

the milk. Under consumption the impetus for product innovation were rather weak and the efficiency of milk processing was rather low. After the privatisation the overwhelming Hungarian milk production capacities were privatised by Western European investors. There were some hopes concerning the privatisation of milk factories to agricultural producers, but they did not have money either for buying or for financing the production. In some cases milk producers got a minority ownership, but this was not enough to get through their interests. The multinational enterprises follow three different strategy #types: cost-leadership, differentiation and focussing local producers. Rapid development was resulted by introduction of ISO 9000 norms, and HACCP systems. Important problem of Hungarian milk sector was the limited purchasing power of Hungarian consumers. It makes a limited possibility for product development. 80% of domestic purchasing of milk and milk-products are marketed through the 6 largest commercial companies. These companies

utilise the tactic of predatory pricing or economic pressure in process of determination of prices. The government lays not enough weight on the handling of this problem, because this price-pressure strategy of commercial companies is a good tool in fighting against the inflation. This is a short-term possibility however, because the 1,5-2,0 real profit level does not gives technical upgrading. The EU joining means new threats and possibilities for milk processing sector. The most important threat is the introduction of milk-quota system. It is an open #end question, whether after the EU joining the multinational enterprises, producing in Hungary. The higher costs and the export-defence system of non-EU members is an important factor of decision, in second case the loss of Hungarian workplaces, processing capacities and the lower level of material and human infrastructure are the most important factors.

Key Words: Milk industry, Marketing, Multinational enterprises

Ruminant Nutrition Ruminal Fermentation

723 Ruminal fluid effects on in vitro digestion kinetics of corn starch. F. M. Fickett* and M. S. Allen, Michigan State University, East Lansing.

Effects of rumen fluid on digestion kinetics of corn starch in vitro were evaluated. Four ruminally-cannulated cows were used in an experiment using a crossover design for diet with a 2 x 2 x 2 factorial arrangement of treatments. Rumen fluid was from cows offered diets formulated to 21% and 32% dietary starch, and collected at two times relative to feeding (one hour before, or two hours after feeding). Periods were 14 d in length for which 12 d were allowed for diet adaptation and 2 d for rumen fluid collection. One cow from each dietary treatment group was sampled both before and after feeding on each collection day. Substrate treatments were dry corn grain with flourey or vitreous endosperm, ground in a Wiley mill with a 2 mm screen. Amounts of media, blended and filtered rumen fluid, and substrate used for incubations were 40 ml, 10 ml, and 0.25 g, respectively. Flasks were incubated under positive carbon dioxide pressure for 0, 1.5, 3, 6, 10.5, 16.5, and 24 h. Residual starch was determined following incubation. Disappearance curves were fit to a 1-pool exponential decay model with discrete lag using non-linear regression. No interactions of treatments were detected for rate of starch digestion, which was higher for flourey compared to vitreous endosperm (43.6 and 28.5 %/h, $P < 0.001$) and higher after feeding compared to before feeding (41.2 and 30.9 %/h, $P < 0.01$). Rate of starch digestion was not affected by dietary starch concentration. An interaction was observed between dietary starch concentration and endosperm type for digestion lag time ($P < 0.01$). Lag time was greater for vitreous compared to flourey endosperm for the high starch diet (2.33 vs. 1.90 h) but lower for the low starch diet (1.87 vs. 2.76 h). Lag time was greater ($P < 0.01$) when rumen fluid was collected after meals compared to before meals (2.53 h and 1.90 h). Effect of time relative to feeding on rate of digestion is attributed to differences in enzyme activity of rumen fluid, which was not different for diets varying in starch concentration.

Key Words: Starch digestion kinetics, Rumen fluid, Corn endosperm type

724 Comparison of fermentation parameters in ruminal fluid collected from lactating dairy cows at different production levels. S. A. Martin*¹, T. G. Nagaraja², T. C. Jenkins³, S. E. Ives², H. J. Strobel⁴, J. Sullivan⁵, K. Murphy⁵, D. Luchini⁵, S. Koenig⁵, and J. L. Klingener¹, ¹University of Georgia, Athens, ²Kansas State University, Manhattan, KS, ³Clemson University, Clemson, SC, ⁴University of Kentucky, Lexington, ⁵Bioproducts, Inc., Fairlawn, OH.

