T229 Measurements of net portal flux of nitrogen (N) compounds in ruminants: First step of a meta-analysis. R. Martineau*[1], I. Ortigues-Marty[2], J. Vernet[2], and H. Lapierre[1], [1]*Agriculture and Agri-Food Canada*, *Sherbrooke, Quebec, Canada*, [2]*Institut National de la Recherche Agronomique*, *Theix, St Genès Champanelle, France*.

A first step in developing mechanistic models of nutrient use by ruminants is to accurately predict net portal absorption (NPA) of nutrients in relation with feed intake and composition. From the FLORA database (FLux of Nutrients through the Organs of Ruminant Animals; Reprod. Nutr. Dev. 46:527-546), all the studies simultaneously reporting apparently digested N and NPA of NH_3, urea, and AA were selected (n = 33 publications; 40 cattle and 71 sheep treatments). It was hypothesized that differences between inputs (digested N + urea-N uptake) and outputs (NH_3-N + total AA-N) across the portal-drained viscera, called NPA residuals, should average 0 assuming that unaccounted N (e.g. salivary N, peptides, nucleic acids) sums to 0. The NPA residuals [g N/(d•kg BW)] were different from 0 at the 95% confidence level (CL). Single corrections were applied to standardize the reported observations: NPA of urea-N and NH_3-N on plasma were corrected to a blood basis (hematocrit; 25%: sheep; 27%: cattle); NPA of NH_3-N measured with the Berthelot reaction were corrected to a glutamate dehydrogenase (GDH) basis (× 1.38); and finally, NPA of α-amino-N were corrected to a total AA-N basis (× 1.35). With all corrections applied, NPA residuals were not different from 0 but variability did not improve. These results suggest that before performing a meta-analysis on reported NPA, some corrections would need to be applied to better reflect the biology and chemistry of the nutrients absorbed.

Table 1.

NPA residuals (n = 111), g N/(d•kg BW)	Mean	SD	Min	Max	95% CL
No corrections	0.092	0.118	–0.31	0.44	± 0.022
With all corrections	–0.023	0.132	–0.39	0.33	± 0.025
When single corrections are made:					
- urea-N (blood basis; 22 corrections)	0.099	0.121	–0.31	0.52	± 0.023
- NH_3-N (blood basis; 21 corrections)	0.073	0.135	–0.34	0.39	± 0.025
- NH_3-N (GDH basis; 99 corrections)	0.036	0.120	–0.39	0.38	± 0.023
- AA-N (total AA-N basis; 81 corrections)	0.047	0.116	–0.31	0.38	± 0.022

Key Words: Portal, Nitrogen, Ruminant

T230 Diversity of rumen bacteria as revealed by multivariate analysis of 16S rDNA gene sequences. K. L. Liu[1], J. Q. Wang*[1], D. P. Bu[1], S. G. Zhao[1,2], H. Y. Wei[1], and L. Y. Zhou[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Gansu Agricultural University*, *Gansu, China*.

To improve our understanding of the composition of rumen microbial community and factors may affect its composition, 2023 16S rDNA sequences with length >1200 bp were retrieved from Ribosomal Database Project II and then subject to multivariate analysis. Using 97% minimum similarity as the threshold for any pair of sequences in a operational taxonomic unit (OTU), a total of 915 bacterial OTU were identified, among them 90% represented sequences from yet uncultured species. The terminal Chaol richness estimates suggested that the rumen microbial community could consist of 1720 species. Land distribution analysis by fLAND software indicated that *Firmicutes* and *Bacteroides* were the dominant groups in rumen, accounting 91% of the total taxa. *Cytophaga-Flavobacterium- Bacteroides* (CFB) bacterium from rumen appeared to be the farthest away from the common ancestor of the division, suggesting a strong host selection pressure and co-evolution. To investigate factors may affect the composition of rumen microbial community, six 16S rDNA libraries which totaled 905 sequences were collected from database. Principal component analysis (PCA) and hierarchical clustering were performed by UniFrac. On PCA plots, libraries from cows clustered together and separated clearly with that from castrated cattle, indicating host sex as the most important factor shaping the composition of rumen bacterial community. In addition, dietary forage: concentrate ratio was identified as the second important factor.
Acknowledgement: Research funded by Ministry of Science and Technology (2006BAD04A08).

Key Words: Rumen Microbial Community, 16S rDNA Gene, Multivariate Analysis

T231 Screening of ureases from a bovine rumen metagenomic library. K. L. Liu[1], J. Q. Wang*[1], D. P. Bu[1], S. G. Zhao[1,2], Y. X. Zhu[3], H. Y. Wei[1], L. Y. Zhou[1], and Z. Y. Dong[3], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Gansu Agricultural University*, *Gansu, China*, [3]*State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences*, *Beijing, China*.

Lack of sufficient protein forage has promoted the utilization of urea as a protein supplement in ruminant forage, which could be hydrolyzed by urease secreted by ureolytic bacterium in rumen. To get more information about rumen urease gene sequence, gene cluster structure, protein structure and function, rumen contents of a Holstein cow were collected and used as raw materials to construct a metagenomic library. A series of ways to clean bacterium cells and make plugs were developed, and high molecular weight DNA fragments with the size in excess of 2 Mb were finally extracted. After digestion with *Hind* III and separated by pulse field gel electrophoresis, DNA fragment ranged from 50-100 kb were collected and ligated to pCC1BAC vector to construct the library. The metagenomic library consisted of 153, 600 clones with the total capacity estimated to be 857 Mb. To screen ureolytic active clone from the library, a urease screen agar that take advantage of the visible phenotype of urease activity was employed. Eight clones were identified as ureolytic positive. Restrictive digestion analysis and enzymatic analysis suggested a high level of diversity existed among these clones. Further study addressing urease gene sequence, gene cluster structure, protein structure and function characterization is currently on the way.
Acknowledgement: Research funded by Ministry of Science and Technology (2006BAD12B03).

Key Words: Metagenomic, Rumen BAC Library, Urease Screening

T232 Effect of fatty acids and malic acid on total gas production and methane release by batch culture. L. Liu, J. Q. Wang*, D. P. Bu, S. J. Liu, K. L. Liu, H. Y. Wei, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

The objective of this study was to determine the effect of different concentrations of malic acid (0, 5, 10 mM) and degree of unsaturation of the fatty acids (stearic acid-SA; oleic acid-OA; linoleic acid-LA; linolenic acid-LNA) on total gas production and methane release using an automated cumulative gas production estimation system *in vitro*. The substrate (500 mg) contained alfalfa hay and corn (50:50). Rumen fluid was collected from two ruminally fisulated lactating cows. Substrate was incubated in 60 mL fermentation fluid (rumen fluid to McDougall buffer ratio was 1:2). Individual fatty acids were dissolved in 0.5 mL ethanol and added in fermentation fluid at 3.5% of diet DM. Each treatment contained four flasks. Incubations were carried out in batch cultures at 39°C for 36 h two times at a two-week intervals. The gas samples were collected by gas collecting bags and then methane content determined with a gas chromatograph. The results showed that total gas production and methane release decreased ($P < 0.01$) when the degree of unsaturation of the fatty acids increased. In contrast, total gas production increased ($P < 0.01$), whereas methane release decreased ($P < 0.01$) with malic acid level increment. The interaction of fatty acids and malic acid were characterized by decreased ($P < 0.01$) methane release. Results suggested that addition of unsaturated fatty acids and malic acid could inhibit methane release, and fatty acids-mediated depression in methane release was associated with the degree of unsaturation of the fatty acids.

Acknowledgement; Research supported by Ministry of Science and Technology (2006BAD12B03).

Table1: Effect of fatty acids and malic acid on total gas production and methane release.

		Tgas,mL	CH_4,mmol
	SA	77.86[a]	0.41[a]
FA	OA	70.74[b]	0.37[b]
	LA	53.33[c]	0.23[c]
	LNA	50.31[c]	0.22[c]
	O	61.53[b]	0.42[a]
MA(mM)	5	64.36[b]	0.34[b]
	10	70.22[a]	0.24[a]
	SEM	1.86	0.005
P	FA	<0.001	<0.001
	MA	0.006	<0.001
	FA×MA	0.163	<0.001

Key Words: Malic Acid, Methane Release, Total Gas Production

T233 The use of simultaneous models for estimate in vivo nutrient digestibility of alfalfa hay and barley grain. H. Jahani-Azizabadi, M. Danesh Mesgaran*, R. Valizadeh, and H. Nasirimoghadam, *Ferdowsi University of Mashhad*, *Mashhad, Iran*.

In vivo total tract nutrients digestibility [Dry matter (DM), Crude protein (CP), Organic matter (OM) and Digestible organic matter in dry matter (DOMD)] from alfalfa hay and barley grain were evaluated using a direct calculation (difference method) or simultaneous models. Eight Balochi lambs (49.5 ± 3.5 kg, body weight) were fed experimental diets made of four alfalfa hay: barley grain ratio (DM basis) as 1.0:0.0, 0.75:0.25, 0.50: 0.50 and 0.25: 0.75 in a 4×4 repeated Latin squares design. Each period consisted of 28 d with 7 d for feces collection. Nutrient concentrations of feed samples and feces were determined using standard procedures. For direct calculation, the data of first and third diets were used. Simultaneous models were provided using Polynomial regression models of SAS. Apparent DM, CP, OM and OMD digestibility of alfalfa hay and barley grain calculated by direct calculation were 0.61& 0.78, 0.74 & 0.69, 0.64 & 0.80 and 0.59 & 0.79, respectively. When simultaneous models were considered, a linear equation was significant ($P < 0.01$) for all nutrients. Therefore, the nutrient digestibilities of the feed samples were determined using this model (Table 1). Results of the present study might suggest that the simultaneous models appeared to give reasonable estimate rather than direct calculation.

Table 1. Simultaneous models and mean nutrient digestion coefficients of barley grain and alfalfa hay

			digestion coefficient	
Nutrient	Simultaneous models	R2	Barley grain	Alfalfa hay3
DM	Y=61.204(±0.66) +0.183(±0.04) X	0.88	79.5	61.2
CP	Y=73.99(±0.66) + 0.003(±0.04) X	0.45	74.2	74
OM	Y=64.615(±0.63) + 0.177(±0.041)X	0.87	82.3	64.6
DOMD	Y= 59.45(±0.60) + 0.191(±0.037)X	0.91	78.5	59.4

1-Y= Nutrient digestion coefficient,2- X= Barley level in diets (%),3- When X= 0.0

Key Words: Simultaneous Models, Digestibility, Barley Grain

T234 In situ ruminal degradability of soybean and sunflower by-products. R. H. de T. Buschinelli de Goes*[1], R. de C. M. Tramontini[2], G. D. de Almeida[2], S. T. Cardim[2], J. Ribeiro[2], L. A. de Oliveira[2], and F. Morotti[2], [1]*Universidade Federal da Grande Dourados*, *Dourados, MS, Brazil*, [2]*Universidade Estadual de Maringá*, *Umuarama, PR, Brazil*.

