradability, rumen fermentation

W324 A meta-analysis of continuous culture rumen fermentation and digestibility data. A. N. Hristov*[1], C. Lee[1], R. A. Hristova[1], P. Huhtanen[2], and J. L. Firkins[3], *[1]The Pennsylvania State University, University Park, [2]Swedish University of Agricultural Sciences, Umeå, Sweden, [3]The Ohio State University, Columbus.*

A meta-analysis was conducted to investigate variability in ruminal fermentation and nutrient digestibility in continuous culture (CC) experiments in comparison with in vivo data. One hundred and 80 CC studies representing 1,074 individual treatments were used in the analysis. Studies were classified into 2 groups based on the type of CC used: CC system specified as RUSITEC (Rumen Simulation Technique) and non-RUSITEC CC systems. The CC data were compared with a data set of in vivo trials (INVIVO) with ruminally-cannulated lactating dairy cows (a total of 366 individual cow data). Data were analyzed using the MIXED procedure of SAS with study as random effect. Average total VFA concentration for RUSITEC and non-RUSITEC was 68 and 80% ($P < 0.001$) of INVIVO concentrations: 79.0 (SD = 30.9), 93.8 (36.3), and 116.9 (19.8) m*M*, respectively. Average concentration of acetate was also lower ($P < 0.001$) for the CC data sets compared with INVIVO and that of propionate was considerably lower for RUSITEC compared with INVIVO ($P < 0.001$), but butyrate concentrations were similar between CC and INVIVO. Average acetate:propionate ratios were lower ($P < 0.001$) for the CC data sets compared with INVIVO (2.69, 2.61, and 2.94, respectively). Variability in the VFA data was generally the highest for non-RUSITEC (higher CV and variance; $P < 0.001$), followed by RUSITEC, and was the lowest for INVIVO. Digestibilities of neutral-detergent fiber (NDF) and particularly organic matter (OM) were lower ($P < 0.001$) in the CC data sets compared with INVIVO; average NDF digestibility was 34.2 (15.9), 45.5 (17.2), and

53.0 (11.6) % for RUSITEC, non-RUSITEC, and INVIVO. A possible explanation for the considerably lower NDF digestibility with RUSITEC vs. non-RUSITEC could be the lower microbial enzymatic activities within the nylon bag vs. the surrounding rumen reported for the in situ procedure. This analysis demonstrated that in CC, total VFA and acetate concentrations were lower, protozoa were low or absent, and OM and NDF digestibilities were lower than in vivo. Overall, variability was much greater for CC vs. in vivo experimental data.

Key Words: continuous culture, rumen fermentation, meta-analysis

W325 Amount and digestibility of NDF affects rumen nutrient pool sizes and passage kinetics of dairy cows. K. W. Cotanch,* C. Kokko, H. M. Dann, J. W. Darrah, and R. J. Grant, *William H. Miner Agricultural Research Institute, Chazy, NY.*

Ruminally cannulated Holstein cows (n = 8, 91 ± 11 d in milk) were used in a replicated 4 × 4 Latin square design study with 21-d periods to evaluate rumen pool size and turnover of organic matter (OM) and neutral detergent fiber (aNDF) of cows fed different sources and amounts of corn silage using a rumen evacuation technique. Conventional corn silage (CCS) and BMR corn silage (BMRCS) contained 37 and 39% aNDF with a 24-h aNDF digestibility (NDFD) of 38 and 51%, respectively. Treatments were 1) LCCS: 53% forage, 39% CCS, 32% aNDF, and 56% NDFD; 2) HCCS: 68% forage, 55% CCS, 36% aNDF, and 54% NDFD; 3) LBMR: 50% forage, 36% BMRCS, 32% aNDF, and 62% NDFD; and 4) HBMR: 64% forage, 50% BMRCS, 35% aNDF, and 60% NDFD. Intake was measured during d 14 to 21. Ruminal contents were evacuated manually 4 h after and before feeding on d 20 and 21, respectively. Total rumen contents were weighed and volume determined. Ten percent of rumen contents were collected, subsampled, and analyzed for OM and aNDF content. Data were analyzed as a replicated Latin square with the MIXED procedure of SAS with fixed effects of diet, period, and replicate. Cow within square was the random effect. Intake of dry matter (DMI) and aNDF were affected by treatment. Ruminal digest volume and mass were greatest for HCCS and lowest for LBMR. Pool size of OM did not differ among treatments but turnover time of OM was greater for HCCS than LBMR and HBMR. Pool size of aNDF was greater for HCCS than LBMR; LCCS and HBMR were intermediate. Turnover time of aNDF was greater for LCCS and HCCS than HBMR. The amount and digestibility of forage aNDF from corn silage affect nutrient pool size and passage kinetics of dairy cows.