The objective of this study was to compare ruminal fermentation parameters and ciliated protozoal populations in lactating cows at three different production levels. Ruminal contents were collected via stomach tube from Holstein cows at a commercial dairy in California. The ruminal contents were obtained approximately 1.5 h after feeding from five cows within each of three different production levels (low = 13,736 kg, medium = 16,479 kg, high = 19,716 kg). Immediately after collection of each ruminal fluid sample pH was measured and aliquots of samples were fixed in formal-saline for protozoal enumeration. Volatile fatty acids, malate, lactate, ammonia, and protein were also determined. When ruminal fluid samples from each production level ($n = 5$) were analyzed, pH was lower ($P < 0.05$) for the low producing cows compared

to the medium and high groups. Concentrations of acetate, propionate, butyrate, isovalerate, and valerate were lower ($P < 0.05$) in ruminal fluid from the medium and high producing cows. Ammonia concentrations were lower ($P < 0.05$) in the high producing cows. When compared to the ruminal fluid from the low production cows, lactate and protein concentrations were numerically lower and the acetate:propionate ratio was numerically higher in the medium and high production samples. There were no significant differences in protozoal populations between the three production levels with the exception of *Entodinium* numbers being lower ($P < 0.05$) in the medium production group compared to the low production group. While it is unclear what specific factors are responsible for these differences between production groups, our results suggest that cows at the medium and high production levels had higher ruminal pH and lower concentrations of most fermentation end products compared to the low producing cows. These differences may be associated with greater ruminal turnover and(or) absorptive capacity in the medium and high production animals.

Key Words: Rumen, Fermentation, Dairy cattle

725 Dose-response effects of propionate infusion on feeding behavior and plasma metabolites in lactating dairy cows. M. Oba* and M. S. Allen, Michigan State University, East Lansing, MI.

Three experiments were conducted to evaluate dose-response effects of intra-ruminal infusion of propionate on DMI. Infusion treatments were mixtures of sodium propionate and sodium acetate, at ratios of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0, infused into the rumen continuously for 18 h starting 6 h before feeding. Dose-response effects of propionate on DMI and plasma metabolites, and their relationship were summarized. In experiment 1, DMI decreased and plasma glucose concentration (PG) increased linearly as propionate infusion increased. In experiment 2, DMI did not decrease at lower rates of propionate infusion which were associated with greater increases in PG, but DMI decreased at higher rates of propionate infusion associated with a much lower marginal response in PG. Marginal response in DMI (kg/12h) per mmol/min of propionate infusion was negatively related to PG across treatment means for these experiments ($r^2 = 0.26$; $P < 0.01$; marginal DMI response = $13.6 - 0.23 \times \text{PG}$). We speculated that hypophagic effects of propionate are lower when propionate is extensively utilized for gluconeogenesis and greater when the marginal effect of infused propionate on PG decreases, increasing oxidation in the liver. One inconsistency is that cows in early stage of lactation in experiment 3 decreased DMI linearly at lower rates of propionate infusion despite a greater marginal increase in PG. However, plasma concentration of β -hydroxybutyrate was greatly reduced at lower rates of propionate infusion for cows in early stage of lactation in that experiment. At lower rates of propionate infusion, propionate might have stimulated complete oxidation of acetyl CoA in the liver while partially utilized for gluconeogenesis. These observations were consistent with our hypothesis that propionate decreases feed intake in lactating dairy cows by stimulating oxidative metabolism in the liver.

Key Words: Propionate, Oxidative metabolism, Plasma glucose

726 Metabolism of stable isotopically labeled elaidic acid to stearic acid and other *trans* monoenes by ruminal microbes. J. Proell, E. E. Mosley, and T. C. Jenkins*, *Clemson University, Clemson, SC.*