The ruminal degradation of the dry matter (DM) and crude protein (CP) of the sunflower and soybean crushed, originating from of the cold compressing for the extraction of vegetable oil, was evaluated by the in situ technique, using three rumen fistulated zebu steers at pasture. The foods were grounded though 2mm screen and incubated directly in the rumen in nylon bags, in the times of 72, 48, 36, 24, 18, 12, 06, 03, and 0 hour. The potential degradation (PD) for the disappearance of DM, in the different incubation times were adjusted by a no-linear regression by Gauss-Newton's method, according to the equation PD = A+B*(1-exp-ct), being PD = potential degradabilility, A = soluble fraction, B = potentially degradable fraction, c = degradation rate of the fraction B, and t = time of incubation. The effective degradability was calculated by the formula: ED=a+(b.c)/(c+k), where k = rate of passage of 5%/h. The sunflower crushed contents a dry matter (DM) of 90.0% and crude protein (CP) of 22.0%, the soybean crushed presented 90.5% DM and 53.0% CP. The potentially degradable fraction of DM by the sunflower crushed was of 40.2% and 26.2% for to DM and CP, what provided medium ruminal degradation. The effective degradability for

the sunflower crushed was of 51.0% and 38.7%, with a soluble fraction of 22.4% and 29.9%, for to DM and CP. The soybean crushed presented soluble fraction of 36.6% and 20.7%, for to DM and CP, with a effective degradability of 72.5% and 69.9%, with a potentially degradable fraction of 66.2% and 81.5%, for DM and CP respectively. The sunflower crushed presented low values for the degradation rate of the fraction "b" of 2.5%/h for CP due to the high oil tenor (10%) and the high value of ADF (20.8%) that might have interfered in the degradation of this food, while the soybean crushed presented medium values of 7.5%/h for the degradation rate of CP. The ruminal degradation of the nutrients by the studied foods presented low soluble contents for the sunflower crushed different from the soybean crushed that presented extensive degradations rates for the insoluble fraction, that the sunflower crushed.

Key Words: Chemical Composition, Sunflower Crushed, In situ

T235 Rumen phosphorus metabolism in sheep. R. S. Dias[1], T. Soares[2], R. M. P. Pardo[3], J. C. Silva Filho[3], D. M. S. S. Vitti[2], E. Kebreab*[4], and J. France[1], [1]*University of Guelph*, *Guelph, Ontario, Canada*, [2]*Centro de Energia Nuclear na Agricultura*, *Piracicaba, Sao Paulo, Brazil*, [3]*Federal University of Lavras*, *Lavras, Minas Gerais, Brazil*, [4]*University of Manitoba*, *Winnipeg, Manitoba, Canada*.

The objective of this work was to study the effect of different levels of P on its presence in saliva and the rumen. Moreover, other factors such as specific activity of P in rumen, saliva and plasma were assessed to provide information on P metabolism in ruminants. Twelve Santa Inês male sheep, weighting 30 kg were fed a basal diet comprising roughage, concentrate mixture (cassava flour, soya bean meal, and urea) and a mineral mixture. The treatments consisted of the basal diet supplemented with different amounts of monoammonium phosphate to provide 0, 2, 4 and 6 g P/animal/d, representing treatments T0, T2, T4 and T6, respectively. The % P in dry matter was considered highly deficient, deficient, adequate and excessive respectively. Animals were injected with ^{32}P and thereafter samples of blood were collected over 7 d, and rumen fluid and saliva samples were collected 96 and 144 h after injection. Phosphorus intake affected P in ruminal fluid, whereas P in saliva was not affected. The values for ruminal P turnover and endogenous entry calculated from P measurements were all affected by P intake. The percentage of endogenous P entering the rumen decreased with increased levels of P intake indicating lower endogenous P secretion for higher dietary P. The specific activity of P in saliva, rumen fluid and plasma were also all affected by P intake, and were statistically different between treatments, though they were numerically similar when compared with each other. The possibility of presence of an endogenous ruminal P source besides saliva is discussed (Smith et al. 1955). It is concluded from the results that P in ruminal fluid is maintained at adequate levels to attend the needs of micro-organism regardless of P intake.

Smith, A. H., M. Kleiber, A. L. Black and C. F. Baxter. 1955. Transfer of phosphate in the digestive tract. II. Sheep. J. Nutr. 57:507-527.

Key Words: Rumen, Phosphorus, Metabolism

T236 Evaluation of modeling procedure for fitting in situ degradation profiles. G. I. Zanton* and A. J. Heinrichs, *The Pennsylvania State University*, *University Park*.

In situ degradation of feeds is a common methodology used for describing the digestion rate and potential for ruminant feedstuffs. When the population estimates of parameters of nonlinear equations are required, nonlinear mixed modeling (**NLMM**) of in situ data may be more appropriate than a two-stage approach [**TS**; in which individual kinetic parameter estimates (**PE**) are obtained in the first stage and population estimates are obtained in the second by averaging], since NLMM allows information in common across subjects to be shared during estimation. Therefore, the objective of this experiment was to evaluate the accuracy and precision of PE determined by TS and NLMM using simulation techniques. Four in situ profiles were represented: long lag with degradation rates of 0.05 and 0.02/h and moderate lag with degradation rates of 0.12 and 0.05/h. For all analyses, a minimum of 3200 degradation profiles were simulated with time points representing 0,1,2,4,8,16,24,48,72, and 96 h of ruminal incubation. Parameters were simulated with a systematic source of variation associated with animal and period effects and normally distributed, random residual error. Under these conditions, acceptable levels of bias and precision were found for some models evaluated. Bias of PE of the models examined was different from 0 (P<0.05) for more parameters (2.23X) with TS than NLMM, whereas PE were determined with lower precision for TS than NLMM 3.25X more. Study of the lagged, exponential model revealed that PE bias for TS were more sensitive to enhanced residual variation, level of replication, and sampling schedule than NLMM. When residual variation was low, precision of PE was not substantially affected by estimation procedure; when residual variation was high, precision of PE was generally improved by NLMM (>3X, maximally). Averaging skewed individual animal PE, resulted in considerably biased estimates of population parameters. From the results of these simulations it is concluded that, in most cases, NLMM is more appropriate than TS for producing unbiased, precise PE.

Key Words: In situ, Nonlinear Mixed Modeling, Ruminant

T237 Assessment of free amino acid supplementation on rumen microbial efficiency and nitrogen metabolism using a continuous culture system. M. A. Brooks*, J. H. Porter, and M. S. Kerley, *University of Missouri*, *Columbia*.

This purpose of this experiment was to study microbial efficiency (MOEFF) and nitrogen (N) metabolism in the rumen environment when differing levels of free crystalline amino acids were added to the diet. A continuous culture system with a fractional dilution rate 0.06 was set up to accommodate 6 treatments with 4 fermenters per treatment. Treatments contained a ground corn, soybean meal, and soybean hull basal diet with additional levels of an equal mix of L-Lys, L-Arg, and DL-Met added to the diet at varying levels (0%, 3%, 6%, 12%, 18%, and 6% blood meal as a positive control). After a 4 day acclimation, ammonia and pH of the fermenters were analyzed 1 h before feeding and 4 h after feeding for 3 days. At the end of study, N and organic matter (OM) were analyzed for the diets, bacteria, and effluent and MOEFF calculated. Ammonia concentrations rose as the free AA supplementation in the diets increased (P < 0.001). MOEFF was increased by protein supplementation (P < 0.001) compared to the free AA diets, and MOEFF responded quadratically (P < 0.05) to free AA supplementation. We concluded that peptide N was needed to maximize MOEFF. Free AA supplementation in the diet does not appear to supply peptides required for maximizing MOEFF. Our data agreed with previously published data that concluded AA degradation rate was more rapid than AA outflow

at feasible feeding levels. OM digestion was greatest for the 3% AA treatment ($P < 0.05$). The culture pH, measured 4 h after feeding was different among treatments ($P < 0.001$); however, the ranged within only 0.1 pH unit. Diet formulations will continue to move in the direction of formulating for AA requirements. In this process, provisions for the form of microbial N requirement must be met.

Key Words: Microbial Efficiency, Amino Acids, Rumen Fermentation

T238 Effect of pH on rumen fermentation and biohydrogenation of extruded soybean and linseed fatty acids in continuous culture. M. C. Fuentes*[1], S. Calsamiglia[1], V. Fievez[2], and M. Blanch[1], [1]*UAB*, *Bellaterra, Spain*, [2]*Ghent University*, *Belgium*.

Eight dual-flow continuous culture fermenters (1320 mL) were used in two replicated periods of 7 d (4 d adaptation plus 3 d sampling) to study the effects of pH (6.4 vs. 5.6) and diet (Soybean (SB) vs. Linseed (LS)) on rumen microbial fermentation, lipolysis and biohydrogenation, and DNA concentration of bacteria involved in lipolysis and biohydrogenation processes. Diets had a 40:60 forage to concentrate ratio (17.3 % CP, 29.2 % NDF) and similar ingredient composition, differing only in the fat supplement, with SB having extruded soybean (5.4 % DM) and LS having extruded linseed (6.2 % DM). Low pH reduced OM and NDF digestibility, ammonia N concentration and flow, bacterial N flow and CP degradation, and increased non-ammonia and dietary N flow. Low pH reduced linoleic (LA) and linolenic acid (LNA) apparent biohydrogenation (LA = 0.44 vs. 0.82; LNA = 0.54 vs. 0.84) and lipolysis (LA = 0.92 vs. 0.99; LNA = 0.94 vs. 0.99). Low pH reduced C18:0, t11-C18:1, c9,t11-conjugated linoleic acid (CLA), and increased t10-C18:1, C18:2, C18:3, t11,c15-C18:2 and t10,c12-CLA proportions in the effluent. Low pH reduced *Anaerovibrio lipolytica* (1.85 vs. 67.0 pg / 10 ng total DNA) and *Butyrivibrio* VA subgroup (315.5 vs. 1148.5 pg / 10 ng total DNA) and increased *Butyrivibrio* SA subgroup DNA concentrations (1907.0 vs. 1215.5 pg / 10 ng total DNA). Linseed diet increased ammonia N concentration and flow and tended (P = 0.06) to increase CP degradation. Lipolysis of LA and LNA was higher in LS compared with SB diet (LA = 0.98 vs. 0.92; LNA = 0.98 vs. 0.94). Linseed reduced C18:2 and t10,c12-CLA, and increased C18:3 and t11,c15-C18:2 proportions in the effluent. Concentration of *A. lipolytica* DNA was higher (43.4 vs. 25.6 pg / 10 ng total DNA) and that of *Butyrivibrio* VA subgroup was lower in LS compared with SB diet (652.5 vs. 811.5 pg / 10 ng total DNA). Low pH inhibited lipolysis and biohydrogenation. Extrusion of soybean seems to protect fat against lipolysis more than extrusion of linseed.