Table 1.

Item	LCCS	HCCS	LBMR	HBMR	SE
DMI, kg/d	29.0^{a}	26.5^{b}	29.3^{a}	29.2^{a}	0.7
aNDF intake, kg/d	9.4^{b}	9.5^{b}	9.3^{b}	10.3^{a}	0.2
Ruminal digesta, L	123^{ab}	128^{a}	113^{b}	119^{ab}	3
Ruminal digesta, kg	106^{ab}	112^{a}	98^{b}	105^{ab}	3
Ruminal pool size, kg					
OM	13.00	12.49	12.14	12.64	0.59
aNDF	8.32^{ab}	8.45^{a}	7.64^{b}	8.36^{ab}	0.41
Ruminal turnover rate, %/h					
OM	8.95^{ab}	8.31^{b}	9.44^{a}	9.57^{a}	0.51
aNDF	4.84^{b}	4.76^{b}	5.12^{ab}	5.52^{a}	0.30
Ruminal turnover time, h					
OM	11.4^{ab}	12.2^{a}	11.0^{b}	10.9^{b}	0.5
aNDF	21.1^{a}	21.4^{a}	20.3^{ab}	19.0^{b}	1.1

$^{ab}P \leq 0.05$.

Key Words: corn silage, NDF digestibility, passage

W326 Orchard grass forage effects on bacterial communities and long-chain fatty acid profiles in the rumen of Holstein heifers. R. Mohammed[1,2], G. E. Brink[1], D. M. Stevenson[1], K. A. Beauchemin[2], and P. J. Weimer*[1], *[1]USDA-ARS, US Dairy Forage Research Center, Madison, WI, [2]AAFC, Lethbridge Research Center, Lethbridge, AB, Canada.*

The aim of this study was to determine if ruminal bacterial community composition (BCC) and long chain fatty acid (FA) profiles differed in heifers grazing orchardgrass pasture (OP) versus those fed hay (OH) harvested from the same field at the same stage of maturity. Five ruminally cannulated Holstein heifers were allotted to OP or OH with three 28-d periods. Three of these heifers were offered OP, OH and OP successively in the 3 periods while the other 2 heifers remained on OP for all periods. Ruminal digesta were collected on 3 consecutive days near the end of each period. Microbial DNA was extracted from solid and liquid phases of digesta, amplified by PCR using domain-level bacterial primers, and subjected to automated ribosomal intergenic spacer analysis (ARISA). Total ruminal volatile fatty acids (m*M*) and acetate (mol/100 mol) were greater for OH than for OP, while butyrate was less. The ARISA profiles for the OP and OH diets formed distinct clusters, indicating that ruminal BCC was affected by diet. Branched-chain FA (% total FA methyl esters) in rumen digesta (13:0 *anteiso*, 14:0 *iso*, 15:0 *iso*, 15:0 *anteiso* and 18:0 *iso* were greater for OH than OP. Total *trans* (*t*)-18:1, *t*11- and *t*10-18:1 were greater for OP than OH. Conjugated linoleic acid (CLA) isomers (*c*9, *t*11-CLA and *t*10, *c*12-CLA) were not influenced by the diets. Relative population size (RPS) of *Butyrivibrio fibrisolvens* and *Megasphaera elsdenii* in the liquid phase of rumen digesta (fraction of total 16S rRNA gene copy number), were not affected by diet. Regression analysis of ruminal *t*10-18:1 and RPS of *M. elsdenii* was not significant ($P = 0.59$; n = 8). Unexpectedly, the relationship between ruminal *t*11-18:1 and RPS of *B. fibrisolvens* was negative ($r^2 = 0.52$; $P = 0.04$; n = 8) suggesting that there are likely yet unidentified polyunsaturated FA biohydrogenating rumen bacteria. The difference in ruminal chemistry and BCC in heifers fed OP and OH reflects possible alterations in substrate availability to the rumen microflora due to differences in the form of diet (fresh vs. conserved forage).