A previous study (Mosley et al. 2002. *J. Lipid Res.* In Print) showed that oleic acid was converted by mixed ruminal microbes to stearic acid and also converted to a multitude of *trans* octadecenoic acid isomers. This study was conducted to trace the metabolism of one of these *trans* C18:1 isomers upon its incubation with mixed ruminal microbes. Unlabelled and labelled (^{13}C -18- *trans*-9 C18:1) elaidic acid were each added to three in vitro batch cultures of mixed ruminal microbes. Samples were taken at 0, 12, 24, and 48 h and analyzed for ^{13}C enrichment in component fatty acids by gas chromatography-mass spectroscopy. Enrichments were corrected for natural ^{13}C abundance in unlabelled cultures and then triplicate enrichments were analyzed by t-test to determine if they differed from zero. At 0 h of incubation, enrichment was 37% ($P < 0.01$) for *trans*-9 C18:1, but not significant for stearic acid or any *trans* C18:1 isomer. By 48 h of incubation, ^{13}C enrichment was 18% ($P < 0.01$) for stearic acid and ranged from 7 to 30% ($P < 0.01$) for all *trans* C18:1 isomers having double bonds between carbons 6 through 16. Two additional cultures were run to determine if movement of the double bond from elaidic acid to other *trans* C18:1 isomers required the presence of the ruminal microbes. One culture contained unlabelled elaidic acid and the other contained labelled (^{13}C -18- *trans*-9 C18:1) elaidic acid, but neither were inoculated with ruminal microbes. After 48 h, ^{13}C enrichment was only detected in the original elaidic acid. This study shows that ruminal microbes transform elaidic acid to stearic acid consistent with the process of biohydrogenation. Unexpectedly, the ^{13}C label from elaidic acid was also found in a multitude of *trans* C18:1 isomers suggesting that ruminal microbes have the capacity to move the *trans* double bond in elaidic acid from carbon 9 to other carbon positions. Because double bond movement only occurred in the presence of ruminal microbes, the movement is more likely an enzymatic process involving one or more microbial isomerases than it is a nonenzymatic process such as double bond migration.

Key Words: Biohydrogenation, Ruminal Microbes, *Trans* monoenes

727 Effects of pH on nutrient digestion and microbial fermentation in a dual flow continuous culture system fed a high concentrate diet. P.W. Cardozo, S. Calsamiglia*, and A. Ferret, *Universitat Autònoma de Barcelona.*

Eight 1325-mL dual flow continuous culture fermenters were used in two replicated periods (9 days) to study the effects of pH on microbial fermentation and nutrient flow when fed a high concentrate diet. All fermenters were fed 95 g/d of a 10 to 90 forage to concentrate diet (18% crude protein, 20% neutral detergent fiber). Treatments were 8 different pH, ranging from 4.9 to 7.0 (in 0.3 unit increases) and were assigned randomly to fermenters within period. Fermenters were maintained at 39°C, with liquid and solid dilution rates at 10 and 5 %/h, respectively, and pH was controlled by infusion of 3 N HCl or 5 N NaOH. Results were analyzed for linear (L), quadratic (Q) and cubic (C) effects ($P < 0.05$). The increase in pH resulted in a L increase in the true digestion of organic matter (OM), neutral detergent fiber and acid detergent fiber, and a L decrease in true digestion of dry matter. Effects were L for total volatile fatty acid (highest at pH 6.1, 129 mM; and lowest at pH 4.9, 100 mM), for the proportion of acetate (highest at pH 7.0, 62%; and lowest at pH 4.9, 36%), propionate (highest at pH 5.5, 51%; and lowest at pH 7.0, 21%) and branched-chain VFA (highest at pH 7.0, 5.9%; and lowest at pH 5.5, 0.3%), and for the acetate to propionate ratio (highest at pH 7.0, 3.0; and lowest at pH 4.9, 0.8). Effects were Q for ammonia N concentration (highest at pH 6.7, 6.7 mg N/dL), and bacterial N flow (highest at pH 7.0, 1.3 g/d). The pH resulted in a C effects for dietary N flow (highest at pH 5.8, 2.3 g/d; and lowest at pH 6.7, 1.6 g/d), crude protein degradation (highest at pH 6.1, 24.1%; and lowest at pH 5.8, 13.4%), and the efficiency of microbial protein synthesis (highest at pH 7.0, 31.0; and lowest at pH 5.8, 18.4 g N/kg OM truly digested, respectively). Results indicated that the fermentation profile of a high concentrate diet was optimized at pH between 6.1 and 7.0, and that most measurements followed a linear response.