Key Words: pH, Lipolysis, Biohydrogenation

T239 Effect of pH and level of concentrate in the diet on biohydrogenation intermediates in a dual flow continuous culture. M. C. Fuentes*, S. Calsamiglia, and P. W. Cardozo, *UAB*, *Bellaterra, Spain*.

Milk fat depression in cows fed high grain diets has been related to an increase in trans-C18 fatty acids (FA) in milk. Trans-C18 FA are produced as a result of the incomplete biohydrogenation of dietary polyunsaturated FA in the rumen. There seems to be a confounding effect between ruminal pH and quantity of concentrate in the diet on biohydrogenation intermediates production. In the current experiment, the effect of pH (6.4 vs. 5.6) and two different forage to concentrate ratios (F:C) in the diets (70:30 F:C vs. 30:70 F:C) on rumen microbial fermentation, effluent FA profile and DNA concentration of bacteria involved in lipolysis and biohydrogenation processes was investigated in continuous culture. The study consisted of two experimental periods of 8 d (5 d adaptation plus 3 d sampling), with a 2 x 2 factorial arrengement of treatments. Both diets had a similar FA profile (44.0 % C18:2, 9.77% C18:3 of total FA). Low pH reduced OM and NDF digestibility, ammonia N concentration and flow, CP degradation and tended to reduce bacterial N flow, and increased non-ammonia and dietary N flow. Low pH decreased C18:0, t11-C18:1 and c9,t11-conjugated linoleic acid (CLA) and increased t10-C18:1, C18:2n6, C18:3n3 and t11,c15-C18:2 concentrations in the effluent. Low pH reduced *Anaerovibrio lipolytica* (32.7 vs. 72.1 pg / 10 ng total DNA) and *Butyrivibrio* VA subgroup DNA cocentrations (588 vs. 1394 pg / 10 ng total DNA). The 30:70 F:C diet increased OM and NDF digestibility, non-ammonia and dietary N flow, and reduced ammonia N concentration and flow. The 30:70 F:C reduced t11-C18:1, c9,t11-CLA and tended to reduce (P < 0.10) t10-C18:1, and increased C18:2n6, C18:3n3 and t11,c15-C18:2 compared with the 70:30 F:C diet. Moreover, the 30:70 F:C diet increased *A. lipolytica* DNA concentration (65.2 vs. 39.7 pg / 10 ng total DNA). Results confirm that the low pH is responsible for the accumulation of biohydrogenation intermediates which cause milk fat depression, and not the concentrate of the diet.

Key Words: pH, Concentrate, Fatty Acid Biohydrogenation

T240 Comparison of *in vitro*, *in situ*, and *in vivo* methodologies to assess nutrient digestibility in ruminants. L. E. Sims*[1], N. A. Pyatt[2], P. H. Doane[2], and S. S. Block[2], [1]*Oklahoma State University*, *Stillwater*, [2]*ADM Research Center*, *Decatur, IN*.

This study compared *in situ*, *in vitro*, and *in vivo* methods of estimating digestibility of substrates having variable starch content. Starch content of substrates was 65% (corn; high), 39% (corn-forage; med-high), 28% (forage-corn; med-low) or 22% (corn fiber; low). Lactating dairy cows and beef steers were used for *in situ* evaluation. *In vitro* digestibility was determined using the Ankom Daisy System with ruminal inoculum from steers fed high-starch diets or lactating dairy cattle fed moderate starch diets. The mobile bag method was used to estimate postruminal digestibility of residue collected from *in situ* and *in vitro* incubations. The med-high and med-low substrates were fed as complete diets to 20 lambs to assess *in vivo* total tract digestion. Rate and extent (48 hr) of ruminal *in situ* DM, CP, NDF, and ADF digestion was greater for dairy versus beef whereas rate and extent of starch digestion was less. *In vitro* rate of DM digestion was similar whether beef or dairy ruminal fluid inocula was used but extent was greater for dairy. Across substrates, *in vitro* DM digestion estimates were about five to 10 percent greater versus *in situ* estimates. Med-high and med-low substrates had similar (P>0.10) *in vivo* total tract digestibility. Ruminal (*in situ* or *in vitro*) and post-ruminal (mobile bag) digestibility or the sum of ruminal and post-ruminal estimates were compared with *in vivo* total tract digestion of med-high or med-low substrates. *In vitro* and *in situ* methods gave similar relative ranking among substrates but rate and extent of nutrient digestion differed. Selected methods can provide comparable estimates of nutrient digestion obtained in more time-consuming and expensive *in vivo* methods.

Table 1. Substrate Starch Content

Item	High (Corn)	Med-High (Corn-forage)	Med-Low (Forage-corn)	Low (Corn Fiber)
Ruminal DM digestion				
In situ – Beef (48 hr)	82.0	72.0	73.4	42.3
In situ – Dairy (48 hr)	90.4	78.9	83.3	60.7
Ankom – Beef Inocula	96.6	90.2	88.7	76.9
Ankom – Dairy Inocula	97.8	91.7	90	81.3
Total tract DM digestion				
Lamb in vivo	—	75.0	74.8	—
In situ residue in mobile bag	76.0	80.2	79.3	43.9
Ankom residue in mobile bag	90.6	87.8	86.6	57.8

Key Words: In vitro, In situ, Digestibility

T241 Effect of an enzymatic extract from *Agaricus bisporus* on *in vitro* digestibility of cell wall and dry matter. A. Ayala-Martinez*[1], S. S. Gonzalez-Muñoz[2], C. Vazquez-Gonzalez[1], G. Mendoza-Martinez[3], M. Meneses-Mayo[2], and O. Loera-Corral[4], [1]*FMVZ-UNAM*, *Mexico D.F.*, [2]*Colegio de Postgraduados*, *Montecillo, Edo. Mexico, Mexico*, [3]*UAM Xochimilco*, *Mexico D.F.*, [4]*UAM Iztapalapa*, *Mexico D.F.*

The objective of this trial was to determine the effect of a commercial enzymatic extract (CE) from *Trichoderma* spp. and an extract from *Agaricus bisporus* (AE) on residual NDF, ADF and DM *in vitro* digestibility (IVDMD) of alfalfa, crop residues, barley straw, Taiwan grass and orchard grass. The experimental design was completely randomized with a factorial arrangement of treatments (5 × 3); an analysis of variance was performed and means were compared with Tukey test ($P<0.05$). For alfalfa residual NDF there were differences ($P<0.05$) between the treatment without extract, AE and CE at: 1) 12h: 91.8a, 79.45b, 75.4b; 2) 24 h: 83.9a, 72.1b, 71.4b; 3) 48 h: 68.7a, 49.9b, 59.9b; ± 1.1%. For alfalfa residual ADF there were differences ($P<0.05$) between the treatment without extract, AE and CE at: 1) 12 h: 75.1b, 95.7a, 82.2b; 2) 24 h: 74.0b, 94.0a, 59.0b; 3) 48 h: 66.2b, 95.7a, 50.0b; ± 1.1%. There were differences of DM *in vitro* digestibility ($P<0.05$) between the treatment without extract, AE and CE: 1) crop residues at 72 h (52.2b, 66.2a, 58.2a; ± 1.2%); 2) barley straw at 24 h (36.8b, 41.39a, 42.65a; ± 1.02%), 48 h (38.0b, 44.09a, 45.2a; ± 1.02%) and 72 h (41.2b, 48.1a, 48.9a; ± 1.02%); 3) Taiwan grass at 48 h (30.07b, 40.1a, 41.0a; ± 1.03%) and 72 h (31.9b, 46.2a, 42.3a; ± 1.03%). No differences were found for alfalfa and orchard grass ($P>0.05$). Therefore, it may be concluded that *in vitro* digestibility of residual NDF, ADF and DM, for different forages, do not change when using a commercial enzymatic extract from *Trichoderma* spp. or an extract from *A. bisporus*.

Key Words: Fibrolytic Enzymes, Cell Wall Digestibility, Fungi

T242 In situ dry matter degradation parameters of treated and untreated Sainfoin (*Onobrichis vicifolia*) a tanniferous legume forage. H. Khalilvandi*, K. RezaYazdi, M. Dehgan-Banadaki, N. Vahdani, and H. R. Khazanehei, *University of Tehran*, *Karaj, Tehran,Iran*.

Condensed tannins are phenolics belonging to the plant secondary compounds, witch bind to plant proteins and other nutrients. Sainfoin is tannin rich, temperate legume forage, which its CT concentration fluctuated from 2.5 to 7.7 % of dry matter. Detrimental effects of CT are more probable in the case of high tannin concentration (more than 40 g/ kg DM).

In order to investigating different chemicals to improving degradation characteristics of sainfoin, an in situ experiment carried out using 3 ruminally fistulated Holstein cows. Samples of forage were chopped 3-5 cm length, and then treated with solutions of $KMnO_4$ (0.03 M), NaOH (0.05 M), sodium bicarbonate (0.1 M), wood ash (180 g/L) with forage to reagent volume ratio of 1:4 (W/V). and one water soaking considered as blank for 6th and 7th treatment. 5% solution of PEG (6000 MW) was sprayed to forage with 1:1 ratio and urea (20g/ 100 ml/1 kg of DM) treatment applied respectively. All of above treatments carried out in 25°C temperature, for 20 min, with hand shaking. Urea treatment was done using adhesive rubber to create anaerobic conditions for 1 week. Treated forages then expose to 40°C temperature in a forced air oven, for 48 hour. All forage samples grind to pass 2 mm screen size (Wiley mill). Followed grinding, these feeds were sieved to remove particles > 0.45 mm. 5 g of forage samples were weighed into nylon bags (10×20 cm) with 53 µm pore size, to create sample size: surface area of 12.5 mg/cm2. Duplicates were incubating for 4, 8, 12, 24, 48, 72 and 96h in ventral rumen.

Results showed that PEG treated forage have greater rapidly soluble fraction (a), but PEG could not increase potentially degradable fraction (b). Rate of degradation of b fraction was highest for wood ash (0.10240), and lowest for $KMnO_4$ (0.05113). Effective degradability in different rumen dilution rates were high for PEG treated Sainfoin compared to others. Treatment with $KMnO_4$ resulted in lowering effective degradability.