Key Words: conjugated linoleic acid, orchardgrass, rumen bacterial communities

W327 Silicone plastination of rumen models: A room temperature technique. H. C. Puch,* K. B. Cunningham, and D. C. Brown, *LongView Animal Nutrition Center, Land O'Lakes Purina Feeds, Gray Summit, MO.*

The objective of this work was to create demonstration models of rumen tissues that are preserved and realistic compared with fresh tissues. Silicone plastination is a process that replaces tissue and cellular fluids with silicone polymers. Using this method, rumen tissue specimens can be preserved that are lightweight, flexible and anatomically correct. Random Holstein calves at 12, 20, or 24 weeks of age were euthanized and the reticulo-rumens collected for preservation. The full reticulo-rumen was placed on its right side, and an incision made around the outer circumference. The reticular fold, cranial and caudal pillars are cut at their mid points so that the reticulo-rumen can lay flat creating a symmetrical left and right side. The left side portion of the rumen was first dehydrated in cold 100% acetone at −24 C. Each rumen is stretched and attached to a customized polypropylene frame and placed into the vacuum chamber filled with silicone (NCS10 and NCS6, supplied by R.W. Henry, DVM, 1455 A. R. Davis Road, Seymour, TN. 37865). The lab made welded vacuum chamber is made from 6.5 mm steel with a chamber 810 mm long × 460 mm wide × 150 mm deep and has 170 mm

× 130 mm × 12 mm thick acrylic window in the top. Silicone replacement of tissue acetone occurs slowly in the vacuum chamber by decreasing pressure. Vacuum is controlled by adjusting inlet needle valve. Visually, the acetone will start to boil/vaporize at 22 cm Hg. Vacuum is held 22 cm Hg for 24 h. Each day the vacuum pressure is decreased by 1/2 for 3 d and the vacuum is 2 cm Hg. On d 4 the needle valve is completely closed and the vacuum held for 24 h or until acetone stops vaporizing. The rumen is removed from the silicone, allowed to drain, air dry for 24 h and removed from stretching frame. Final end chain polymerization is done by spraying tissue with the polymerizing catalyst (NCS3) and held in a closed plastic bag for 24 h. Visual differences can be observed in the plastinated rumens at different weeks of age in, papillae size, shape, color appearance and any abnormalities. This method creates preserved tissues that are ideal for teaching and demonstrations.

Key Words: model, plastination, rumen

W328 Techniques for sampling and measuring total two-dimensional surface area of rumen papillae. H. C. Puch*[1], K. M. O'Diam[2], and K. M. Daniels[2], *[1]LongView Animal Nutrition Center, Land O' Lakes Purina Feeds, Gray Summit MO, [2]Ohio Agricultural Research and Development Center, The Ohio State University, Wooster.*

The aim was to develop an objective method for determining total 2-dimensional (T2D) surface area of rumen papillae which can effectively be used to study rumen development. Eight Holstein steers were fed defined amounts of texturized grower concentrate with ad libitum access to alfalfa hay from 12 to 24 wk of age. Steers were euthanized at 24 wk to evaluate rumen development. The full reticulo-rumen was placed on its right side and an incision made around the outer circumference. The reticular fold, cranial and caudal pillars were cut at their mid points, so that it could be laid out flat creating a symmetrical left and right side. The right side was sampled to measure papillae development. Tissue sections (2x12 cm) were cut from 4 regions (caudal dorsal blind sac, caudal ventral blind sac, cranial dorsal and cranial ventral) and individually stapled to labeled 15 cm wooden tongue depressors and preserved in fixative for later histological analyses (data not shown). Ten representative papillae were dissected from each rumen tissue section and adhered to millimeter graph paper to determine length (L) and width (W). T2D surface area was calculated for each papilla, using an oval equation [2*(L/2*W/2 * 3.14)]. For comparison, T2D surface area of the same papillae was also determined via a digital image analysis program (CellSens Imaging; Olympus Corp.). In this method, papillae images were traced and the 2D area x2 determined. Both measurement techniques were not different (pooled across region: 32.2 vs. 31.6 mm2, SE 5.3). However, the 4 regions of caudal dorsal blind sac, caudal ventral blind sac, cranial dorsal and cranial ventral had different papillae surface area of 28.4, 30.9, 26.5, and 42.0 mm2, SE 5.4, respectively ($P < 0.001$). Both methods are effective in quantifying T2D surface area of rumen papillae; the digital method has an advantage in that it does not assume the shape of each papilla to be an oval. Both methods are helpful in evaluating dietary influences on rumen papillae size. Due to regional differences in papillae area, each region must be examined in future studies to fully characterize papillae surface area response to dietary changes.

