## **Ruminant Nutrition: Rumen Function and Digestion**

M350 Comparison of three marker systems and three sites of digesta sampling to estimate the rumen outflow in bulls fed with corn silage or sugar cane. P. P. Rotta,\* S. de C. V. Filho, L. F. C. Silva, F. A. C. Villadiego, and E. M. Galindo, *Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.* 

The aim of this study was to evaluate the rumen outflow and the rumen and intestinal digestibility of dry matter and digesta components in crossbred bulls by use of sampling in 3 places (abomasum, omasum or reticulum) evaluating 3 marker systems (single, double or triple) with bulls feeding corn silage or sugar cane in different ratios. Eight crossbred bulls fitted with ruminal and abomasal cannulas and with 24 mo, weighting  $353 \pm 36.94$  kg were separated in  $2.4 \times 4$  Latin squares design. The experimental diets were: 1) 60% of corn silage + 40% of concentrate; 2) 40% of corn silage and 60% of concentrate; 3) 60% of sugar cane + 40% of concentrate; 4) 40% of sugar cane + 60% of concentrate. Eight digesta samples were collected in each experimental period with intervals of 9 h. At the end of each experimental period the samples were pooled and they were appointed to the 3 marker systems (single system: sample without separation; double system: filtered sample; and triple system: filtered and centrifuged sample). The whole reticular digesta composition was different (P < 0.0001) in comparison with the others to ether extract (EE), neutral detergent fiber (NDF) and indigestible NDF. The digesta sampled at the abomasum and omasum presented similar (P >0.05) values to rumen outflow to dry matter (DM), organic matter (OM) and NDF. Similarly, the abomasal and omasal digesta were similar (P > 0.05) to estimate of ruminal digestibility of DM, OM, crude protein (CP) and NDF. Digesta sampled at abomasum and omasum presented similar (P > 0.05) values to estimations of intestinal digestibility of OM, CP and NDF. The omasal sampling is recommended as an alternative to abomasal sampling. Due to no difference among the marker systems to ruminal and intestinal digestion for NDF and intestinal digestion for CP, any marker systems can be used. Whereas that there are few differences to rumen outflow and digestions by estimating with double and triple marker systems, it is recommended the use of double marker system (indigestible NDF and Co-EDTA), that can be accessible to any research center.

Key Words: abomasal digesta, ruminal digestibility, omasal digesta

M351 Comparison of in situ nylon bag protocols for analysing ruminal degradation of dry matter and crude protein in forages. H. van Laar\*<sup>1</sup>, J. Doorenbos<sup>1</sup>, J. D. Steckley<sup>2</sup>, and J. A. Metcalf<sup>2</sup>, <sup>1</sup>Nutreco R&D, Boxmeer, the Netherlands, <sup>2</sup>Nutreco Canada Agresearch, Guelph, ON, Canada.

This experiment compared degradation of forages between different in situ nylon bag protocols in 2 laboratories. In laboratory 1 fresh forages were cut to <2cm, 5g DM was weighed into nylon bags and incubated by time point in the rumen of 3 fistulated cows for 0, 3, 8, 16, 32, 56, 96 and 336 h in a net bag with a 1.5kg weight. In laboratory 2 forages were dried and ground over a 2 mm sieve, 5g DM was weighed into polyester bags and incubated in the rumen of 4 fistulated cows for 0, 1, 2, 4, 8, 16, 24 and 72 h attached to a 5 foot long nylon belt with a 1kg weight at the end. Forages were 5 grass silages, 5 grass hays, 5 haylages, 5 mixed hays (both a mix of alfalfa and grass) and 10 corn silages. After incubation samples were pooled by time point within cow and analyzed for dry matter and crude protein content. Degradation characteristics,

Washable (W), Degradable (D), Undegradable (U) and degradation rate (K<sub>d</sub>) were estimated with PROC NLIN of SAS. Effective degradability (ED) was calculated assuming a passage rate of 0.045/h. Differences between laboratories were analyzed by a paired t-test. PROC REG of SAS was used to estimate linear regression coefficients and r-square. For DM and CP (all forages combined), U and residues at 0, 8 and 16 h were lower (all P < 0.02) whereas W and ED were higher (all P< 0.0001) for laboratory 2. D fractions for DM and CP did not differ whereas  $K_d$  for DM was higher (P < 0.05) in laboratory 2. The  $r^2$  of the comparison between laboratories for U, D, W, K<sub>d</sub> and ED was 0.29, 0.07, 0.74, 0.23, and 0.87 for DM and 0.73, 0.09, 0.53, 0.41, and 0.59 for CP. When analyzed by forage type, correlation for ED of DM and CP between laboratories was good ( $r^2 > 0.5$ ) only for grass hay (0.99, 0.96), haylage (0.92, 0.88) and mixed hays (0.94, 0.55), whereas correlations were poor ( $r^2 < 0.5$ ) for grass and corn silages. The different in situ procedures result in a higher ED for laboratory 2, with acceptable correlation between degradation parameters taking all forages together; however, correlation between laboratories is not acceptable for all individual forage types.

Key Words: in situ, forage, rumen degradation

M352 Methane emissions and diet digestibility for sheep offered diets varying in fat content and forage quality. E. J. Mc Geough,\* Y.-H. Chung, K. A. Beauchemin, S. M. McGinn, and T. A. McAllister, Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada.