Key Words: Sainfoin, In situ Degradability, Tannin

T243 Accuracy of the n-alkanes technique for intake estimates in beef cattle fed with palisade grass (*Brachiaria brizantha* cv. Marandu). J. A. S. Morais[1], T. T. Berchielli[1], M. F. S. Queiroz*[1], A. Keli[2], A. de Vega[2], R. A. Reis[1], C. López[2], S. F. Souza[1], and G. Fiorentini[1], [1]*Faculdade de Ciencias Agrarias e Veterinarias/UNESP*, *Jaboticabal, São Paulo, Brazil*, [2]*Facultad de Veterinaria, Universidad de Zaragoza*, *Zaragoza, Spain*.

Animal performance is mainly determined by diet intake and digestibility, being the estimation of those parameters a difficult and not yet fully resolved task in grazing conditions. The objective of this research was to evaluate the n-alkanes methodology as markers to estimate the dry matter intake (DMI) in eight Nellore steers grazing palisade grass. Animals, in metabolism cage, were fed twice daily on (30 or 60 days regrowth) at 2.0% body weight. Animals were dosed twice daily during twelve days with paper pellets containing equal amounts of octacosane (C28), dotriacontane (C32) and hexatriacontane (C36). From day 10 to day 15 spot faecal samples were collected, directly from the rectum, at the same time of alkane dosing. N-Alkanes profiles in samples of grass, orts and faeces were extracted following the technique of the

ethanolic saponification for fourteen hours with alkane analysis carried out by gas chromatograph. The DMI estimates were not affected by the forage regrowth period (P > 0.05). Only the pair of alkanes C32/C33 did estimate adequately the DMI, underestimating the total intake at just 8%, while the pairs C31/C32 and C35/C36 under and overestimated the forage DMI in -15.3 and +18.8%, respectively, (P < 0.05). The n-alkanes methodology presented potential to estimate the forage DMI in tropical conditions.

Table 1. Forage dry matter intake (DMI) observed and estimated with C31/C32, C32/C33 and C35/C36 alkane pairs in steers fed with tropical forage (São Paulo – Brazil).

	Regrowth age, days		Mean	SEM [1]
Methods	30	60		
Actual DMI	4.20	4.32	4.26 [b]	0.147
Estimated DMI				
C31/C32	3.50	3.72	3.61 [c]	
C32/C33	3.60	4.15	3.88 [bc]	
C35/C36	4.46	5.65	5.06 [a]	

[1] Standard error of the mean; [abc] Means values within columns with different superscripts are significantly different (P<0.05).

Key Words: Nellore, Palisade Grass

T244 The effect of non fibre carbohydrate on in vitro first order NDF disappearance of alfalfa. M. Danesh Mesgaran*, F. Rezaii, and A. R. Heravi Mousavi, *Ferdowsi University of Mashhad*, *Mashhad, Iran*.

The aim of this study was to determine the effect of supplementing sucrose or starch on in vitro first order NDF disappearance model of alfalfa. Samples of alfalfa were ground to pass 0.75 mm screen and dried at 80 ËšC for 48 h. One gram of non-supplemented or non-fiber carbohydrate supplemented samples (70 mg/g DM of feed sample as starch or sucrose) were incubated in a medium containing 40 ml cell-free rumen fluid, 60 ml mineral mixtures and 5 ml of cloth-cheese strained rumen fluid in a 200 ml bottle. Each bottle was finely bubbled with CO2. Rumen fluid was obtained from 4 Holstein steers fed corn silage, alfalfa hay, wheat straw, barley grain and soybean meal (3.4, 2.4, 0.8, 1.6 and 0.8 kg/d DM, respectively). Bottle of each sample was incubated for 24, 48, and 96 h at 39°C (n=4). Then, bottle content was filtered through a 22 Âµm filter paper. Unfiltered NDF was determined. Data were analyzed using GLM procedure of SAS and applied to a non-linear first order model [D(t)= D(i).exp (-k. time)+ I; where D(t) is potentially digestible fraction of DM , D(i) is potentially digestible residues, k is fractional rate constant of digestion (h-1) and I is indigestible fraction]. Indigestible fraction of NDF of alfalfa hay was significantly (p< 0.05) increased when it was supplemented with starch (0.63, 0.64 and 0.76 for alfalfa, alfalfa+sucrose and alfalfa+starch, respectively). The lowest constant rate of digestion was observed when sucrose (0.007) was added to alfalfa. Constant rate of digestion of alfalfa and alfalfa+starch was 0.01 and 0.02, respectively. Results of the present study showed that the first order fractional rate of digestion of NDF of alfalfa might be influenced by source of NFC. Therefore, there is a need to determine the associated effect of a feed and nature of NFC on fractional rate constant of digestion and indigestible fraction for each forage or a composed diet.

Key Words: NDF, Model, Disappearance

T245 Ruminal and post-ruminal protein disappearance of high oil sunflower meal treated with formaldehyde or sodium hydroxide. T. Mohammadabadi, M. Danesh Mesgaran*, and M. R. Nasiri, *Excellence Center for Animal Science, Ferdowsi University of Mashhad*, *Mashhad,Iran*.

This study was conducted to evaluate the effect of formaldehyde or sodium hydroxide on in situ ruminal disappearance and in vitro intestinal digestion of high oil (165 g/kg DM) sunflower meal (SM). Samples were: Untreated SM (USM), sodium hydroxide treated SM (SHSM, 40 g/kg DM), formaldehyde treated SM (F30SM and F60SM, 30 and 60 g/kg DM, respectively). Ruminal disappearance of sample was determined using four fistulated Holstein steers (400±12 Kg, body weight). Samples were weighed into nylon bags (19×12 cm, pore size 48 μm, n=6) and incubated in rumen for12 h. An in situ/ in vitro enzymatic 3-step procedure was conducted to determine post-ruminal disappearance of the samples. In this procedure, a part of ruminal-undegraded nitrogen (after 12 h rumen incubation) was included in pepsin and pancreatin to determine post-ruminal protein disappearance of the sample. DM content of all intact and incubated samples was determined using air-forced oven (65°C, 48 h). Nitrogen concentration of the samples was determined using Kjeldahl method. Data were analysed using GLM procedure of SAS. Results indicated that ruminal DM and CP of F60SM was significantly (P < 0.05) lower (0.42 and 0.39, respectively) than USM (0.7 and 0.65, respectively). Formaldehyde and sodium hydroxide caused an increase in post-ruminal CP disappearance (0.44, 0.4, 0.33 and 0.27 for F60SM, F30SM, SHSM and USM, respectively). Total tract CP digestibility for F60SM, F30SM, SHSM and USM was 0.83, 0.85, 0.88 and 0.93. It was concluded that both formaldehyde and sodium hydroxide caused an increase in the ruminal and post-ruminal CP disappearance of high oil content sunflower meal.
Key Words: High Oil Sunflower Meal, 3-Step, Disappearance

T246 The effect of feed iodine supplementation on milk production traits in dairy goats. A. Nudda*[1], M. Decandia[2], G. Epifani[2], G. Battacone[1], G. Spanu[1], and G. Pulina[1,2], [1]*University of Sassari*, *Sassari, Italy*, [2]*AGRIS Sardegna*, *Sassari, Italy*.

Iodine requirements are higher for goats than for other ruminants. An adequate supply of dietary iodine can prevent iodine deficiency disorders in goats and increase the iodine content in milk. However, the effects of iodine-enriched diets on milk production traits of lactating goats have been poorly investigated. This work aimed to determine the effect of iodine supplementation to dairy goats on milk yield and composition. Thirty crossbred dairy goats were divided into 3 homogeneous groups. Each goat was supplemented with potassium iodide (KI) at 0 (control group), 400 (group 1), or 950 μg of KI/day (group 2). The dose of KI (76.5% of iodine) was dissolved in water and administered every day for 8 weeks. Milk yield and milk composition (fat, protein, urea) were recorded weekly. Milk yield was not influenced by KI supplementation and averaged 1229, 1227 and 1179 g/d per head in groups 0, 1 and 2, respectively. Milk fat content was higher (P≤0.01) in group 1 (4.12%) compared to group 0 (3.78%) and group 2 (3.84%). The protein content was similar in groups 1 and 2 (on average, 3.43%), and tended to be higher than in the control group (3.36%). On the contrary, the milk urea concentration was significantly lower in the groups that received KI supplementation (32 and 33 mg/dl in groups 1 and 2, respectively) than in the control group (37 mg/dl). In conclusion, the doses of KI used in this study did not influence milk yield and had favorable effects

on urea and, to a lower extent, protein contents in goats, suggesting an improvement of rumen activity, mainly of nitrogen utilization.
Acknowledgements: Research supported by the Ministry of University and Research (FISR grant).

Key Words: Iodine, Dairy Goat, Milk

T247 An examination of the intake and digestibility characteristics of ground ear maize for beef cattle. P. O'Hanlon, D. A. Kenny, T. M. Boland, G. P. Keane, and M. B. Lynch*, *UCD School of Agriculture, Food Science and Veterinary Medicine*, *University College Dublin, Ireland*.

Feed is the largest single cost in cattle production and as demand for cereal grain for non-agricultural purposed increases, elevating feed grain costs will impact on profitability. Ground ear maize (GEM) is a novel approach to potentially utilising the maize plant as a high energy forage based substitute for grain based diets and essentially consists of the corn and cob mix with a portion of the leaf material retained. The objective of this short term experiment was to compare the intake and digestibility characteristics of GEM with barley grain as a feed source for high producing beef cattle. GEM was ensiled for two months in round plastic wrapped bales. Young beef bulls (n=24) with a mean ± sd liveweight of 356 ± 20 kg were blocked on liveweight and age and randomly allocated within block to one of two treatments (1) GEM-based diet (GEM) (2) Barley-based diet (BAR). All animals were individually fed and soybean meal and barley straw were added to make both diets isonitrogenous (13.5% CP), isofibrous (27% NDF) and isoenergetic (17 MJ GE/kg DM). GEM was fed as a TMR, while concentrate and straw were offered separately for BAR. Feed intake was recorded for 14 days and diet digestibility was determined using the chromic oxide technique with faecal samples were collected on days 13 and 14. Animals on GEM had higher DMI ($P = 0.07$) compared with BAR. However, animals on BAR had higher ($P < 0.01$) digestibility of neutral detergent fibre, organic matter and dry matter and a tendency towards higher digestibility of N ($P = 0.09$) compared with those on GEM. In conclusion, these data indicate that ground ear maize is worthy of further investigation as a substitute for grain based diets. Longer term studies are required to assess effects on animal production and carcass quality.

Key Words: Ground Ear Maize, Beef Cattle, Feed Intake

T248 Comparison of procedures to detach particle-associated microbes from ruminal digesta in Rusitec fermenters. M. E. Martínez, M. J. Ranilla*, S. Ramos, M. L. Tejido, C. Saro, and M. D. Carro, *Universidad De LeÓN, Campus De Vegazana*, *León, Spain*.