Key Words: papilla, rumen, dairy steer

W329 Changes in rumen bacterial communities and rumen chemistry in primiparous Holstein cows during the periparturient period. **R. Mohammed[1,2], D. M. Stevenson[2], P. J. Weimer[2], G. B. Penner*[3], and K. A. Beauchemin[1],** *[1]AAFC, Lethbridge Research Center, Lethbridge, AB, Canada, [2]USDA-ARS, US Dairy Forage Research Center, Madison, WI, [3]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

The objectives were to study the changes in: 1) rumen bacterial community composition (BCC) and fermentation as influenced by feeding regimen and period; and 2) pH and VFA profiles among selected cows with minimum (stable) and maximum variation (unstable) between pre- and post-parturient periods. Fourteen Holstein heifers paired by expected calving date and BCS were allotted to 1 of 2 prepartum feeding regimens: low concentrate regimen (2 diets ranging from forage:concentrate, F:C = 80:20 to 54:46); or a high-concentrate regimen (4 diets ranging from F:C = 68:32 to 46:54). All cows received the same lactation diet postpartum. Microbial DNA extracted from 58 rumen digesta samples collected prepartum (d −50, −31, −14) and postpartum (d +14, +52) and amplified by PCR were subjected to automated ribosomal intergenic spacer analysis (ARISA). Changes in rumen chemistry (pH, VFA, and acidosis indicators at d −54, −35, −14, −3, +3, +17, +37, +58) were analyzed using principal component analysis (PCA). The ARISA profiles did not show a diet effect; however, 4 out of 14 cows showed a shift in BCC from the prepartum to the postpartum period. The PCA profiles did not show a diet effect for rumen fermentation variables. However, 4 cows (stable) showed minimal changes while 4 other cows (unstable) had greater changes in rumen fermentation when PCA profiles of individual cows were examined. The remaining cows had a PCA profile intermediate between the stable and the unstable fermentation profiles. Cows with a stable profile had greater minimum rumen pH, mean pH, less bouts under pH 5.5 and 5.2, and less duration and area of moderate (pH <5.5) and acute ruminal acidosis (pH <5.2) compared with cows with an unstable profile. Milk fat content tended to be greater for cows with a stable profile than for those with an unstable profile, with no difference in milk yield or FCM. Substantial changes in rumen chemistry occurred before and after calving with only small changes in BCC. Rumen chemistry during the periparturient period was not affected by feeding regimen but varied considerably among cows.

Key Words: ruminal acidosis, ruminal bacterial communities, periparturient period

W330 Detection of the methanol dehydrogenase structural gene *mxaF* in rumen fluid by PCR. E. T. Kim*[1], C. S. McSweeney[2], S. S. Lee[1], S. C. Kim[1], and S. H. Kang[2], *[1]Division of Applied Life Science (BK21 Program), Gyeongsang National University, Jinju, Gyeongnam, Republic of Korea, [2]CSIRO Livestock Industries, Queensland Bioscience Precinct, St Lucia, Qld, Australia.*