The objective of this study was to determine DMI, diet DM digestibility (DMD) and methane (CH<sub>4</sub>) emissions for sheep offered diets varying in fat content and forage quality. The study was a replicated (4 square) Latin Square design with 4 experimental treatments based on 2 levels of fat inclusion (low fat = 21.5, high fat = 59.6 g/kg DM) and 2 basal forages varying in nutritive quality. Fat was derived from the inclusion of canola meals and oil. To achieve variability in diet quality, oat hulls (LQ) and beet pulp (HQ) were chosen as the basal forages, accounting for 400 and 500 g/kg of diet DM, respectively. Twenty 4 Canadian Arcott sheep (16 male and 8 female), initial BW 60.4 (SD 4.80) kg, were assigned to 1 of the 4 treatments. Within treatment, sheep were assigned to 1 of 2 groups based on LW and sex to facilitate measurements in climate-controlled open circuit respiration chambers over 2 subsequent 4 d periods within each period of the Latin square. Each period consisted of 17 d of dietary adaptation followed by 4 d to measure CH<sub>4</sub> production and DMD. Three sheep were assigned to each chamber and housed individually in metabolism crates. Apparent total tract digestibility was determined by collecting total feces and urine for 4 d from group 1. Data were analyzed using PROC MIXED of SAS. Improving forage quality reduced DMI, on average 16.2%, (P < 0.05)for both low and high fat diets, but addition of fat did not affect DMI (P = 0.17) within forage type. Dry matter digestibility was also higher (P = 0.001) for HQ than LQ forage (on average 19.7%) regardless of dietary fat level. Increasing the fat content of LQ diets increased (P <0.05) DMD, however DMD of the HQ diet was not affected (P = 0.64) by fat content. Improving the quality of forage in the diet increased (P < 0.001) CH<sub>4</sub> emissions (per kg DMI) from both low fat (13.1–15.0 g/ CH<sub>4</sub>) and high (14.0–16.5 g/CH<sub>4</sub>) fat diets. Fat content did not affect CH<sub>4</sub> output (P = 0.17) for LQ diets, however CH<sub>4</sub> emissions from HQ diets were increased (9.2%; P < 0.05) with the higher level of fat inclusion.

Thus, dietary supplementation of fats may not be an effective means to mitigate  $CH_4$  emissions in high quality forage diets.

Key Words: fat, forage quality, methane

M353 Leucaena diets with or without polyethylene glycol affecting rumen fermentation and methane emission in sheep. Y. Soltan\*<sup>1,2</sup>, A. Morsy<sup>1,3</sup>, R. Lucas<sup>1</sup>, S. Sallam<sup>2</sup>, H. Louvandini<sup>1</sup>, and A. Abdalla<sup>1</sup>, <sup>1</sup>Centre for Nuclear Energy in Agriculture, University of Sao Paulo, Piracicaba, Sao Paulo, Brazil, <sup>2</sup>University of Alexandria, Alexandria, Egypt, <sup>3</sup>Animal Production Research Institute, Cairo, Egypt.

This study evaluated the effect of leucaena (Leucaena leucocephala) diet with or without polyethylene glycol (PEG) on rumen fermentation and methane (CH4) emission in vivo. Six adult rumen cannulated Santa Inês sheep ( $70 \pm 2.5$  kg of BW) divided in to 3 experimental diets completing a double Latin square design [3 treatments, 3 periods (17 d each), 6 animals] as follow: control (CNTRL-70% Tifton hay (Cynodon spp.) + 21% ground corn, 9% soybean meal); leucaena diet that consisted of the control diet replaced by 0.50 of Tifton hay with leucaena (LEUC -123 and 8.8 g/kg DM of total tannins and condensed tannins respectively) and leucaena diet plus 20 g/day/animal of PEG (LPEG). Leucaena diets (LEUC and LPEG) decreased (P < 0.0001) the CH4 emission per kg digested organic matter by 0.14 and 0.11, respectively as well as decreased (P < 0.0001) the rumen acetate:propionate ratio compared with CNTRL, however LPEG increased butyrate (P = 0.0034) when compared with the LEUC. No differences (P > 0.05) were detected for rumen pH or protozoa count for all the experimental diets while LEUC showed the lowest (P < 0.0001) rumen ammonia concentrations (20.4, 19.6 and 24.3 (SEM = 1.350) mg/100 mL respectively for CNTRL, LEUC and LPEG). These results showed that leucaena diets either with or without PEG addition reduced methane emission by manipulating rumen fermentation.

**Key Words:** tannins, polyethylene glycol, methane

M354 Biometrics digestive tube of kids suckled up to 60 days fed different goat milk replacers. L. S. Knupp, M. I. Marcondes,\* C. S. Cunha, T. S. Oliveira, J. G. L. Regadas Filho, J. C. M. Lima, L. C. Lacerda, and C. M. Veloso, *Universidade Federal de Viçosa, Viçosa, MG, Brazil.* 

There are few studies on the development of the digestive tract of goats. The study of non-carcass parts is important, because they tend to vary with diet, directly affecting carcass yield and weight gain. This work aimed to evaluate the biometrics of the digestive tract of goats suckled up to 60 d fed different goat milk replacers. Twenty-four Saanen (12) and Alpine Brown (12) kids were randomly assigned to treatments and were slaughtered at 60 d. They were offered coast-cross hay, starter ad libitum, and one liter of milk, or replacer, per day. The treatments were: goat milk (GM), cow milk (CM), fermented cow colostrum (FC) and lactal - commercial milk replacer (LAC). The organs were separated and weighed without digesta. The parameters analyzed were: Rumen, omasum, abomasum, small and large intestine. Data were analyzed using a complete randomized design, using the initial weight as a covariate. When a treatment effect was observed, the Tukey test was applied to evaluate the least squares means, using a significance level of 5%. The treatments did not affect any of the evaluated parameters (P > 0.05). The averages of rumen weight for kids who received CM, LAC, GM, and FC, respectively, were: 0.231, 0.185, 0.207, 0.163 kg; the average omasum weights were: 0.019, 0.012, 0.015, 0.009 kg; the average abomasum weights were: 0.090, 0.077, 0.085, 0.082 kg; the average small intestine weights were: 0.358, 0.296, 0.297, 0.262 kg; and the average large intestine weights were: 0.179, 0.263, 0.157, 0.129 kg. We concluded that all evaluated goat milk replacers can be used for feeding goats up to 60 d without affecting the development of their digestive tract. Supported by CNPq/INCT-CA.

Key Words: abomasum, omasum, rumen

M355 Comparative influence of solvent extracted-peanut meal and soybean meal on apparent digestibility of diets for finishing lambs. L. R. Flores\*1, A. Camacho¹, N. E. Villalba², J. J. Lomelí¹, and R. Barajas¹, ¹FMVZ-Universidad Autonóma de Sinaloa, Culiacán, Sinaloa, México, ²Agricola Ganadera Mojolo, Culiacán, Sinaloa, México.