Three different detachment procedures (DP) were evaluated for their ability to remove particle-associated microbes from incubation feed residues in Rusitec fermenters fed a 70:30 concentrate:alfalfa hay diet. Concentrate and hay were incubated in separate nylon bags, and incubation residues were treated independently. In the methylcellulose procedure (MET) feed residues were incubated at 38°C for 15 min with saline solution (0.9% NaCl) containing 0.1% methylcellulose with continuous shaking. In the stomacher procedure (STO) residues were mixed with saline solution and homogenized with a stomacher for 5 min at 230 rev min-1. In the freezing procedure (FRE) residues were immediately frozen at -20°C for 72 h, thawed at 4°C, and mixed with saline solution. All solutions were added at a rate of 3 mL/g of residue. Common to all treatments was storing at 4°C for 24 h after the treatment, homogenization, filtration, and resuspension of residues two times in the treatment solutions. Filtrates were centrifuged at 20,000 g for 25 min at 4°C to obtain microbial pellets. Microbial removal was estimated indirectly from removal of ^{15} N. Each procedure was repeated in three consecutive days (n=3). There were no feed x DP interactions (P=0.15 to 0.91) in any variable. Both detaching efficiency and total recovery were affected by the DP ($P<0.001$), STO presenting the greatest values (mean values for both substrates of 63.2 and 25.5%, respectively), and FRE the lowest ones (44.3 and 18.4%). There were no differences (P=0.71) among the pellets obtained by the different DP in their N content, but pellets from MET had greater ($P<0.05$) ^{15}N enrichments than those from STO and FRE. Pellets detached from hay presented greater ($P<0.001$) N content and ^{15}N enrichments than those obtained from concentrate (mean values of 63.3 vs 55.7 mg N/g dry matter, and 0.152 vs 0.132% atoms in excess). Results suggest that STO was the most effective method to detach ruminal microbes from both concentrate and hay incubated in Rusitec fermenters

Key Words: Adhesion, Ruminal Microbes, ^{15}N

T249 Effects of varying levels of fish oil, fed as a calcium salt, on rumen fermentation and biohydrogenation in continuous culture. C. M. Klein*[1], T. C. Jenkins[1], and K. D. Murphy[2], [1]*Clemson University*, *Clemson*, [2]*Virtus Nutrition, Lancaster, PA*.

Fish oils, including docosahexaenoic acid (C22:6, DHA) and eicosapentaenoic acid (C20:5, EPA) have been found to have human health benefits and are used as fat supplements in ruminants. Transfer of omega-3 fatty acids from the ruminant diet, to meat and milk products, depends on their escape from microbial biohydrogenation in the rumen. Fatty acids are often fed as calcium salts to reduce biohydrogenation and lessen the negative effects on fermentation. To determine if fish oil protection was enhanced when incorporated in a matrix of palm oil fatty acids, this study examined the effects of varying the ratio of fish oil and palm oil calcium salts on ruminal fermentation and biohydrogenation. Ruminal microorganisms maintained in continuous culture were exposed to diets with 5% added fat as soybean oil or as calcium salts of fish oil and palm fatty acids in three combinations (45/55, 75/25, and 90/10). A control diet and the four fat diets were fed to fermentors in a 5x5 Latin square with 10 d periods. As expected, the acetate/propionate ratio decreased ($P < 0.05$) when soybean oil was added to the diet (1.78 for the control and 1.30 for SBO). The acetate/propionate ratios for the fish oil diets were not different from the SBO diet (1.22, 1.07, and 1.07 for the 45FO, 75FO, and 90FO diets, respectively). Of the three combinations of fish oil tested, the 45FO and 75FO did not differ in the amounts of EPA (43.8% and 40.8%) or DHA (40.8% and 33.3%) that disappeared. There were however, differences in EPA and DHA lost between 75FO and 90FO. Losses of EPA (59.8%) and DHA (55.8%) were greater ($P < 0.05$) for FO90 compared to either 45FO or 75FO. These results indicate that rumen protection of DHA and EPA is diminished when fish oil comprises more than 75% of a fish oil/palm oil calcium salt mix.

Key Words: Biohydrogenation, Fish Oil, Continuous Culture

T250 By-product of biofuels processing in the feeding of ruminant. J. A. G. Azevedo[1,2], D. S. Pina[2], N. K. P. Souza[2], J. C. M. Lima[2], A. S. Oliveira[2], C. V. Xavier[2], S. C. Valadares Filho[2], and H. J. Fernandes*[3,2], [1]*Universidade Estadual de Santa Cruz - FAPESB*, *Ilhéus, Bahia, Brazil*, [2]*Universidade Federal de Viçosa*, *Viçosa, Minas Gerais, Brazil*, [3]*Universidade Estadual do Mato Grosso do Sul- FUNDECT*, *Brazil*.

This experiment was carry out aiming to evaluate the effects three levels (0.0, 8.0 and 24.0; and 0.0, 5.0 and 16.0 % of dietary DM) of inclusion of two by-products: sunflower (*Helianthus annus*) and turnip (*Raphanus sativus*), respectively, on the intake and apparent total tract digestibility of DM, OM, CP, EE, NFC and NDF. Twelve crossbred heifers with averaging BW 263.0 ± 34.0 kg were used. The experiment was performed in two periods of 15 days, in which the last three days were used to make total fecal collection. The animals were held at individual sheltered pens of approximately 3 m2 and fed twice daily at 08:00 and 16:00 h, allowing for up to 10% of orts. The corn silage was the only roughage in the diet, in which was incorporated a mix of urea/ammonium sulfate (9:1) aiming to maintain the same dietary CP levels among the diets (13.0% of DM). The animals were allocated in six 2×2 Latin square design in a simple reversion arrangement and all statistical analyses were performed using PROC MIXED of SAS. The intake and apparent digestibility of DM in the diet contain sunflower ranged 34 and 18% respectively, with the average of 1.8% BW and 62%, respectively. In the diet contain turnip, the DMI ranged from 1.2 to 2.6% BW and the apparent digestibility of DM ranged from 57.8 to 66.9%. The quadratic effects ($P < 0.05$) were observed by the levels of sunflower on the intake of DM, OM, CP, EE, NDF and apparent digestibility of EE. The levels of turnip affected quadratically ($P < 0.05$) the intake and apparent digestibility of EE. These results indicated that the level of inclusion of sunflower by-product that maximizes the intake of nutrients is the 7%, however the maximum level (16% of DM) did not affect the apparent digestibility of all nutrients with exception the EE. The maximum level of inclusion of turnip by-product (24% DM) did not affect either intake or apparent digestibility of DM, OM, CP, NFC and NDF.

Key Words: Alternative feed, Sunflower, Turnip

T251 In vitro gas production kinetics of biofuels by-products. J. A. G. Azevedo[1,2], D. S. Pina[2], J. C. M. Lima[2], N. K. P. Souza[2], C.V. Xavier[2], A. S. Oliveira[2], S. C. Valadares Filho[2], and H. J. Fernandes*[3,2], [1]*Universidade Estadual de Santa Cruz*, *Ilheus, Bahia, Brazil*, [2]*Universidade Federal de Viçosa*, *Viçosa, Minas Gerais, Brazil*, [3]*Universidade Estadual do Mato Grosso do Sul- FUNDECT*, *Brazil*.

This study was conducted aiming to evaluate the in vitro gas production digestion kinetics of biofuels by-product (sunflower, turnip, palm and castor). Four samples of each by-product were pre-dried in a forced air over (60°C) and ground in a Wiley mill (1 mm screen). Samples (200mg) were weighed into 100 mL graduated glass syringes with pistons lubricated with Vaseline. Buffered mineral solution was prepared and placed in acclimatized room at 39°C under continuous flushing of CO_2. The ruminal fluid inoculun used in all experiment was obtained from the rumen fistulated heifers fed with corn silage and concentrate (70:30 DM ratio). Rumen fluid was transferred into pre-warmed thermo flasks, filtered through four layers of cheesecloth and flushed with CO_2. Rumen fluid was added to the buffered mineral solution (inoculum), which was maintained in acclimatized room at 39°C under continuous flushing of CO_2. Inoculum (30 mL) was dispensed into syringes containing by-product samples. The clip was closed, the initial volume recorded, and gas readings for each syringe were manually recorded at 2, 4, 6, 8, 10, 12, 24, 26, 28, 30, 32, 36, 48, 52, 54, 56, 60 and 72 h after incubation. The recorders volumes were corrected for a blank incubation (i.e., buffered rumen fluid without sample). The rate and extent of gas production was determined by fitting of gas production profile with dual-pool logistic by Gauss-Newton algorithm (SAS NLIN procedure). The final gas volume yields from nonfiber carbohydrate (NFC) were highest to turnip and sunflower by-product (23.5 and 18.0 mL), while the gas yielded from fibrous carbohydrate (FC) were highest to castor and palm by-product (19.3 and 17.2 mL). The degradation rate of the NFC and FC to palm, sunflower, castor and turnip by-product were 0.0912 and 0.0255; 0.1533 and 0.0390; 0.1824 and 0.0267; 0.1297 and 0.0306 h-1, respectively. The highest lag time was observed to palm by-product (5.08 h). It was concluded that the NFC presented the higher digestion rate than FC as expected. Therefore, by-product with high levels of NFC could provide more energy supply than by products with high levels of FC.

Key Words: Alternative Feed, Digestibility, Fermentation Kinetics

T252 Bacterial diversity in rumen fluid samples collected via oral lavage or rumen cannula. J. Pisel, S. L. Lodge-Ivey*, J. Browne-Silva, and M. B. Horvath, *New Mexico State University*, *Las Cruces*.

There are inherent limitations associated with cultivation-based methods for characterizing the composition of bacterial communities in the rumen. Cultivation-independent methodologies are available. Denaturing gradient gel eletrophoresis (DGGE) of amplified fragments of 16SrRNA genes has become a widely used tool to assess the diversity of complex bacterial communities in a variety of environments. A study was conducted to determine if sampling the rumen contents ruminally via a cannula or orally via lavage tube would yield similar DGGE profiles of the bacterial community. Two species of ruminally cannulated animals were used for this study (cows n=2; sheep n=3). All animals were allow ad libitium access to feed. Cattle were allowed fed a poor quality forage consisting of baled unprocessed sorghum-sudan straw (5% CP, 68% NDF; DM basis) while sheep were maintained on chopped alfalfa (18% CP, 40% NDF; DM basis). Ruminal fluid was collected once a week for 4 weeks from each animal using a plastic tube equipped with a suction strainer with a handheld suction pump through the rumen cannula or oral cavity. DGGE analysis and principal component comparison demonstrates that yield of bacterial diversity was not different between the two sampling methods ($P = 0.15$). However, species and/or diet sampled did influence the number of bands in the DNA band pattern in the DGGE analysis. Sheep had fewer bands per lane on DGGE gels which is equated to less diversity than cattle ($P < 0.01$; 25.1 vs 30.0). Additionally, when samples were grouped according to DNA band patterns groups were most stable according to individual animal and species rather than sampling method. These data indicate that collecting samples via a lavage tube or rumen cannula is more influenced by species and individual animal than sampling method. This knowledge will allow for sample collection from a greater population of animals and a reduction in cost associated with developing and maintaining ruminally cannulated animals.