Key Words: Microbial fermentation, pH

728 Advancements in the quantification of protozoal nitrogen flow to the duodenum using molecular-based analyses. J. T. Sylvester*¹, S. K. R. Karnati¹, Z. Yu¹, C. J. Newbold², B. A. Dehority¹, M. Morrison¹, and J. L. Firkins, ¹*The Ohio State University, Columbus, OH, USA,* ²*Rowett Research Institute, Bucksburn, Aberdeen, UK.*

Attempts to quantify protozoal protein in duodenal contents have been inaccurate due to a lack of marker specificity. Current microbial markers (i.e., purines) do not separate bacteria from protozoa; therefore, procedures to measure microbial production and intra-ruminal recycling due to protozoal lysis have been limited. The current objectives are to report on progress made towards development of a molecular-based assay using 18S rDNA as a marker for protozoal N quantification. Rumen fluid was isolated from approximately 2.5 kg of rumen digesta. The particulate fraction was washed with anaerobic buffer to enhance protozoal recovery, and the wash was added to the rumen fluid. After incubation at 39°C for 45 min, the flocculent scum layer was aspirated. After fixation in 1% formalin, protozoa were enriched, washed, and isolated using centrifugation and filtration. The extraction method had linear DNA recovery for both formalin-treated and non-treated samples, and genomic DNA was successfully isolated from enriched protozoa, rumen fluid, and duodenal digesta. Ciliate protozoal specific PCR primer sets were designed to amplify a 1.5-kb fragment of the small subunit rRNA gene by conventional PCR methods, which was confirmed by electrophoresis and ethidium bromide staining and via subsequent cloning and sequencing. A second set of primers was designed and verified for real-time PCR (RT-PCR) to amplify an approximate 300-bp fragment standardized against amplified and purified PCR product containing the 1.5-kb fragment from each sample. After comparing predicted rDNA copy numbers generated by RT-PCR with microscopic counts of enriched protozoa and rumen fluid, protozoal flow to the duodenum can be estimated. More work is needed to optimize DNA recovery during purification and account for variability among replicates prior to in vivo comparison to the standard use of purines as a microbial marker.

Key Words: Protozoal N, SSU rRNA, Real-time PCR

729 Effect of medium pH on microbial crude protein yield, pH, and neutral detergent fiber digestion from fermentation of neutral detergent fiber and sucrose in vitro. L. Holtshausen*¹ and M. B. Hall¹, ¹*Dept. of Animal Sciences, University of Florida.*

The effect of medium pH on yield of microbial crude protein (MCP), pH, and NDF digestion in fermentations of NDF and sucrose with mixed ruminal microbes was examined in two 24 h batch culture fermentations in 50 ml tubes fitted with gas release valves. Initial media pH were 6.8 (N) and 5.6 (A) for the control (Goering/Van Soest medium) and citric acid treatment (4.4 ml of 1M citric acid solution/100 ml of Goering/Van Soest medium), respectively. Substrates were isolated bermudagrass neutral detergent fiber (iNDF) and a 50:50 blend of iNDF plus sucrose (SuNDF) (240 mg of substrate/tube). Fermentation tubes for each substrate and medium were destructively sampled at 0, 4, 8, 12, 16, 20 and 24 hours and analyzed for MCP, pH, and residual NDF. MCP yield was estimated as trichloroacetic acid precipitated crude protein (TCACP) corrected for medium and substrate TCACP at 0 h and the mean of fermentation blanks for the specific hour. All values presented are least squares means. Significance was declared at $P < 0.05$. MCP yield from SuNDF and temporal pattern of yield differed between media. Maximum MCP yield was recorded at 12 h for N and 20 h for A. Maximum MCP yield was almost double for N (19.2 mg) compared to A (10.9 mg). NDF digestibility and magnitude of pH change differed between media and between substrates within each medium. At their minima, the difference in pH between iNDF and SuNDF was greater for A (6.07 at 0 h and 5.25 at 8 h, respectively) than for N (6.99 at 0 h and 6.71 at 4 h, respectively). For N, 24 h NDF digestion was greater for SuNDF (42.4%) than for iNDF (26.4%) as substrate. The reverse was true for A, (% NDF digestion for SuNDF: 2.2% and iNDF:7.8%). As compared to N, NDF digestion and MCP yield were decreased in A. Inclusion of sucrose improved NDF digestion at neutral pH, but decreased NDF digestion at acidic pH. We conclude that in vitro media pH affects NDF digestion, MCP yield, and magnitude of pH change with SuNDF substrate, and the presence of sucrose may increase NDF digestion at neutral pH.