Four hair lambs  $22.3 \pm SE 0.5$  kg were used to compare influence of solvent extracted-peanut meal and soybean meal on apparent digestibility of diets for finishing lambs. In an experiment with a Crossover design, lambs were assigned to consume one of 2 diets in that consisted the treatments: 1) Diet containing 19.6% CP, integrated (DM basis) with soybean meal 27%, corn 57%, corn straw 8.3%, sugar cane molasses 5.0%, and 2.7 of mineral premix (SBM); or 2) Diet containing 21.4% CP, where SBM was completely substituted by solvent extracted peanut meal (SEPM). The crude protein content of SEPM was 52.3% and its ether extract value was 2.2%. Each 11-d experimental period was integrated for 7 d adaptation phase and 4 d total feces collection phase. Lambs were fed 95% of appetite to prevent refusals. Dry matter intake, crude protein intake, dry matter excreted in feces, and crude protein excreted in feces were similar between treatments (P > 0.30). Apparent digestibility of dry matter was no affected by treatments (P = 0.78) with mean values of 82.2 and 82.7% for SBM and SEPM, respectively. Crude protein apparent digestibility was similar in both protein-meal sources (P = 0.80), with means values of 83.3 and 83.9% for soybean meal-diet and peanut meal-diet, respectively. Digestible energy of diets was unaffected by treatments (P = 0.78). Using substitution method and soybean meal as ingredient of reference, true CP-digestibility of peanut meal was calculated in 95% and its digestible energy content in 3.8 Mcal/ kg of DM. It is concluded, that peanut meal can substitute soybean meal completely in finishing lambs diets without altering nutritional value of the diet. The true CP-digestibility of peanut meal is 95% and its digestible energy content is 3.8 Mcal/kg of DM.

Key Words: digestibility, lambs, peanut meal

M356 Development of a new marker for utilization in digestibility studies. C. C. C. Couto Filho<sup>1</sup>, E. O. S. Saliba\*<sup>1</sup>, M. N. Pereira<sup>2</sup>, N. M. Rodriguez<sup>1</sup>, and N. N. Morais Júnior<sup>3</sup>, <sup>1</sup>Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, <sup>2</sup>Universidade Federal de Lavras, Lavras, MG, Brazil, <sup>3</sup>Instituto Federal de Educação Ciência e Tecnologia do Espírito Santo, Colatina, ES, Brazil.

A new marker using nanoparticles was developed by a research group at the Federal University of Minas Gerais. This trial was conducted to evaluate the estimates of fecal output and digestibility in dairy cattle with this new marker. Ten Holstein cows were used in the trial, which had consisted of 14 d for adaptation to the diet, 10 d of adaptation to chromic oxide and 2 d to nanomarker. The markers were also provided during the collection period. The markers were administered separately in capsules containing 500 mg of nanomarker and 10 g of chromic oxide. The fecal output and the digestibility were determined by total collection and by different markers, by sampling at intervals for 6 h, directly from the rectum of each animal, between fifteenth and nineteenth days.

Feces for the analysis of the nanomarker were collected during 24 h, starting on the sixteenth day. The dry matter content (AOAC, 1995), chromic oxide (Williams et al., 1962) and the nanomarker (near infrared reflectance spectroscopy - NIRS) in feces was determined. The statistical design was randomized blocks, with the animals being the blocks. The chromic oxide overestimated fecal output and consequently underestimated digestibility, fact which can be evidenced by the incomplete recovery of this marker in the feces (Table 1). In contrast, the results obtained with the nanomarker does not differ from the total collection method, showing complete fecal recovery (Table 1). The highest velocity dispersion of nanoparticles in the gastrointestinal tract provided a better homogenization of the material in the digesta, allowing a steady state faster. Thus, the nanomarker can be used as an marker in digestibility trials to replace the total collection.

**Table 1.** Fecal output, fecal recovery and digestibility by different techniques

Technique	Fecal output (kg)	Fecal recovery (%)	Digestibility (%)
Total collection	6.6 <sup>a</sup>	100.0a	72.1 <sup>a</sup>
Nanomarker	6.5a	100.8a	71.9 <sup>a</sup>
Chromic oxide	9.0 <sup>b</sup>	139.3 <sup>b</sup>	62.2
SEM	0.17	2.88	0.93
P value	< 0.001	< 0.001	< 0.001

 $^{a,b}\mbox{Means}$  followed by different letters in the same column differ (P < 0.05) by Tukey test.

**Key Words:** fecal output, markers, nanotechnology

M357 Effects of vitamin B<sub>12</sub> on in vitro rumen fermentation and microbial enzyme activity. Y. X. Li, J. K. Wang, Y. M. Wu, and J. X. Liu,\* Institute of Dairy Science, MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, China.

This study was conducted to investigate the effects of vitamin B<sub>12</sub> (VB<sub>12</sub>) on rumen fermentation and microbial enzyme activity in an in vitro study with a 3 × 3 factorial arrangement of treatments. Substrates were designed with 3 ratios of forage and concentrate (C/F, 35:65, 50:50 and 65:35) and 3 doses of VB<sub>12</sub> (0, 40 and 90 ng/mL). Rumen fluid was collected from 3 rumen cannulated Hu sheep fed a diet consisting of Chinese wildrye hay and concentrate mixture (60:40) twice daily. Cobalt was not added into the incubation medium (mixture of rumen fluid and buffer solution) so that it did not interfere in the effect of vitamin  $B_{12}$ . Gas pressure measurements were recorded at 2, 4, 6, 9, 12 and 24 h of incubation and then converted to gas volume. Ammonia N, pH, volatile fatty acids, microbial protein, and activities of carboxymethylcellulase, xylanase and amylase were determined at 24 h incubation. Data were analyzed using the GLM procedure of SAS. Terminal pH varied within normal range (6.52 to 6.65) regardless of treatment or substrate. With the increasing C/F ratio and additional doses of VB<sub>12</sub>, gas production at 24 h incubation was enhanced (P < 0.01) and VB12 had a linear effect. Addition of VB<sub>12</sub> to the substrate at C/F ratio of 35:65 did not have effects on all the rumen fermentation parameters (P > 0.05), while ammonia-N, volatile fatty acids, and microbial protein increased (P < 0.05) at 40 and 90 ng/mL VB<sub>12</sub> when the C/F ratio was at 50:50 and 65:35. Activities of carboxymethylcellulase, xylanase and amylase increased (P < 0.05) at 40 and 90 ng/mL VB<sub>12</sub> for the C/F ratio of 35:65 and 50:50, and at 90 ng/ mL VB<sub>12</sub> for C/F ratio of 65:35. Although the C/F ratio and VB<sub>12</sub> dose had varying effects on in vitro rumen fermentation, the results of this study indicate that addition of VB<sub>12</sub> can improve rumen fermentation

through enhancing microbial enzyme activity with pronounced effect at higher C/F ratio.