Key Words: DGGE, Rumen Sampling, Rumen Microbiology

T253 Image analysis and microscopy in animal by-products characterization. A. Campagnoli, C. Paltanin, L. Maggioni, G. Savoini, V. Dell'Orto, F. Cheli, and L. Pinotti*, *Department of Veterinary Sciences and Technology for Food Safety, Veterinary Medicine Faculty*, *Milan, Italy*.

Aim of this study was to evaluate the potential of microscopic methods in association with computer image analysis to identify the source of meat and bone meal. For this purpose reference samples (SAFEED-PAP Project; VSA, University of Milan) containing ruminant (bovine and ovine) and non-ruminant (pig, rabbit, chicken, turkey) meat and bone meals were analysed by microscopic method (as described in Commission Directive 2003/126/EC). Through a CCD camera and an image analysis software (Image-for Plus 4.5.1, USA), 890 bone fragment lacunae images at X40 were obtained. Images have been enhanced and processed in order to obtain for each lacuna a monochrome mask on which 26 dimensioned and 4 no-dimensioned shape variables (constructed by combining the various size variables so that dimension units cancel out) were measured. Data were analysed by a cross-validated non-parametric classification method (kernel model applied in prediction by PROC DISCRIM of SAS statistic software). The results obtained indicated that: (i) the variables that best discriminated ruminants from non-ruminants were almost exactly the same as reported previously for mammalian and poultry; (ii) 87.54% of ruminant lacunae were correctly classified, and 12.46% incorrectly as non-ruminant. However 47.06% of non-ruminant lacunae were correctly classified (and 52.94% incorrectly classified as ruminant). To conclude, the use of microscopic methods in association with computer image analysis to identify ruminant from non-ruminant material in feedstuffs appears promising although further improvements (e.g. more defined statistical methods) are required.
This work is funded by the European Commission, within the framework of the FOOD-CT-2006-036221 Project SAFEED-PAP

Key Words: Meat and Bone Meals, Bone Lacunae, Image Analysis

T254 Influence of a diet enriched in extruded linseed on fatty acid composition of goat cheese. A. Nudda*[1], G. Battacone[1], M. Addis[2], A. Pirisi[2], A. Mazza[1], and G. Pulina[1,2], [1]*University of Sassari*, *Sassari, Italy*, [2]*AGRIS Sardegna*, *Sassari, Italy*.

This study aimed to assess the effect of long-term dietary supplementation with extruded linseed on goat milk and cheese fatty acid composition.
Forty-eight crossbreed dairy goats were allocated to 2 groups: one was fed the control diet (CON) and the other one was supplemented with 200 g of extruded linseed (LIN) which supplied 70 g/d of fat. The trial lasted 8 weeks. Bulk milk from each experimental group was collected weekly to produce a soft cheese type. Fatty acids were determined in milk and in 1-day and 20-day-old cheese. The FA content did not differ between milk and cheese. The cis-9, trans-11 CLA was higher in cheese produced from LIN milk than in cheese made from CON milk (1.22 vs. 0.85 g/100 g of fat; P<0.01). The concentration of cis-9, trans-11 CLA in LIN cheese produced from supplemented-goat milk increased gradually until the fifth week (1.81 g/100 g of fat) of the trial and decreased afterwards. The C18:1 trans11 content was significantly higher in LIN cheese than in CON cheese (2.82 vs. 1.60 g/100 g of fat; P<0.01). The content of linolenic acid (LNA) was much higher in LIN cheese than in CON cheese (1.6 vs. 0.67 g/100 g of fat; P<0.01)and remained stable during all the experimental period. The CLA and LNA concentration did not differ between 1-day and 20-day-old cheeses. Goat diet supplemented with a fat source rich in LNA for a long period resulted in an enhanced CLA content in cheese compared with the control diet. However, the concentration of CLA did not remain stable throughout the fat supplementation period.
Acknowledgements: Research supported by the Ministry of University and Research (FISR grant).

Key Words: Goat Cheese, Linseed, Fatty Acid

T255 Relationship between vaccenic acid content of ruminal bacteria and duodenal bacteria. S. J. Liu, J. Q. Wang*, D. P. Bu, S. Liang, L. Liu, H. Y. Wei, L. Y. Zhou, and K. L. Liu, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*.

Isomers of Conjugated linoleic acid (CLA), especially *cis* -9, *trans* -11 CLA and *trans* -10, *cis* -12 CLA have a wide range of benefits on human health. It was proved that some unique microorganisms could absorb vaccenic acid (VA) to reduce biohydrogenation, followed increasing CLA synthesis by Δ^9-desaturase in mammary gland and adipose tissue. However, few reports have referred to the relationship between vaccenic acid of ruminal bacteria and duodenal bacteria. The objective of this study was to evaluate relationship of vaccenic content between mixed ruminal bacteria and duodenal bacteria in beef cattle. Four steers with ruminal cannulas were randomly assigned to control (CK, without additional oil supplement) or CK with 3% sunflower oil plus 1% fish oil (SF1), 2.5% sunflower oil plus 1.5% fish oil (SF2) and 2% sunflower oil plus 2% fish oil (SF3) in a 4×4 Latin square with isonitrogenous and 12-wk durational periods (forage to concentrate ratio was 65:35). Ruminal and duodenal digesta were collected every 4 h over a 24 h period on d 20, and d 21 of each experimental period and pooled for each animal, and pooled samples were stored at -20°C for bacteria separation according to different centrifuge method and fatty acids analysis was described by M. Sönnichsen. Regression analysis adopted the linear regression procedure of SAS8.2.
Our study showed that there existed linear regression relationship of vaccenic acid content between mixed ruminal bacteria and duodenal bacteria in beef cattle ($y = 1.15x + 0.09$; $R2 = 0.87$, $P < 0.0001$). In the linear regressive equation, vaccenic acid content from duodenal bacteria (y value) was higher than that from rumen (x value). In conclusion, ruminal bacteria can effectively inhibit vaccenic acid hydrogenated in rumen, which increase vaccenic acid concentration of duodenal bacteria.
Acknowledgement: Research supported by Ministry of Science and Technology (2006DFB32160).

Key Words: Ruminal Bacteria, Vaccenic Acid, Hydrogenation

T256 Isolation of prominent lipolytic rumen bacteria. N. A. Krueger*, R. C. Anderson, T. R. Callaway, T. S. Edrington, and D. J. Nisbet, *USDA, ARS, Food and Feed Safety Research Unit*, *College Station, TX*.

Ruminant-derived foods contain high proportions of saturated fats, a result of ruminal biohydrogenation which rapidly saturates and thus limits the availability of free unsaturated fatty acids for assimilation. Strategies to enrich ruminant-derived foods with unsaturated fatty acids are desired as these are considered beneficial for good human health.

Lipolysis is a pre-requisite for biohydrogenation because saturase enzymes act only on free fatty acids. We conducted a descriptive study to isolate and characterize prominent lipolytic bacteria from the bovine rumen. Serial dilutions ($10^{-1 \text{ to } -10}$) of ruminal fluid (from a pastured cannulated cow) were inoculated to roll tubes or plates containing buffered rumen fluid based agar supplemented with triolein, olive oil or linseed oil (2.5% wt/vol) as substrate for lipolytic bacteria. Medium within plates also contained rhodamine B dye specific for detection lipase expressing colonies. Inoculated roll tubes were incubated under 100% CO_2 and plates under N_2:CO_2:H_2 (90:5:5) for up to 7 days. Four colonies exhibiting characteristic zones of clearing and 15 colonies exhibiting fluorescence were isolated media inoculated with $10^{-4 \text{ to } -10}$ ml of rumen fluid indicating that some were among the predominant flora containing 10^{10}colonies/ml. Biochemical and 16S rRNA gene sequence characterization revealed that none of these isolates were *Anaerovibvibrio lipolytica* or *Butyrivibrio* spp., bacteria considered major contributors to ruminal lipolysis but not isolated in this study. Recovered isolates were identified as *Clostridium chauvoe, Clostridium sporogenes, Proprionibacterium acnes, Proprionibacterium avidum* and *Staphylococcus epidermidis*. Results indicate that the ruminal population of lipolytic organisms is more diverse than previously thought and that efforts targeting this activity as a way to protect unsaturated fat from biohydrogenation will likely need to target more than just *A. lipolytica* or *Butyrivibrio* spp.

Key Words: Biohydrogenation, Lipolysis, Rumen Bacteria

T257 Biohydrogenation of vaccenic-1-^{13}C acid by ruminal microbes in vitro. E. E. Mosley* and M. A. McGuire, *University of Idaho*, *Moscow*.

Efforts in the human food industry have focused on the elimination of trans fatty acids (TFA) from foods. Thus, it is vital for producers to limit the amount of TFA in beef and milk. Typically, the major trans isomer in beef and milk fat is vaccenic acid (VA). Understanding the rumen microbial modifications of VA will provide necessary information concerning biohydrogenation pathways. Our objective was to determine if VA is isomerized to other 18:1 isomers and if the isomerization and or biohydrogenation to stearic acid is altered by other fat sources. Rumen in vitro cultures (25 ml) containing ^{13}C-labeled VA at 0.25 mg/ml alone or combined with stearic acid, oleic acid, linoleic acid, linolenic acid, cis-9, trans-11 conjugated linoleic acid, corn oil or fish oil at varying concentrations (0.5, 1.0, or 1.5 mg/ml) were run under standard conditions. After 48 h, cultures were frozen, freeze dried, and direct methylated using methanolic-HCl and sodium methoxide. Fatty acid methyl esters (FAME) were converted to dimethyl disulfide derivatives (DMDS). The FAME and DMDS were analyzed by GC-MS. Significant ^{13}C enrichment was found in stearic acid, cis- and trans-9, 12, 13, 14, 15, 16, and trans-10 18:1 isomers. All concentrations of fish oil inhibited the biohydrogenation of VA to stearic acid and increased the isomerization of VA to various 18:1 isomers when compared to cultures containing only VA ($P<0.0001$). Cultures containing fish oil or oleic acid at 0.5 mg/ml contained the greatest enrichment for trans-13, 14, and 15 when compared to all other treatments ($P<0.0001$). The biohydrogenation of VA by ruminal microbes in vitro involves the formation of positional isomers of cis- and trans-18:1 and stearic acid. Identifying the extent and profile of the TFA produced from VA in the rumen will allow the beef and dairy industries to be proactive in identifying new ways to provide more healthful products to the consumer. Supported in part by the Idaho Agricultural Experiment station and National Research Initiative Competitive Grant nos. 2006-35206-16819 from the USDA Cooperative State Research, Education, and Extension Service, and NIH-NRRI grant P20 RR15587.