Key Words: sucrose, nonfiber carbohydrates, fermentation

730 Enhancing ruminal concentrations of conjugated linoleic acid and trans vaccenic acid. E. S. Kolver*, M. J. de Veth, J. R. Roche, and A. Chand, *Dexcel (formerly Dairying Research Corporation), Hamilton, New Zealand.*

Four continuous culture fermenters were used to test the hypothesis that supplementation of a pasture diet with unsaturated C18 fatty acids would increase ruminal synthesis of conjugated linoleic acid (CLA) and trans vaccenic acid (t11-C18:1; TVA). High quality pasture (control) was fermented and supplemented with either oleic, linoleic, or linolenic acid (3.3% of DM) according to a 4 x 4 Latin square design. Digesta samples were collected during the last 3 d of each of the four 9-d experimental periods. Ruminal concentrations of CLA and TVA in the control treatment were 0.09 mg/g ruminal DM, and 1.75 mg/g ruminal DM, respectively. Linoleic and linolenic acid increased ($P < 0.001$) ruminal concentrations of CLA by 15- and 5-fold, and TVA by 9- and 4-fold, respectively. Oleic acid increased ($P < 0.001$) TVA concentrations 2-fold, but did not change CLA concentrations. The predominant CLA isomers (as a percentage of CLA isomers) were t9,t11-CLA (39%; control), t9,t11-CLA and c9,t11-CLA (38% and 38%, respectively; oleic treatment), c9,t11-CLA (67%; linoleic treatment), and c9,c11-CLA (44%; linolenic treatment). Low ruminal concentrations of t10,c12-CLA were observed in the control (0.007 mg/g ruminal DM), with increased ($P < 0.05$) concentrations only observed in the linoleic treatment (0.155 mg/g ruminal DM). Treatments did not differ in true DM digestibility and microbial synthesis, and fiber digestibility in supplemented treatments was the same or higher ($P < 0.05$) than the control. These results can be used to predict the ruminal CLA and TVA response to lipid feed supplements. In addition, a new pathway is proposed that yields CLA intermediates from the biohydrogenation of linolenic acid. Linolenic acid is the predominant fatty acid in pasture. These results suggest that the production of CLA and TVA from linolenic acid may be the reason why high concentrations of milkfat CLA have been reported for cows fed pasture.

Key Words: CLA, Ruminal, Pasture

731 Nutrient yields from in vitro fermentations of sucrose and neutral detergent fiber by mixed ruminal microorganisms. M. B. Hall*¹ and P. J. Weimer², ¹*Dept. of Animal Sciences, University of Florida*, ²*U.S. Dairy Forage Res. Ctr., Agricultural Research Service, USDA, Madison, WI.*

The effect of level of sucrose on nutrient yield by mixed ruminal microbes was evaluated in vitro in two 24 h fermentations in sealed vials. Isolated bermudagrass (*Cynodon dactylon*) NDF (130 mg) were incubated with sucrose (Suc) (65, 130, or 195 mg) in Goering/Van Soest buffer with 15% ruminal inoculum (total volume 32 ml/vial). Vials for each level of sucrose were destructively sampled at 0, 4, 8, 12, 16, 20, and 24 h and analyzed for microbial crude protein (MCP), dextran, organic acids, and residual sucrose, glucose (Glc), and fructose (Fru). To estimate MCP, vial contents were precipitated at a concentration of 20% trichloroacetic acid (TCA), and filtered to collect unfermented NDF and precipitate. Collected residues were analyzed for crude protein (CP) as combustion N x 6.25. MCP was estimated as TCA-precipitated CP corrected for the TCA-precipitated CP content of substrates at 0 h, and the mean of fermentation blanks from each hour. Significance was declared at $P < 0.05$. Least squares mean values are reported. MCP yield increased linearly with increasing Suc, but MCP mg/Suc mg declined linearly. MCP yield peaked at the 8 h sampling, and exhibited a slow decline through 24 h. No Suc was detected in the media at 0 h. Fru declined to undetectable levels by 4 h. Glc exhibited a similar decline, but was detected through 4 or 8 h. Dextran yield increased linearly with increasing Suc and peaked at 4 h. The proportion of Suc converted to dextran decreased with increasing Suc. pH at 24 h declined linearly with increasing Suc (6.28, 6.14, and 5.94, respectively). Organic acids increased rapidly to 4 h then linearly but more slowly through 24 h. Organic acid yield at 24 h increased linearly with Suc. At 24 h, molar percentages of acetate decreased linearly, propionate increased quadratically, and butyrate increased linearly with increasing Suc. Lactate was transiently observed at 4 h. Lactate yield increased quadratically with increasing Suc. Total nutrient yield mg (MCP+dextran+organic acids)/Suc mg at 24 h decreased linearly as Suc increased (1.17, 0.89, and 0.79, respectively). Thus, increasing Suc supplementation increased nutrient yield,

decreased yield of nutrients/Suc mg, and altered the profile of organic acids produced.