**Key Words:** microbial enzyme activity, rumen fermentation, vitamin B<sub>12</sub>

M358 Urinary recovery of purine derivatives and microbial production in Nellore cattle. A. M. Barbosa\*1, R. F. D. Valadares², S. de C. V. Filho², D. do S. Pina³, and M. A. Fonseca²⁴, ¹Universidade Federal da Bahia, Salvador, Bahia, Brazil, ²Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, ³Universidade Federal do Mato Grosso, Sinop, Mato Grosso, Brazil, ⁴Texas A&M University, College Station.

The objectives of this study were to estimate the abomasum proportion of purines recovered as purine derivatives (PD) in urine, and verify the effect of intake level on microbial production (Nmic) in Nellore cattle. The animals were fed with one diet with different levels of DM offer. The trial was performed using 8 cannulated (rumen and abomasum; moreover 4 of those with ileum cannula) heifers with average BW of 258±20 kg. The animals were allocated on individual stalls and fed twice a day. The statistical analyses were performed using 2 balanced Latin square designs (4 x 4). Treatments were constituted of four levels of DM offer: 1.2, 1.6, 2.0 and 2.4% of BW. The average of RNA apparent digestibility coefficient obtained was 75.63%, but it was not affected (P>0.05) by the levels of DM offered in diet. The daily flux of RNA, and the Nmic into the abomasum as a function of DM offered in the diet presented quadratic behavior. Estimates were maximum of 1.50 mmol/ kg<sup>0.75</sup> and 55.20 g Nmic for the DMI of 2.43 and 2.45% of BW. Daily urinary excretion of PD, in mmol/kg<sup>0.75</sup>, increased linearly (P>0.05) with the level of DM offered in diet. When the excretion of PD, expressed in mmol/day basis, was related with TDN intake, kg/day, was observed (P>0.0001) an excretion of 32.15 mmol for each kg of TDN consumed. Microbial production estimated by PD in urine as a function of the level of DM offered in the diet, presented linear behavior (P>0.05). The recovery of the absorbed PD in urine was 0.86.

Key Words: level of intake, RNA, ruminant nutrition

M359 Methane emission potential, chemical composition and degradability of banana crop wastes for ruminants. L. N. Oliveira\*<sup>1</sup>, S. L. S. Cabral Filho<sup>1</sup>, and L. C. Geraseev<sup>2</sup>, <sup>1</sup>University of Brasília, Brasília, Federal District, Brazil, <sup>2</sup>Federal University of Minas Gerais, Montes Claros, Minas Gerais, Brazil.

Five substrates, consisting of banana leaves hay, banana pseudostem hay, coastcross hay and 50% substitution levels of coast-cross hay to leaves or pseudostem banana hay were evaluated for the chemical composition, cumulative gas production, methane emission potential and degradability. The semi-automated in vitro gas production technique was employed to determine the cumulative gas production with 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 48, 72 and 96 h after incubation in ruminal fluid of sheep and cattle. Each sample included 3 repetitions per inoculum, with 6 replicates to evaluate the dry matter degradability in 2, 6, 12, 24, 48 and 96 h. After 24 and 48 h the gas was storage to evaluate the methane concentration using gas chromatograph. The experimental design was completely randomized in  $5 \times 2$  factorial, with substrates and inoculum factors. Data for cumulative gas production, degradability and volume of methane were subjected to variance analysis and the means compared by Tukey test (5%). The fermentation kinetics parameters was estimated using the France model. The banana leaves hay had the highest crude protein content (13.8%), while in pseudostem hay this nutrient level was lower (3.5%). This last substrate had small values of neutral detergent

fiber (64.6%) and acid detergent fiber (36.2%) and high proportion of non-fibrous carbohydrates (28.4%), when compared with the coastcross hay. After 24 h fermentation, the pseudostem hay had cumulative gas production higher than others substrates (P < 0.05), reflecting their greater effective degradability (76.3%). The lowest rate of effective degradability was observed for the coastcross hay (46.3%), resulting in low gas production. The 50% addition level of leaves or pseudostem banana hay to the coastcross hay improved the fermentative quality of the substrate, being observed increase in effective degradability, up 10.6%, and in maximum potential gas production, up 14.7 mL/g of dry matter. The methane emission was lower (P < 0.05) for banana leaves and coastcross hay, both not differing from each other (P > 0.05).

Key Words: alternative forages, methane, ruminant nutrition

M360 Corn grain or citrus pulp associated with glycerin in Nellore feedlot steers: intake and ruminal fermentation. V. R. Fávaro,\* J. M. B. Ezequiel, J. R. Paschoaloto, M. T. C. Almeida, A. P. D'Áurea, A. C. Homem Junior, and V. C. Santos, São Paulo State University, Jaboticabal, São Paulo, Brazil.