This study was conducted to demonstrate the potential possibility of methane oxidation in rumen by the *mxaF* gene, a functional gene (methanol dehydrogenase (MDH)), involved in methane oxidation pathway. The rumen fluid was collected before morning feeding from fistulated Holstein cows maintained on Leucaena and Rhodes grass diets, and on Lucerne diet. The DNA sample was amplified by PCR with the *mxaF* target primers. The expected DNA size band from PCR products was observed, and then cut for sequencing analysis. The PCR products of gDNA were ligated into the pGEM®-T Easy Vector according to the protocol of the TA Cloning Kit. Briefly, after the transformed cells had been incubated with isopropylthiogalactoside (IPTG), 192 random clones of white colonies were sequenced by an ABI 3130xl Genetic Analyzer. The amplicon sequences were compared with sequences of reference organisms from Greengenes and NCBI. The result from sequencing analysis matched *Methylobacterium sp.* which is extremely important because they can use methanol and methylamine as well as acetate, propionate and butyrate compounds to grow. *Methylobacterium*

sp. is common in soil and on surfaces of leaves and other plant parts, and has the ability to utilize methane and other more complex organic compounds as carbon and energy sources. *Methylobacterium sp.* is known as a facultative methylotroph. *MxaF* gene, one of functional genes involved in methane oxidation pathway, was detected in rumen fluid in present study

Table 1. The result of sequencing analysis of mxaF-target from the rumen fluid

Classification	Identity (%)	
Methanol dehydrogenase alpha subunit (mxaF) gene (Methylobacterium brachiatum)	99	(527/533)
Methanol dehydrogenase alpha subunit (mxaF) gene (Methylobacterium platani strain KCTC12901)	98	(533/544)
Methanol dehydrogenase alpha subunit-like (mxaF) gene (Methylobacterium aquaticum strain DSM 16371)	98	(545/558)
Methanol dehydrogenase alpha subunit (mxaF) gene (Methylobacterium hispanicum strain DSM 16372)	97	(516/532)

Key Words: methane oxidation, methanol dehydrogenase, MxaF gene

W331 Evaluation of DM, NDF, and starch ruminal degradabilities of corn silage hybrids: A three-year study. D. R. Ouellet*[1], G. F. Tremblay[3], and A. F. Mustafa[2], *[1]Dairy and Swine R&D Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [2]Dept. of Animal Science, Ste-Anne de-Bellevue, QC, Canada, [3]Soils and Crops R&D Centre, Agriculture and Agri-Food Canada, Québec, QC, Canada.*

This experiment evaluated the DM, NDF, and starch ruminal degradabilities of 6 corn silage hybrids (Garst 8707 MP, Maizex leafy 2, Mycogen TMF 94, Novartis G 4106, Pickseed Exel, Pioneer 38T27) seeded at 5 different locations in Ontario, Canada, and in 3 consecutive years. Each year, corn hybrids were ensiled in duplicate in 20-L mini-silos which were sampled after more than 100 d of fermentation. All frozen silage samples were placed in nylon bags and incubated for 0 (wash only) or 16 h into the rumen of 2 fistulated cows to determine the ruminal degradation characteristics using double-point estimation (Vanzant et al. 1996 JAS 74:2773). Effective ruminal degradabilities (ED) of nutrients were estimated assuming a fractional rate of passage of 0.06 h^{-1}. There was no interaction between hybrids and production years for all parameters. Silage DM concentration varied between 32 and 36% and was affected by hybrids and years (32.4^a, 32.7^a, and 34.1^b % for year 1, 2, and 3, respectively). Silage NDF and starch concentrations were also affected ($P < 0.05$) by hybrids (46.2 to 49.2 and 26 to 32% of DM, respectively) and years. Degradation rates of the potentially degradable DM, NDF, and starch were not affected by hybrids averaging 1.7, 1.1, and 8.8% h^{-1}, respectively. Degradation rate of DM was affected by the production year (1.5^a, 2.2^b, and 1.3^c % $h^{-1}$for year 1, 2, and 3, respectively); NDF degradation rate was affected by year in the same manner. The ED of DM was affected by hybrids (53.1^a, 53.8^{ab}, 54.3^{ab}, 54.9^{ab}, 55.3^b, and 56.9^c for Garst 8707 MP, Pickseed Exel, Novartis G 4106, Pioneer 38T27, Maizex leafy 2, Mycogen TMF 94, respectively). The ED of NDF and starch were similar among hybrids averaging, 23.0% (SE = 1.4) and 90.1% (SE = 0.7), respectively. Years affected ED of DM (51.7^a, 57.1^b, and 55.5^c% for 1, 2, and 3, respectively), NDF (24.5^a, 25.9^a, and 18.6^b% for year 1, 2, and 3, respectively) and starch (90.6^a, 89.0^b, and 90.6^a% for year 1, 2 and 3, respectively). Results indicates that the production year have a significant effect on DM, NDF, and starch concentrations of silage, which in turn affected degradation rate and ED of these parameters except for degradation rate of starch. Degradation rates and ED of NDF and starch were not affected by hybrids, but the hybrid Mycogen TMF 94 had the greatest ED of DM.