Key Words: sucrose, nonfiber carbohydrates, fermentation

732 Effects of mild heat challenge on rate of passage and rumen fermentation in lactating dairy cattle. K. M. Spurlin*, J. Porter, M. Ellersieck, and J. N. Spain, *University of Missouri - Columbia.*

An environmental chamber study was conducted to evaluate passage rate and rumen fermentation of cows exposed to various levels of heat challenge. Six fistulated, lactating cows were used to compare 4 cooling strategies. Treatments were 24 h (24H), 12 h nighttime (12N; fans on between 1900 - 0700), 12 h daytime (12D; fans on between 0700 - 1900), and no fan cooling (NO). Treatments were administered during consecutive 14 d periods arranged as a 4x4 Latin Square. Periods were comprised of a 6 d thermoneutral (TN; constant 20C) period, 3 d step-up, and a 5 d heat challenge (HC). During HC, maximum ambient temperature was held at 33C from 1400 - 1800 and lowered to 23C from 0200 - 0600. Feeding occurred at 0600, 1400, and 2200 h daily with refusals and water consumption measured prior to each subsequent feeding. At the morning feeding on days 4 and 12 of each period, 110 g of Cr-mordanted alfalfa hay, 200 g of Yb-labeled soyhulls, and 200 ml of Co-EDTA were dosed into the rumen of each cow. Samples of whole rumen contents were collected at 0 (immediately before dosing), 4, 7, 10, 13, 16, 19, 22, 28, 32, 38, and 54 h post-dosing. Fluid was analyzed for pH and concentrations of ammonia, VFA, and Co. Solid contents were analyzed for Cr and Yb by atomic absorption spectroscopy. Cooling treatments were not different, thus comparisons are between TN and HC. Hay, soyhull, and liquid passage rate were 4.9, 7.4, and 11.6%/h during TN, and were 4.1, 6.7, and 11.1%/h during HC, respectively. The rate of passage for hay and soyhulls declined 16.8 and 10.2% ($P < 0.05$), respectively, during HC. Particle size influenced both absolute passage rate and the reduction due to HC. Dry matter intake was not different on day of dosing indicating heat stress directly reduced passage rate, which may contribute to gut fill and a subsequent reduction of DMI. Rumen pH reflected an acute response to HC compared to TN with the initial difference influenced by changes in intake patterns. This was followed by a chronic decline in rumen pH. Both acute and chronic responses were noted for ammonia and VFA concentrations with ammonia levels increased during chronic HC. Rumen function is altered during HC by means related to, but not solely controlled by changes in DMI.

Key Words: Heat stress, Passage rate, Fermentation

733 Characterizing volatile fatty acids and other gases in a rumen closed in vitro fermentation system. Jarett Spinhirne¹, Jacek Koziel*¹, and Norbert Chirase^{1,2}, ¹*Texas Agricultural Experiment Station, Texas A&M University*, ²*West Texas A&M University.*

Fermentation characteristics of feedstuffs in vitro reflect their metabolism in the rumen and more importantly, the kinetics of that metabolism. Qualitative and quantitative characterization of these volatile organic products in ruminal fluids involves rigorous liquid sampling and sample preparation procedures. A new method for the rapid sampling and characterization of the headspace gases of closed in vitro cultures using solid phase microextraction (SPME) was evaluated for ruminal fluid and ruminal fluid with feed containing a new feed additive. One min samples were collected every h for 27 h using a DVB/Carboxen/PDMS 50/30 SPME fibers followed by immediate analysis on a GC-MS. Trends of quantities of specific detected gases were plotted and compared for ruminal fluid and rumen fluid with additive cultures over the time of the experiment. Acetic, propionic, butyric, isobutyric, isovaleric, and valeric acids were detected in the headspace. This finding is consistent with current knowledge because low molecular weight volatile fatty acids (VFAs) have been used to determine the energetic efficiency of microbial fermentation in the ruminant. In addition, several other new compounds including hexanoic acid, toluene, dimethyl disulfide, nonanal, octanal, and pentadecane were also identified. Gas quantities above ruminal fluid with feed were always greater than those of ruminal fluid alone except for samples obtained 1 to 2 h post incubation. SPME technology facilitated rapid sampling and immediate analysis to identify specific end products of microbial digestion in the headspace. These end products are frequently used for predicting diet quality, effectiveness of feed additives in selecting against specific