In most studies glycerin has been evaluated as an energy source replacing the corn of the diet. In Brazil, citrus pulp represents an interesting alternative for feedlot cattle, due to its nutritional characteristics, good availability and lower price when compared with corn. The objective of this research was to evaluate the association of corn grain or citrus pulp with glycerin in diets for Nellore feedlot steers on dry matter intake, rumen pH and rumen ammonia concentration. Five rumen cannulated Nellore steers (420  $\pm$  20 kg BW) were used in a 5x5 Latin square design. Experimental diets consisted of 30% corn silage and 70% concentrate (corn grain, citrus pulp, soybean hulls, urea and glycerin) and were labeled as (DM basis): diet with no added glycerin (CON), 30% of corn grain with 10% of glycerin (CG10), 25% of corn grain with 15% of glycerin (CG15), 25% citrus pulp with 10% of glycerin (CP10), 20% of citrus pulp with 15% of glycerin (CP15). To determine pH and ammonia concentration, rumen fluid samples were taken through the rumen cannula at the time of feeding and, 1, 2, 4, 6 and 8 h after feeding. Statistical analysis was performed using the MIXED procedure of SAS. Orthogonal contrasts were used to determine the effect of 0% glycerin vs glycerin treatment, CG10 vs CG15, CP10 vs CP15 and corn grain vs citrus pulp treatments. For pH, and ammonia data the time of harvest was included as a repeated measure. The rumen pH and ammonia concentrations were not influenced (P > 0.05) by the treatments. The mean values of pH and ammonia concentration were respectively 6.38 and 17.99 mg/dL. DM intake decrease (P < 0.009) when corn grain treatments was compared with citrus pulp treatments, the mean values were 7.27, 7.55, 7.24, 5.50 and 6.56 kg/day respectively for CON, CG10, CG15, CP10 and CP15. These data suggest that the use of glycerin at 10 or 15% with corn or citrus pulp does not affect the rumen pH neither ammonia concentration but, diets with more citrus pulp proportion causes decrease in dry matter intake.

Key Words: biodiesel, byproducts, feedlot cattle

M361 Assessment of the in vitro fermentation pattern of native forage from the Brazilian semiarid region. D. K. A. Silva\*<sup>1,2</sup>, L. O. Tedeschi<sup>2</sup>, M. A. Fonseca<sup>3,2</sup>, N. F. De Paula<sup>3,2</sup>, K. P. Pereira<sup>1</sup>, G. R. Medeiros<sup>4</sup>, J. C. B. Dubeux Junior<sup>1</sup>, and D. P. V. Silva<sup>1</sup>, <sup>1</sup>Federal Rural University of Pernambuco, Academic Unit of Garanhuns, Garanhuns, Pernambuco, <sup>2</sup>Texas A&M University, Department of Animal Science, College Station, <sup>3</sup>Federal University of Viçosa, Department

of Animal Science, Viçosa, Minas Gerais, Brazil, <sup>4</sup>National Institute of Semiarid, Campina Grande, Paraíba.

This work was aimed to evaluate the in vitro gas production (IVGP) and the fermentation kinetic parameters of 6 Brazilian native forages from the semiarid region in 2 seasons (PY) of the year: rainy (RS) and dry (DS) seasons. The forages analyzed were Calotropis procera (AS), Croton sonderiamus Müll Arg (M), Caesalpinia pyramidalis Tul. (CA), Macroptilium martii Benth. (OO), Ipomoea glabra Choisy (JIT), and Herissantia crispa (L.) (MB). The statistical analyses were performed using a random coefficient model assuming PY as a random variable and forage as a fixed variable. The IVGP technique was used to determine the total gas production (TGP). The results indicated that only PY affected TGP (P < 0.05) (Table 1). JIT had a greater value during the RS than DS season. During the RS period, AS and CA had the greatest and M had the lowest TGP values. During the DS period, AS and OO presented the greatest while JIT and M had the lowest TGP values. The greatest fractional rate of fermentation (kf h<sup>-1</sup>) was observed for AS, which also had the greatest lag time (1,351  $\pm$  0,24), suggesting that microbes needed more time to start the fermentation of AS but they were able to ferment it quickly. This is likely because either AS had greater amounts of nonfiber carbohydrates or a specific group of secondary compounds that interfered on the in vitro fermentation. These results indicated that these forages, except for JIT, have the potential to be used as alternative feed sources with no disadvantage in the ruminal fermentative aspects throughout the year under the Brazilian semiarid region.

Table 1. In vitro gas production and fermentation parameters of Brazilians semiarid native forages

	Forages						
PY	AS	CA	JIT	OO	MB	M	
Rainy season IVGP (mL/100 g DM)	26.83 ±1.09 <sup>A</sup> ,a	25.31 ±1.08 <sup>A,b</sup>	24.65 ±0.92 <sup>A,ab</sup>	23.05 ±0.99 <sup>A,b</sup>	24.07 ±1.08 <sup>A,b</sup>	17.25 ±0.99 <sup>A,c</sup>	
Dry season IVGP (mL/100 g DM)	$^{26.60}_{\pm 0.99^{A,a}}$	$^{22.91}_{\pm 0.99^{A,b}}$	$^{19.18}_{\pm 0.92^{B,c}}$	$^{24.24}_{\pm 0.92^{A,ab}}$	$^{22.73}_{\pm 0.92^{A,b}}$	$19.87 \\ \pm 1.22^{A,c}$	
$kf(h^{-1})^1$	$0.165 \pm 0.01^{a}$	0.099 ±0.01 <sup>cd</sup>	0.106 ±0.01 <sup>cd</sup>	0.106 ±0.01°	$0.133 \pm 0.01^{b}$	0.086 ±0.01 <sup>b</sup>	

 $^{\rm a-d}Values$  within rows with same lowercase letters do not differ (P > 0.05).  $^{\rm A,B}Values$  in columns (for IVGP) with same uppercase letters do not differ (P > 0.05).

<sup>1</sup>Fractional rate of fermentation.

Key Words: Brazilian semiarid, IVGP, lag time

M362 An in vitro evaluation of rumen fluid type on hydrogen sulfide production of common beef cattle feedstuffs. K. L. Neuhold\*1, J. J. Wagner², K. S. Sellins¹, and T. E. Engle¹, ¹Colorado State University, Fort Collins, ²Southeast Colorado Research Center, CSU, Lamar, CO.