Key Words: corn silages, forage degradation

W332 Rumen microorganisms growth as a function of the concentration of corn silage and soybean meal in culture medium. C. P. Ghedini[1], R. P. Lana[1], A. S. Oliveira[2], D. C. Abreu*[1], R. M. Paula[1], C. J. Silva[1], and P. E. P. Barros[3], *[1]Universidade Federal de Viçosa, Viçosa, MG, Brazil, [2]Universidade Federal do Mato Grosso, Sinop, MT, Brazil, [3]Universidade Federal de Lavras, Lavras, MG, Brazil.*

The objective was to assess the effect of levels of corn silage and soybean meal on the ruminal microbial growth. Vials containing 6 mL of artificial saliva, 24 mL of inoculum and substrate (half corn silage, half soybean meal) in weights of 1.2; 0.6; 0.3; 0.1; 0.05 and 0 g were incubated in triplicate at 39°C for 72 h. Optical density (OD-600nm) and pH were measured at 0, 6, 12, 18, 24, 48 and 72 h of incubation. The experiment was analyzed as completely randomized design as a function of substrate concentration, time of incubation and the respective interaction. Microbial growth has reached the maximum value ($P < 0.01$) in the highest concentration of substrate, where it was also noted the lowest pH value (6.75). Regardless of level of substrate, the largest optical density was observed within 24 h incubation ($P < 0.01$) showing that the limiting factor was the substrate, as the pH was always above 6.9. After the time of maximum growth the microbial growth curve followed the hyperbolic model, assimptotic, being analyzed by saturation kinetics model of Lineweaver-Burk, as follows: 1/OD = y = 0.0869 + 0.5573 (1/concentration of substrate), $r^2 = 0.90$. The theoretical maximum growth rate was 1.79 and the substrate concentration required to achieve half of the maximum response was 0.160 g. The pH values were 6.75; 7.48; 7.93; 8.06 and 8.16 for 1.2; 0.6; 0.3; 0.1 and 0.05 g of substrate, respectively, and 7.70; 7.98; 7.80; 8.07; 7.74; 7.09; 7.36 in times of 0, 6, 12, 18, 24, 42 and 72 h of incubation, respectively. The growth of microbial population in rumen depends not only on the availability and substrate concentration, but also on fermentation products accumulation. Under the terms of this work the limiting factor was the availability of the substrate, leading to saturation of microbial growth, following the hyperbolic model. Supported by FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais).

Key Words: fermentation kinetics of saturation, microbial growth, substrate

W333 Passage of liquid and fiber particles in dairy cows fed diets differing in NDF from conventional and bmr corn silages. K. W. Cotanch*[1], C. Kokko[1], H. M. Dann[1], J. W. Darrah[1], R. J. Grant[1], and D. R. Mertens[2], *[1]William H. Miner Agricultural Research Institute, Chazy, NY, [2]Mertens Innovation & Research LLC, Belleville, WI.*

Ruminally cannulated Holstein cows (n = 8, 91 ± 11 d in milk) were used in a replicated 4 × 4 Latin square design with 21-d periods to evaluate mean retention time (MRT) of liquid and fiber particles in cows fed diets varying in aNDF and 24-h NDF digestibility (NDFD) from conventional corn silage (CCS) and bmr corn silage (BMRCS). Treatments were 1) LCCS: 53% forage, 39% CCS, 32% aNDF, and 56% NDFD; 2) HCCS: 68% forage, 55% CCS, 36% aNDF, and 54% NDFD; 3) LBMR: 50% forage, 36% BMRCS, 32% aNDF, and 62% NDFD; and 4) HBMR: 64% forage, 50% BMRCS, 35% aNDF, and 60% NDFD. Samples of feces, haylage, CCS, and BMRCS were extracted in neutral detergent and sieved to obtain fiber particles of defined sizes. Fine fecal particles (1.59 - 4.75 mm), medium haylage particles (1.18 - 4.75 mm), and