rumen microbes and for manipulation of diets to reduce the production of odorous compounds. SPME-based approach could serve as a novel technique for the development of an alternative method for characterization of ruminal fermentation end products, and effects of important variables on their kinetics. Characterization of rumen gases that were not detectable or previously not of interest may open new approaches and applications related to animal (and perhaps in the future human) metabolism.

Key Words: Detection, Ruminal gases, Gas chromatography - mass spectrometry

734 Inhibition of methanogenesis in Methanobrevibacter (Mbr.) smithii cultures and ruminal cultures by p-aminobenzoate (pABA) analogs. B. A. DeMontigny*, R. Dumitru, S. Schroeder, H. Palencia, S. W. Ragsdale, J. M. Takacs, and J. L. Miner, ¹University of Nebraska-Lincoln.

Ruminal methane production causes a loss of 2 to 12% of feed gross energy during digestion. Methane is a greenhouse gas and livestock account for 17% of total methane emissions. Inhibition of ruminal methanogenesis could both increase feed efficiency and mitigate global warming. Methanogenic archaea use a unique pathway to synthesize methane. The enzyme, 4-(β-D-ribofuranosyl) aminobenzene 5'-phosphate synthetase is a promising target for inhibition. A natural substrate of this enzyme is pABA. We hypothesize that analogs of pABA could inhibit methane production in mixed rumen cultures and pure cultures of *Mbr. smithii*. Methanogenesis assays were conducted in 30-hour, 4-ml anaerobic incubations of bovine rumen fluid in buffer containing trypticase, cellobiose and trace nutrients, with 5.4 ml of headspace pressurized (190 kPa) with H₂/CO₂ (80:20). Headspace methane was quantified by gas chromatography (GC) using a silica gel column, thermal conductivity detection (TCD), and adjustment for headspace pressure, and VFA analyses were completed by GC. Three pABA analogs were included in the cultures from 0 mM to 10 mM. Five mM 4-ethylamino-benzoate (EB), 9 mM 4-isopropylamino-benzoate (IB), and 6mM 4-(2-hydroxy-ethylamino)-benzoate (HEB) each completely inhibited methane production. Eight mM IB enhanced (P < 0.02) total VFA production but EB and HEB did not. To determine if these pABA analogs inhibit growth of methanogenic archae as well as inhibit methane synthesis, we grew pure cultures of *Mbr. smithii*, media included EB, IB, or HEB at 0, 1 and 10mM. Absorbance was measured two times daily for one week via spectrophotometry. After 117 hours, headspace samples were analyzed for methane content. At

10mM, all three compounds completely (P < 0.01) inhibited methane production and cell growth. One mM EB and IB each delayed (P < 0.01) the growth curve of *Mbr. smithii* >20hours. We conclude that these pABA analogs inhibit methane production as well as the growth of methanogenic archaea.

Key Words: *Mbr. smithii*, rumen, methane

735 Modeling starch digestion in the rumen: a comparative approach. A. Offner*¹, A. Bach², and D. Sauvant¹, ¹INA P-G INRA, Paris, France, ²Agribbrands, Barcelona, Spain.

Digestibility of starch in the rumen (dR, %) is highly variable. This study was conducted to evaluate and compare three different rumen models (Lescoat and Sauvant LE, 1995; Molly MO, 1999; CNCPS CO, 2001) on their ability to predict the partitioning of starch digestion. To perform this study, an independent dataset with complete data on animal characteristics, rations and starch digestibility was built: 31 references (110 treatments) on dairy cows were pooled. Starch content in the experimental diets ranged from 12.1 to 54.7 % of DM (average starch = 30.1 ± 8.6) and the observed dR ranged from 27.2 to 96.7 % (dR = 65.2 ± 16.2). One single library with complete feed model inputs was created for the 43 feedstuffs used in the references. Thus, the comparative simulations were based on identical inputs. The results were evaluated and compared among models and with observed values. Three parameters were used to estimate the accuracy of dR predictions: the coefficient of determination (R²), the residual standard deviation (rsd, %) and the slope (b). The values were respectively of 0.26, 13.9 % and 0.98 for CO and 0.22, 14.3 % and 0.59 for LE. The regression with MO was not significant. MO tended to underestimate dR (dR_{MO}, % = 62.3 ± 9.7) whereas starch digestibility was rather overpredicted in CO and LE. Statistical analyses were also conducted within references, the parameter values were then of 0.78, 8.8 % and 1.02 for MO, 0.89, 6.1 % and 1.24 for CO and 0.90, 6.0 % and 0.71 for LE. Generally, variations in starch digestibility were poorly predicted by MO but were predicted satisfactorily in LE and CO. LE, which is based on *in situ* values, appeared to be the most accurate model, especially when the experiments with large normalized residuals were removed. This result supports our current work dealing with the development of another model of ruminal starch digestion based on *in situ* data and adjusted with *in vivo* observations.