Rumen fluid from fistulated steers receiving a high roughage (ROU; 50% alfalfa hay, 50% corn silage) or a high concentrate-based diet (CON; 70% rolled corn, and 30% corn silage) was utilized to examine in vitro hydrogen sulfide (H<sub>2</sub>S) production of common beef cattle feedstuffs. Rumen fluid was collected and combined in equal amounts from 2 rumen fistulated steers that had ad libitum access to ROU and mixed at a 3 to 1 ratio of artificial saliva to rumen fluid. Fermentation substrates included: corn, alfalfa, consolidated corn distillers soluble, dried distillers grains (DDG), and wet distiller's grain (WDG). Individual substrates (700mg) were added to separate 125 mL glass serum bottles (in triplicate) with 50 mL of rumen fluid-artificial saliva mixture. Fermentation bottles were capped with an air tight rubber stopper and incubated in a water bath for 24 h at 39°C. After 24 h of incubation, the total volume of gas produced

was measured and a 5-mL gas sample was obtained. Gas samples were then analyzed for H<sub>2</sub>S concentration, after gas sampling, pH and dry matter disappearance (DMD) were determined. This experiment was then repeated using rumen fluid from steers fed CON. A feedstuff by rumen fluid type (ROU vs. CON) interaction (P < 0.001) was detected for µmol of H<sub>2</sub>S produced per mg of DMD. Consolidated corn distillers soluble substrate produced less H<sub>2</sub>S per mg of DMD when incubated with CON compared with ROU rumen fluid while corn, alfalfa, DDG and WDG produce greater µmol of H<sub>2</sub>S per DMD when incubated in CON compared with ROU rumen fluid. Across diets (ROU vs. CON) fermentation of corn produced the lowest pH and fermentation of alfalfa produced the highest pH and across feedstuff ROU had a higher pH when compared with the CON rumen fluid. These data suggest that type of rumen fluid (ROU vs. CON) and feedstuff can influence the production of H<sub>2</sub>S. Understanding factors that influence H<sub>2</sub>S production within the rumen may be useful when formulating beef cattle diets.

Key Words: hydrogen sulfide, in vitro, sulfur

M363 In vitro methane production and dry matter degradability of citral. T. S. Canaes,\* I. C. S. Bueno, F. G. Vilela, A. P. C. Araújo, M. C. B. Santos, B. C. Venturelli, S. N. Macedo, J. E. Freitas Junior, and F. P. Rennó, Sao Paulo University, Sao Paulo, Sao Paulo, Brazil.

Citral, a major component of lemongrass oil has several applications in industry and also antibacterial, antifungal, and anticarcinogenic effects. The aim of this study was to determine the in vitro effect of different concentrations of citral from 2 different industries (sources S<sub>1</sub> and S<sub>2</sub>) on methane production and ruminal degradability of a ruminant diet using a semi-automatic gas production technique. A total mixed ration (50% maize silage and 50% concentrate) was incubated with buffered rumen fluid and different levels of citral (0, 15, 30, 45 and 60µL/mL) at 39°C for 72h. Gas production was measured at 4, 8, 12, 16, 24, 30, 36, 48, 60 and 72h of incubation and the dry matter disappearance at 12, 24, 48 and 72h. Data were analyzed using PROC MIXED of SAS 9.1. Differences were observed for sources (P = 0.017) and doses (P = 0.017) = 0.001). The inclusion of citral increased dry matter degradability (DMD) for  $S_1(DMD = -0.0006S_1^2 + 0.0921S_1 + 21.611$ ;  $R^2 = 0.58$ ) and for  $S_2(DMD = -0.0076S_2^2 + 0.5949S_2 + 20.968; R^2 = 0.99)$ , but the  $S_2$  was more efficient. Unlike what has been expected, the citral, regardless of source, increased production of methane. There was quadratic effect in the methane production to  $S_1(CH_4 = 3.186 + 2.077S_1 - 0.021S_1^2; R^2 = 0.91;$ P = 0.001), 4.69; 23.61; 55.09; 47.73; 52.49 mL/g DMD and S<sub>2</sub> (CH<sub>4</sub> =  $4.228+2.603S_2-0.032S_2^2$ ;  $R^2=0.83$ ; P=0.001), 4.71; 30.96; 64.63; 43.67; 45.37 mL/g DMD, respectively to doses 0, 15, 30, 45, 60, and the interaction of citral doses and sources was significant ( $P \le 0.001$ ). Ammonia-N concentration was not altered with inclusion of citral at different doses. There was significant effect of citral on the methane production and dose containing 45 and 60  $\mu$ L/mL decreased significantly ( $P \le 0.05$ ) the production. The effects of citral would not be nutritionally beneficial to the ruminal energetic metabolism. Fapesp project number 2011/06260-9.

Key Words: degradation, gas production, lemongrass

M364 Estimated microbial production, efficiency and nitrogen balance in sheep fed high concentrate varying amounts of glycerin from soybean biodiesel. R. L. Galati,\* R. S. Gomes, P. G. Paiva, L. S. Cabral, J. T. Zervoudakis, J. G. Abreu, L. R. Rebelo, and M. Zanchetin, Universidade Federal do Mato Grosso, FAMEV/UFMT, Cuiabá, Brazil.

The objectives of this study were to evaluate the effect of glycerin from soybean biodiesel in high-concentrate diets on ammonia concentrations,

nitrogen balance, excretion of purine derivatives, and microbial production (Pmic) and efficiency (Efmic). Animals were housed in individual pens, randomly allocated in a  $5 \times 5$  Latin square design, and the dietary treatments were: 0, 5, 10, 15 and 20% of glycerin on diet DM. The glycerin used was derived from soybean biodiesel production (98% glycerol, 19.2% fat, 0.98% methanol, 0.18% ethanol, 101 mg KOH/g). Five male sheep, castrated and cannulated in the rumen, with initial body weight of  $56.0 \pm 9.02$  kg were used in this study, and fed isoproteic diets (12.5%) CP), containing 25% corn silage and 75% concentrate, on DM basis. For each 50 mL of ruminal fluid collected, 1 mL of 50% sulfuric acid was added to preserve samples during freeze. Feed, refusal, total fecal and urinary output was collected on 16 d through 20 d of each period. Feces were collected with canvas bag and the urine was collected into plastic vessels containing 50 mL of 20% v/v sulfuric acid for each 450 mL of urine to perform the nitrogen (N) balance, estimates of Pmic and Efmic from the total excretion of purine derivatives in urine. Ammonia concentrations were no effect by glycerin (P > 0.11), with an average of 17.24 mg/dL. There was a quadratic effect (P < 0.11) on nitrogen (N) intake and absorption, with maximum values of 9.32 and 9.67% glycerin, respectively, but without influence (P > 0.11) on N retention (10.28 g/d), which corresponded to 56,35% of the absorbed. Fecal and urinary excretion of N were not affected (P > 0.11), with average values of 4.73 and 7.37 g N/d, in the same order. Allantoin, uric acid, xanthine + hypoxanthine excretions (P > 0.11) were 7.68, 0.50 and 0.29 mmol/d, respectively, which corresponded to 89.25, 6.35 and 4.39% of total purines. Purine derivatives excretion (8.48 mmol/d) were not affected (P > 0.11) resulting in a Pmic of 46.03 g/d and Efmic of 45.45 g CP/kg TDN. Inclusions up to 20% glycerin could be recommended without compromising nitrogen balance, microbial production and efficiency in high-concentrate diets to sheep.