small (0.30 - 1.18 mm), medium (1.18 - 4.75 mm), and large (>4.75 mm) particles of CCS and BMRCS were labeled with Cr, La, Sm, Yb, Pr, respectively. Cows were dosed ruminally with a liquid marker (Co-EDTA) and marked particles on d 14 before feeding. Fecal samples were collected from 0 to 168 h after dosing and analyzed for marker by ICP. The MRT were calculated from areas under marker excretion curves, and included ruminal residence time plus post-ruminal transit time, which varied from 6 to 9 h based on first excretion of markers. Data were analyzed as a replicated Latin square with the MIXED procedure of SAS with fixed effects of diet, period, and replicate. Cow within square was the random effect. DMI (kg/d) was lower ($P \leq 0.05$) for HCCS (26.5) than LCCS (29.0), LBMR (29.3), and HBMR (29.2). Small corn silage particles of LCCS had shorter MRT than HCCS, but were not different between LBMR and HBMR. Conversely, medium corn silage particles of HBMR had shorter MRT than these particles in LCCS and HCCS. The lower DMI of HCCS does not appear to be related to marker MRT.

Table 1.

Mean retention time, h	LCCS	HCCS	LBMR	HBMR	SE
Liquid	20.5	20.9	20.7	19.4	0.6
Fine feces	29.5	30.7	31.2	32.2	0.8
Medium haylage	41.8	43.3	42.7	41.9	1.6
Small corn silage	36.1^{y}	39.8^{x}	37.5^{xy}	36.5^{xy}	1.3
Medium corn silage	44.9^{axy}	46.0^{ax}	43.7^{abyz}	42.1^{bz}	1.5
Large corn silage	47.5	48.8	47.9	46.8	1.6

$^{ab}P \leq 0.05$; $^{xyz}P \leq 0.10$.

Key Words: corn silage, NDF, retention time

W334 Effect of silica levels, and its location in the detergent fiber matrix, on in vitro gas production of rice straw. G. S. Cun*[1], G. A. Nader[2], and P. H. Robinson[1], *[1]University of California, Davis, [2]University of California Cooperative Extension, Yuba City.*

Rice straw can be used as a forage for cattle but, due to its very low net energy (NE) value, its use is generally limited to cattle whose intake potential substantially exceeds their energy need. This low NE value, driven by a low digestibility of organic matter (OM), has often been attributed to the high silica (Si) level of rice straw. This series of experiments evaluated how simulated field drying of rice straw, its Si level and location in the detergent fiber matrix, affects in vitro gas production. We also determined how in vitro gas production at 4, 24 and 72h are affected by removing Si from rice straw entirely by growing it in a Si-free hydroponic solution. In Expt. 1, rice plants grown in controlled conditions were analyzed fresh (i.e., within 60 min of harvest), and when fully dried (i.e., 25°C for 7 d), for dry matter, ash, acid detergent fiber (ADF) and acid detergent extracted neutral detergent fiber (ND/ADF) by ND extracting ADF, Si in ADF (ADF-Si) and Si in ND/ADF (ND/ADF-Si), as well as in vitro gas production. Fresh straw had a higher proportion of ADF-Si (539 versus 485 mg/g total Si; $P < 0.01$) and less Si in ND extracted ADF (196 versus 340 mg/g total Si; $P < 0.01$), than the same plants after drying, which may have caused the higher in vitro gas production of fresh straw at all times. However this was not confirmed in Expt. Two where ADF-Si, ND/ADF-Si and total Si were not predictive of any in vitro gas production in 39 field samples of rice straws collected in the Sacramento Valley (CA), possibly due to the relatively narrow range of 3.5 to 6% Si in the samples, or because the location of the Si in the fiber matrix, or Si itself, is simply not predictive of the digestibility of its OM. Indeed, in Expt. 3, rice grown under controlled conditions in Si free or Si containing hydroponic media, in 2 sub-experiments, had similar gas production unit OM. The general lack of impact of rice straw Si levels, either in total or relative to its location in the detergent fiber matrix, on in vitro gas production seems to demonstrate that Si is not causative to the low fermentability of rice straw OM.

Key Words: in vitro, silica, hydroponic