Key Words: Vaccenic Acid, Trans Fatty Acids, Biohydrogenation

T258 WITHDRAWN

T259 Use of inter-organ glycerol fluxes to assess abdominal versus peripheral fat mobilization in transition dairy cows. M. Larsen and N. B. Kristensen*, *University of Aarhus*, *Tjele, Denmark*.

Glycerol flux data from 6 Holstein cows assigned to one of two treatments: No infusion (control; CON) or continuous abomasal infusion (INF) of 1500 g glucose/d (via the rumen) were used to test if fat mobilization differ between abdominal and peripheral tissues. Treatments were initiated at the day of second calving. Cows were fed the same dry-diet and the same lactation-diet, and both were fed in equally sized meals at 8 h intervals. Eight hourly sets of blood samples ($n = 2$ for hepatic vein with CON) were collected simultaneously starting 30 min before the morning feeding 12 days pre partum, as well as 4, 15, and 29 days in milk (DIM). Data was analyzed as a split-plot design and Student's *t*-test was used to test by treatment differences between pre partum and 4, 15 or 29 DIM, respectively. A strong correlation between arterial concentration of glycerol and arterial concentration of non-esterified fatty acids (NEFA) was observed across the data set ($P < 0.01$; $r = 0.88$; Glycerol = NEFA • 0.070 ± 0.002). Except at 4 DIM with CON, the net portal flux and net hepatic uptake of glycerol were relatively constant at a level of 2 ± 1 and 11 ± 2 mmol/h, respectively. From pre partum to 4 DIM, the net portal flux and net hepatic uptake of glycerol tended to increase more ($P = 0.06$) for CON compared with INF (net portal flux: +5 and +1 mmol/h, respectively; net hepatic uptake: +29 and +1 mmol/h, respectively). Assuming no net absorption of dietary glycerol and that glycerol is metabolized solely in the liver, the ratio of abdominal to total fat mobilization was estimated by dividing net portal flux of glycerol by net hepatic uptake of glycerol. The ratio of abdominal to total fat mobilization was not increased ($P > 0.10$) from pre partum to 4, 15 or 29 DIM, or affected by treatment ($P = 0.94$). Thus, the ratio was remarkably constant across the data set (0.18 ± 0.02). In conclusion, the present study indicated similar rates of fat mobilization between abdominal and peripheral adipose tissues in transition dairy cows.

Key Words: Mobilization, Transition, Glycerol

T260 Correlation between UT-B mRNA abundance in ruminal epithelium and net portal flux of urea in transition dairy cows. B. A. Røjen*, P. K. Theil, M. Larsen, and N. B. Kristensen, *Faculty of Agricultural Sciences, University of Aarhus*, *Tjele, Denmark*.

The objective of the study was to test the hypothesis that abundance of UT-B mRNA in ruminal epithelium can explain variation in net portal flux of urea in transition cows. Six Holstein cows fitted with a ruminal cannula and permanent indwelling catheters were assigned to continuous abomasal infusion of 1500 g glucose/d (INF) or control (CON; no

infusion). Cows were fed the same dry and lactation rations in equally sized portions at 8 hour intervals. Eight hourly sets of blood samples were obtained from arterial, portal and hepatic catheters starting 30 min before morning feeding 12 days pre partum, as well as 4, 15, and 29 days post partum. Rumen papillae from the atrium were harvested at each sampling day. Abundance of UT-B mRNA was determined using real time RT-PCR and normalized by GAPDH mRNA abundance. Data was analyzed as a split-plot design using the mixed model procedure of SAS. The net portal flux of urea decreased (being less negative; $P < 0.05$) with INF compared with CON (-75 and -135 ± 13 mmol/h, respectively), and tended to decrease ($P = 0.08$) between 4 and 15 days post partum for INF compared with CON. Post partum abundance of UT-B mRNA was lowered to less than half (0.44) of the pre partum level ($P < 0.05$). No correlation ($P > 0.10$) between UT-B mRNA abundance and net portal flux of urea was detected when both pre- and post partum data were analyzed reflecting the large influence of reproductive stage on the UT-B mRNA abundance. However, weak correlations ($P = 0.04$ to 0.08) between arterial urea concentration and net portal flux of urea with UT-B abundance were observed when analysing only post partum data ($r = 0.487$ and $r = -0.426$, respectively). In conclusion data showed that UT-B abundance in ruminal epithelium appears to be strongly affected by time relative to calving. However, UT-B mRNA abundance only explained a small proportion of the variability in net portal flux of urea across individual cows, treatments, and days in lactation.

Key Words: Urea Transporter, Ruminal Epithelium, Dairy Cows

T261 Hepatic metabolism of alcohols in freshening Holstein cows. B. M. L. Raun* and N. B. Kristensen, *Faculty of Agricultural Sciences, University of Aarhus*, *Tjele, Denmark*.

Eight lactating Holstein cows (26 ± 7 kg milk/d; 14 ± 1 kg dry matter intake/d) implanted with a ruminal cannula and permanent indwelling catheters in major splanchnic blood vessels were used to investigate hepatic alcohol metabolism at day 4 of lactation. The hypothesis was that freshening dairy cows would have a low capacity for hepatic alcohol metabolism. Cows were randomly allocated to a 2 by 2 factorial design with one factor being level of branched chain alcohol (isopropanol from HMBi; 0.26 % of dry matter; Adisseo, France) compared with no addition of isopropanol (calcium carbonate) and the second factor being source of straight chain alcohols ethanol (1.5 % of dry matter) compared with propanol (1.1 % of dry matter). Cows were fed the same dry ration and fed the experimental rations from time of calving. The rations were fed in three equally sized portions daily at 8 hour intervals. Eight hourly sets of arterial, portal vein, and hepatic vein samples were collected. Data was analyzed as a 2 by 2 factorial design with samples within day as repeated measurements using the mixed procedure of SAS. The net portal flux of ethanol increased from 30 ± 8 to 74 ± 9 mmol/h with ethanol compared with propanol ($P < 0.01$) and the net portal flux of propanol increased from 16 ± 6 to 64 ± 5 mmol/h with propanol ($P < 0.001$) compared with ethanol. The net portal flux of isopropanol increased from 2 ± 4 to 13 ± 3 mmol/h ($P = 0.04$) with HMBi compared with calcium carbonate. Numerically net hepatic uptake of all three alcohols mirrored net portal flux and an increased hepatic uptake of propanol and isopropanol could be detected ($P = 0.04$ to $P < 0.001$). In agreement with the responsiveness in hepatic alcohol metabolism to increased alcohol absorption there were not detected any net splanchnic release of ethanol, propanol or isopropanol. In conclusion no limitation in liver capacity for metabolism of ethanol, propanol, and isopropanol could be detected in freshening dairy cows fed rations containing alcohol levels spanning commonly observed variation in silage based rations.

Key Words: Alcohol, Dairy Cow, Metabolism

T262 Use of ARISA to monitor shifts in rumen microbial populations caused by changes in diet. S. E. Stebulis*[1], D. M. Stevenson[2], G. J. M. Rosa[1], and R. R. Grummer[1], [1]*University of Wisconsin*, *Madison*, [2]*USDA-ARS-US Dairy Forage Research Center*, *Madison, WI*.

The objective was to determine whether automated ribosomal intergenic spacer analysis (ARISA) is sensitive enough to detect shifts in rumen microbial populations caused by dietary changes. Six ruminally cannulated, non-lactating, non-pregnant Holstein cows were sampled for rumen contents in a randomized cross-over design. Treatments were either high forage (HF) or low forage (LF) diets offered for ad libitum intake. High forage was composed of 43.7% neutral detergent fiber (NDF) and 31.2% non-fiber carbohydrate (NFC), while LF contained 25.5% NDF and 44.6% NFC. The trial consisted of 3 periods, an adaptation period (30 d), period 1 and period 2 (14 d each). Cows were switched between periods with no adaptation. Treatment sequences, including the initial adaptation period, were HF-LF-HF and LF-HF-LF. Rumen samples were collected 4 h after feeding on d 14 of each experimental period. Bacterial DNA was extracted from each sample, and the ribosomal intergenic spacer region was PCR amplified and used for ARISA. The effects of diet, fraction (liquid vs. solid), period, and cow on changes in rumen microbe population were analyzed using principal components methodology and Chi-squared tests. Rumen pH, analyzed with a mixed model, was significantly greater for HF than for LF (6.7 vs. 6.1 ± 0.08; $P < 0.001$). There were 253 total phylotypes (unique PCR amplicon lengths) detected using ARISA. Of these phylotypes, 25 were unique to an individual cow. There were 12 phylotypes only found in liquid and 3 only in solid fractions, and 16 only in LF and 2 only in HF. Treatment had a significant effect ($P < 0.05$) on 19 phylotypes. Thirty-two phylotypes were affected ($P < 0.05$) by fraction, and 19 were significantly different between cows ($P < 0.05$). Period affected only 6 phylotypes ($P < 0.05$). These results suggest that ARISA is sensitive enough to pick up differences in rumen microbial populations due to diet composition changes, and other factors such as rumen fraction and cow.

Key Words: Microbial Population, Diet, Rumen

T263 Evaluation of n-alkanes, chromic oxide and lignin as indigestible markers to estimate duodenal and fecal flows in lactating dairy cows. S. O. Juchem*[1], E. J. DePeters[1], J. M. Heguy[1], S. J. Taylor[1], and J. E. P. Santos[2], [1]*University of California*, *Davis*, [2]*University of Florida*, *Gainesville*.