Key Words: ammonia concentrations, glycerol, purine derivative

M365 Microbial kinetics, fermentative and chemical characteristics in solid-state fermentation of apple bagasse. O. Ruiz<sup>1</sup>, Y. Castillo<sup>2,1</sup>, C. Angulo<sup>1</sup>, C. Rodriguez<sup>1</sup>, O. Enriquez\*<sup>1</sup>, and C. Arzola<sup>1</sup>, <sup>1</sup>Facultad de Zootecnia y Ecología de la Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, México, <sup>2</sup>División multidiciplinaria de Nuevo Casas Grandes de la Universidad Autónoma de Ciudad Juarez Chihuahua, Nuevo Casas Grandes, Chihuahua, México.

Nutritional characteristics of solid wastes, and its further utilization as an animal feed, can be improved by solid-state fermentation (SSF). This technology is an efficient way to improve degradation of lignocellulosic compounds and enhance the amount and quality of protein fraction. The aim of this trial was to evaluate the effect of incubation time on the fermentative profile and chemical content of fermented apple residues collected after industrial processing for juice extraction. Apple waste was ground and mixed with (as % fed basis) 1.5 urea, 0.2 of ammonium sulfate, 0.9 calcium carbonate and 0.5 of a mineral salt mixture. Calcium carbonate was added to promote a slight alkaline substrate. These ingredients were mixed and then 340g (as fed basis) as substrate were distributed to separate sterile 500mL flasks plugged with cotton and incubated under static conditions at 32°C for 0, 12, 24, 36, 48 and 72h. A completely randomized design was used with 4 replications at each incubation time and data was analyzed with the GLM procedure of SAS. The pH values decreased (P < 0.0001) progressively over time and lactic acid increased (P < 0.0001) through time of incubation. Yeast cell counts (expressed in log10) also increased in the first 48h and decreased subsequently (P  $\leq$  0.0001; 7.02, 7.19, 7.64, 7.78 and 7.26 cfu/mL).Lactobacilli colonies increased to the 36h and then decreased ( $P \le 0.0001$ ; 7.86, 8.71, 9.80, 9.69 and 9.54 cfu/mL). Dry matter content (%) was increased in the first

12h (P<0.001; 18.98, 20.28, 20.08, 20.51, 20.33 and 20.53). Crude and true protein content (%) increased (P<0.0001) also over time (31.7, 33.9, 33.8, 34.9, 35.0 and 35.2; 15.8, 18.8, 21.1, 19.2, 22.6 and 23.9 respectively). Neutral detergent fiber (%) was not affected in the first 12 h (P<0.05), but diminished from 12 to 36 h (P<0.002). According to these results it can be concluded that a 48-h time of fermentation was enough to accomplish an adequate yeast cell growth and obtain an improvement of the protein fraction of fermented residues of the apple industry.

Key Words: apple bagasse, nutritional, solid-state fermentation

**M366** Evaluation external and internal markers for digestibility studies. T. T. Berchielli<sup>1</sup>, R. C. Canesin<sup>1</sup>, D. A. Mota<sup>2</sup>, I. M. Cezinbra<sup>3</sup>, and G. Fiorentini\*<sup>1</sup>, <sup>1</sup>São Paulo State University, Jaboticabal, São Paulo, Brazil, <sup>2</sup>Federal University of Amazonas, Parintins, Amazonas, Brazil, <sup>3</sup>Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

The objective of this experiment was to evaluate complexed chromium (Cr-EDTA) and ytterbium chloride (YbCl<sub>2</sub>) as external markers, and indigestible neutral detergent fiber (INDF) such internal marker digestibility in comparison with total fecal collection (TC). Eight crossbred heifers (Holstein/Zebu) with 202.12 kg  $\pm$  11.54 of BW, were used. The experimental design was a replicated 4 × 4 Latin square. The diets were isonitrogenous (13% CP), and were composed by different protein sources: soybean meal (60% sugar cane, 15% soybean meal, 23.5% corn grain, 1% mineral and 0.5% urea); cottonseed meal (60% sugar cane, 14.2% cottonseed meal, 23.8% corn grain, 1% mineral and 1% urea); peanut meal (60% sugar cane, 13% peanut meal, 25.3% corn grain, 1% mineral and 0.7% urea); and sunflower meal (60% sugar cane, 16.2% peanut meal, 21.75% corn grain, 1% mineral and 0.85% urea). Each period consisted of 7 d of adaptation and 21 d of sample collections. Total feces were collected for 5 consecutive days during each period. Composite diet and fecal samples were analyzed for nutrients (chemical composition), and concentration of chromium and ytterbium was obtained in atomic absorption spectrophotometer and INDF in 11 d ruminal incubation (non-woven textile bags, 20 mg DM/cm<sup>2</sup>). Apparent total digestibilities of all nutrients were lower for INDF compared with Cr-EDTA, YbCl<sub>3</sub> and TC. Digestibility estimated using Cr-EDTA, YbCl<sub>3</sub> or TC were not different (Table 1). In conclusion, using INDF as a digestibility marker yielded greater fecal output estimates and consequently lower apparent digestibility values for all dietary nutrients compared with TC or Cr-EDTA, YbCl3. In the conditions of this experiment, Cr-EDTA and YbCl3 was a more appropriate digestibility marker than INDF.

Table 1. Comparisons of methods for determining total tract apparent digestibility

Item	TC	Cr-EDTA	YbCl <sub>3</sub>	INDF	P-value	SEM
DM	67.62a	66.86a	67.01a	63.62 <sup>b</sup>	< 0.001	4.61
OM	65.23a	64.80a	65.54a	61.30 <sup>b</sup>	< 0.001	6.68
CP	67.20a	65.11a	66.29a	62.57 <sup>b</sup>	< 0.001	8.24
NDF	50.53a	49.07a	48.83a	44.43 <sup>b</sup>	< 0.001	8.06

 $<sup>^{</sup>a,b}$ Means within a row followed by different letters are different (P < 0.05) by Tukey test.