Key Words: Rumen, Modeling, Starch digestion

Ruminant Nutrition Transition Cow

736 Metabolic nutrients for transition dairy cows. D. P. Casper*¹, G. Wernet², and G. B. Ayangbile, ¹Agri-King, Inc., Fulton, IL, ²Purdue University, West Lafayette, IN.

Deficiencies in critical nutrients required for metabolic pathways can lead to metabolic complications during the transition period. The high nutrient requirements for the initiation of lactation, in combination with a reduction in dry matter intake prior to calving, could lead to several possible nutrients becoming deficient. These nutrients were formulated into a pack (PK) consisting of niacin, methionine, rumen protected choline, and several B-vitamins along with yucca. This PK was evaluated during the transition period (3 wk prior and 4 wk after calving) using 26 dry pregnant Holstein cows randomly assigned to one of two treatments. All cows were fed a TMR, prior to calving, consisting of (DM basis) 22.9% corn silage, 9.8% haylage, 18.1% alfalfa hay, 14.9% high moisture corn and 34.3% protein supplement, minerals, and vitamins. After calving, a TMR was fed consisting of 9.8% corn silage, 16.6% haylage, 8.8% alfalfa hay, 29.8% high moisture corn and 35.0% protein supplement, minerals, and vitamins. The treatments were the control (C) TMR and TMR containing the PK at .11 kg/hd/d. During the Pre-Calving period, BW (681.2 and 718.1 kg), BW gain (10.0 and 12.7 kg), dry matter intake (10.1 and 10.5 kg/d), and concentrations of blood glucose (47.7 and 44.3 mg/dl) and non-esterified fatty acids (.53 and .69 meq/l) were similar (P>.10) for cows fed C and PK, respectively. During the Post-Calving period, BW (575.1 and 592.1 kg), BW loss (-42.9 and -27.4 kg), and dry matter intake (14.6 and 13.3 kg/d) were similar (P>.10) for cows fed both C and PK. Yields of milk (

29.2 and 27.4 kg/d), 4% FCM (32.2 and 29.0 kg/d) and solids-corrected milk (27.3 and 24.3 kg/d) were similar (P>.10) for cows fed both C and PK. Percentages of fat (4.71 and 4.62%), protein (2.91 and 2.74%), lactose (4.61 and 4.54%), and total solids (13.13 and 12.80%) were similar (P>.10) for cows fed C and PK. Concentrations of blood glucose (36.7 and 30.2 mg/dl) and non-esterified fatty acids (.51 and .75 meq/l) were similar (P>.11) for cows fed C and PK. Under the conditions of this study it was demonstrated additional nutrients supplied by the PK were not required for optimal performance during the transition period.

Key Words: Choline, Nutrients, Transition cows

737 Effect of timing of sample collection and sample handling on urine pH of close-up dry Holstein dairy cows. P. W. Jardon*, West Central Soy.

The objective of this study was to determine the effect of sample handling on urine pH of close-up dairy cows. Samples were collected from cows (n=31) in the close-up pen on a Wisconsin dairy at 0, 4, 8, and 16 hrs post-feeding for 2 days. Cows were fed a diet balanced for dietary cation-anion difference (DCAD = (Na + K - Cl - S) = -80 meq/kg). All cows had been on the close-up ration for at least 7 days. The TMR was fed once a day. The first few ml of urine were collected separately from the main stream. The pH was measured within one h of collection. The main stream samples from the 0 hr collection on the first day were then split and stored either at 20°C or at 5°C.