The objective was to compare estimates of duodenal and fecal flows obtained from 3 markers, being n-alkanes (ALK; internal, C31 and C33; external, C32 and C36), chromic oxide (CrO; external) and lignin (LIG; internal). Four lactating primiparous Holstein cows cannulated in the rumen and duodenum were utilized in a 4x4 Latin Square design experiment. Each period lasted 14 d, 10 d of adaptation and 4 d of sampling, whereas cows were subjected to 97% feed restriction during the last 4 d to prevent refusals. A new n-alkane slow releasing capsule (CAPTEC, Auckland, NZ) was placed into the rumen at d 0 of each

period in each cow. Chromic oxide was added at 0.11 % of diets DM. All diets had similar chemical composition, and alfalfa hay was the only forage source (47.7 % of DM). Five samples of duodenal digesta (2.5 L) and feces (~500 g) were sampled during the last 4 d of each period at different times in relation to feeding to create an average composite sample. Data were analyzed by the MIXED procedure of SAS, with a model that included effects of cow, period, diet, marker and the marker by diet interaction. DM intake was not affected by dietary treatments (20.1; 18.5; 19.5; 18.8 kg/d). Apparent whole tract digestibility of OM was highest (P<0.01) for CrO (71.9%), intermediate for C31, C33 and LIG (64.0; 65.3; 65.5%), and lowest for C32 and C36 (59.6; 60.7%). Duodenal DM flow was highest (P<0.01) when C32 and C36 dosed ALK were utilized (14.3; 14.4 kg/d), smallest for C31 and C33 dietary ALK (11.0; 10.9), and intermediate for CrO and LIG (12.3; 12.8 kg/d). Forestomach disappearance of NDF-ash free was highest (P<0.05) for C31 and C33 (41.7; 42.1%), intermediate for CrO and LIG (34.8; 32.2%), and lowest for C32 and C36 (24.6; 23.9%). Disappearance of starch was lower (P<0.05) for C32 and C36 ALK (52.4^{c}; 53.2^{c}%), however C31, C33 and CrO provided similar estimates (63.9^{a}; 64.2^{a}; 60.1^{ab} %), whereas LIG was intermediate (58.1^{bc}%). N-alkanes from feed provided estimates of diet digestibility that were close to CrO estimates, and can be utilized as an alternative indigestibility marker.

T264 The use of flow cytometry to assess rumen bacteria in dairy heifers limit fed different forage to concentrate ratios with *Saccharomyces cerevisiae*. G. J. Lascano* and A. J. Heinrichs, *The Pennsylvania State University*, *University Park*.

Counting total viable bacteria using colony-unit forming assays lacks accuracy, as this method only includes culturable bacteria capable of initiating cell division. Thus, viable bacterial counts are often underestimated and total counts unknown. Both live and total bacteria populations can be enumerated using fluorescent characteristics of cell membranes and flow cytometry. The objective of this experiment was to investigate viable and total rumen bacteria counts using LIVE/DEAD Backlight kit (Invitrogen, Corp.) when 3 levels of forage:concentrate (F:C) were fed to heifers at restricted levels of intake with or without the addition of *Saccharomyces cerevisiae* (YC, Yea-Sacc^{1026}, Alltech, Inc). Three cannulated, post-pubertal Holstein heifers (age 18 ± 1 mo) were fed corn silage (CS)-based diets in a 3-period (35 d) Latin Square design. Heifers were fed F:C treatment diets for 21 d, followed by 14 d with YC added (1 g/kg, as fed). High concentrate (HC) TMR (40% CS, 60% grain; 12.6% CP, 25% NDF), medium concentrate (MC) TMR (60% CS, 40% grain; 12.3% CP, 28% NDF), and low concentrate (LC) TMR (80% CS, 20% grain; 12.4% CP, 35% NDF) were fed once/d. Rumen fluid was sampled at -2, 0, 2, 4, 6, 8, 10, 12 h after feeding. Samples were immediately stained with LIVE/DEAD kit and analyzed with a Coulter XL-MCL single laser flow cytometer (Beckman Coulter, Inc.). Mean live bacteria count was not different among treatments (6.75, 4.77, 4.97 × 10^{11} ± 0.53 × 10^{11} cells/mL; P = 0.10) for HC, MC and LC, but YC addition increased number of viable bacteria in all treatments (P < 0.01). There was also a ration by YC interaction with greatest differences in the HC diet (P = 0.01). Total live bacteria counts decreased after feeding (P < 0.01) and began to increase 4 h after feeding. We conclude that feeding different ratios of F:C did not change total viable bacteria population, but YC increased this count in all 3 diets in this experiment.

Key Words: Viable Bacteria Count, Flow Cytometry, Yeast Culture

T265 Updates to the Cornell Net Carbohydrate and Protein System: Effects of changes in feed digestion rates and passage rate assignments on metabolizable energy and protein predictions. E. B. Recktenwald*, D. A. Ross, T. R. Overton, L. E. Chase, P. Huhtanen, and M. E. Van Amburgh, *Cornell University*, *Ithaca, NY*.

Evaluations of the Cornell Net Carbohydrate and Protein System (CNCPS) v6 were made with data from individually fed lactating dairy cows from three independent studies. As implemented, CNCPSv6 accounted for a similar proportion of the variation (86%) in first limiting (ME or MP) milk production as CNCPSv5 but with a lower bias (Tylutki et al. 2007). Further evaluations of observed data from cows fed more MP limiting diets demonstrated large biases in the MP allowable milk (up to 9 kg/d) and this was investigated. Pool sizes and rates of carbohydrate digestion were updated based on the data of Lanzas et al. (2007). In CNCPS v6, NDF degradation rates were linked to NDIN rates to based on the available data on pool degradation characteristics. The soluble protein pools sizes and rates were also evaluated and modified based on the available literature data on protein degradation and flow out of the rumen. For example, the protein A pool rates were decreased from 10,000%/h (Sniffen et al. 1992) to 200%/h. Further, the standardized precipitation method (Licitra et al. 1996) underestimated the peptide content of many forages; thus modifications to the NPN pool were made based on the available feed chemical data. Finally, within the structure of the model the soluble pools (both carbohydrate and protein) were linked to the liquid passage rate equations; they were previously linked to the solids passage rates. Data from 141 observations from individual cows and from means of independent studies were used to re-evaluate CNCPSv6 after these modifications; improvements were demonstrated in ME and MP allowable milk predictions (Table 1).

Table 1.

Prediction	CNCPS Version	Mean	Mean prediction bias	RSMPE	Residual error	Obs vs pred slope
MP	6.0	37.04	-2.57	5.52	4.88	0.73
MP	6.1	41.34	1.66	5.21	4.94	0.74
ME	6.0	44.97	5.35	7.84	5.73	0.66
ME	6.1	44.76	5.08	7.60	5.66	0.67
Most limiting	6.0	36.91	-2.70	5.49	4.77	0.74
Most limiting	6.1	40.60	0.92	4.70	4.61	0.77

Key Words: CNCPS, Metabolizable Protein, Modeling

T266 Dynamics of ruminal fiber digestion of corn milling co-products. L. O. Tedeschi[1], P. J. Kononoff*[2], K. Karges[3], and M. L. Gibson[3], [1]*Texas A&M University*, *College Station*, [2]*University of Nebraska*, *Lincoln*, [3]*Dakota Gold Research Association*, *Sioux Falls, SD*.

The corn-ethanol dry milling industry is a major producer of feedstuffs, namely distillers dried grains plus solubles (DDGS). This industry has also produced new feeds that differ in chemical composition and possibly nutrient availability. The objective of this study was to evaluate the dynamics of ruminal fiber digestion of several co-products. Thirty samples of four corn milling co-products were evaluated in this study:

DDGS, high protein DDGS (HP-DDGS), bran (BRAN), and dehydrated germ (GERM). Alfalfa hay (HAY) was used as a standard feed in the in vitro fermentation dynamics analysis. Neutral detergent residue (without sodium sulfite; NDR) averaged 32.9, 34.8, 21.9, 33.5 % for DDGS, HP-DDGS, BRAN and GERM respectively. The CP and ether extract averaged 30.8 and 11.2 %, 44.6 and 4.18 %, 15.3 and 9.49 %, and 17.4 and 17.4% for DDGS, HP-DDGS, BRAN and GERM, respectively. The feeds were fermented in vitro for a 48 h period. Gas production was measured using a computerized system and data was fitted to an exponential model to compute the fractional degradation rate (kd). Statistical analyses were performed using the random coefficients model assuming an incomplete block design. The TDN for each feed was predicted using a summative equation with the kd of NDR. The kd of NDR and defatted fiber residue (DFR) were significantly different ($P < 0.05$) for DDGS, HP-DDGS, BRAN and GERM, and was estimated to be 6.88 and 8.44 %/h, 11.8 and 11.7%/h, 6.14 and 8.52%/h, 7.24 and 9.14%/h, respectively. Robust regressions were developed to compute kd of NDR using standard chemical analysis. The results suggested that the proportion of fiber digested in the rumen were affected by degree of sample processing and fat removal. Although tabular values for the rate of NDF digestion in the CPM-Dairy model are generally indicative of many feed co-products, these results suggest that the digestion rates of fiber may vary greatly across different types of co-products. Furthermore, the use of model simulations in this study demonstrated that observed differences in fermentability and chemical composition result in differences in the supply of TDN for lactating dairy cows.

Key Words: Fiber, Co-Products, Rumen

T267 Please see abstract 57.

T295 Use of the mobile nylon bag method to determine phosphorus disappearance in common dairy cattle ration ingredients. N. M. Cherry*[1], B. D. Lambert[1,2], and J. P. Muir[1], [1]*Texas AgriLife Research, Stephenville, TX,* [2]*Tarleton State University, Stephenville, TX.*

Phosphorus (P) excretion in manure and has become a major problem facing dairy producers in much of the United States. Excess P released into the environment may pollute surface waters leading to eutrophication and excess algal growth. One approach to reducing P excretion is to avoid excess dietary P. Data regarding P availability in feedstuffs is limited and more precise ways of measuring P availability in the digestive tract are needed. In this experiment the mobile nylon bag method was used to determine the disappearance of dry matter (DM) and P in ground corn, corn silage, alfalfa hay, coastal bermudagrass hay, and Tifton-85 bermudagrass hay in steers after ruminal (24 hrs), ruminal + pepsin/HCL (rumen + PH), and ruminal + pepsin/HCL + intestinal (rumen + PH +I) incubation. Ruminal degradation of both P and DM differed ($P<0.05$) between feedstuffs, and by site of incubation. DM total-tract (rumen + PH + I) availability for ground corn, corn silage, alfalfa hay, coastal bermudagrass hay, and Tifton-85 bermudagrass hay were 90.35. 51.89, 41.66, 69.04, 71.79% respectively. Total tract (rumen + PH + I) P availability for ground corn, corn silage, alfalfa hay, coastal bermudagrass hay, and Tifton-85 bermudagrass hay were 99.22, 92.22, 94.81, 84.55, and 85.36%, respectively. The variability in the availability in P (~15%) indicates that inclusion of a P availability coefficient in ration balancing software could have a measurable impact on subsequent P excretion from dairy cattle. More data concerning P availability as affected by feed ingredient or plant species, maturity and quality are needed to more accurately define P availability in dairy cattle feeds.

Key Words: Phosphorus, Mobile Nylon Bag