Key Words: digestibility, heifers, markers

M367 Addition of fumarate reducing bacteria on in vitro fermentation. L. Mamuad, S. Kim, C. Jeong, and S. Lee,\* Sunchon National University, Suncheon, Republic of Korea.

Fumarate reducing bacteria utilized hydrogen which is one of the main substrate in methanogenesis. This study was conducted to determine if addition of these bacteria on in vitro fermentation can reduce methane, increase volatile fatty acids (VFA) production, and effect on its microbial diversity. The ruminal samples were collected from Holstein cows, mixed with a basal medium (1:3), transferred into 50-mL serum vials containing rice straw and filled under a stream of O2-free N2 gas. Mitsuokella jalaludinii and Veillonella parvula were added to the serum vials and incubated. After incubation, pH, total gas production, VFA, and methane emission were measured. Moreover, diversity of microorganism was determined by DGGE using universal and specific primer. All data were analyzed using SAS (2003). M. jalaludinii significantly reduces methane at 48 h of incubation. In addition, M. jalaludinii significantly increases succinate at 12, 24 and 48 h of incubation followed by V. parvula and then control. Furthermore, total volatile fatty acid was significantly higher in V. parvula at 24 h of incubation. These results suggest that addition of fumarate reducing bacteria to ruminal fermentation reduces methane, increases succinate and total volatile fatty acid, and changes the rumen microbial diversity.

**Key Words:** fumarate reducing bacteria, in vitro, DGGE

M368 Rumen wall morphology of lambs fed high concentrate diets. L. S. Oliveira\*<sup>1</sup>, P. R. Leme<sup>1</sup>, M. R. Mazon<sup>1</sup>, D. M. C. Pesce<sup>2</sup>, S. da Luz e Silva<sup>1</sup>, C. A. Zotti<sup>1</sup>, R. F. Carvalho<sup>2</sup>, and A. P. dos Santos Silva<sup>1</sup>, <sup>1</sup>Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga, São Paulo, Brasil, <sup>2</sup>Pontificia Univerdidade Católica, Poços de Caldas, Minas Gerais, Brasil.

High concentrate diets results in high rumen fatty acids concentrations which stimulates epithelium proliferation of papillae causing parakeratosis and increased occurrence of injury and inflammation. To evaluate different forms of corn grain processing on the rumen wall and papillae morphology 23 Santa Inês × Dorper male lambs with mean BW of 28 kg and 90 d old were fed a high concentrate diet (75% corn, 20% protein and mineral mix and 5% coast cross hay), with whole corn grain (WCG), high moisture corn (HMC) or ground corn grain (GCG). After 65 d of feeding animals were slaughtered and the rumen content (RC) was weighed and its pH measured. The incidence of ruminitis (IR) was classified according to a 0 to 10 scale. Fragments of 3cm<sup>2</sup>were taken from the cranial sac of the rumen and kept in buffer for macroscopic measurements of papillae number (PN), papillary area (PA) and absorption surface (AS). PN was measured by 3 evaluators and PA and AS were measured on scanned images of the papillae and parietal surface fragments using the UTHSCA Image Tool free software. The average BW at slaughter was  $47 \pm 1.44$ kg. There were no significant differences among treatments for ADG (mean = 0.32 kg). The RC was higher for WCG (4.5 kg; P = 0.02) when compared with GCG (3.5 kg) and HMC (3.4 kg) with no differences between GCG and HMC. The rumen pH was smaller (P < 0.0001) for WCG (5.4) when compared with GCG (6.5) and HMC (6.6) with no differences between the last 2 treatments. There was no difference among treatments in IR (mean = 0.48%) and PN (mean = 43 papilla/cm<sup>2</sup>). The treatment WCG, expressed as a percentage of rumen wall surface, had greater PA (95%; P = 0.04) then HMC (93%) but was similar to GCG (94%) with no differences between GCG and HMC. The AS was greater for treatment WGC (19cm<sup>2</sup>) than for HMC  $(14\text{cm}^2; P = 0.05)$  with no difference between GCG  $(17\text{cm}^2)$  and HMC treatments. Although WCG may be interesting because the grain needs

no processing and has a small effect on rumen wall morphology, it may result in an unfavorable rumen environment.

Key Words: feedlot, ruminant, sheep

M369 Growth rate of mixed ruminal bacteria as a function of energetic substrate concentration in bath culture. T. S. de Oliveira,\* R. de Paula Lana, V. S. de Oliveira, T. M. de Oliveira Alves, and G. L. R. Filho, *Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil* 

The objective of this study was to evaluate levels of sucrose on microbial growth. Roll tubes were filled with 15.6 mL of buffer Chen, 1 mL of inocula (rumen fluid from fistulated steer at pasture), 1 mL of Trypticase or hydrolyzed casein (1 g/L as final concentration) and 2.4 mL of sucrose solutions (0.00, 0.37, 0.75, 1.5, 3.0, 6.0, 12.0, and 24.0 g/L), in triplicate. The pH was 5.56 at 0 h and 4.6 after 6 h, and 5.83 in 0 g/L and 4.18 above 1.5 g/L of sucrose. The optical density (OD-600 nm) and microbial protein (MP-595 nm) tended to stabilize after 12 h and 6 g/L of sucrose, with no interaction between them. The OD (6 h to 24 h mean values) was curvilinear (hyperbolic), as a function of sucrose concentration, followed the saturation kinetics of Michaelis-Menten, which is typical of enzymes systems, and was described by the following Lineweaver-Burk equation: 1/OD = 0.508\*(1/sucrose) + 1.15,  $R^2 = 0.99$ . The theoretical maximum microbial growth (1/a = kmax) was 0.87 OD and the concentration of sucrose to reach half of kmax (b/a = Ks) was 0.44 g/L of sucrose. The microbial growth was affected by incubation time, substrate concentration, and culture medium acidification.

Key Words: Lineweaver-Burk, Michaelis-Menten, saturation kinetics

M370 Nutrient digestibility of pregnant WAD ewe fed Mexican sunflower leaf meal (MSLM) based diets. A. H. Ekeocha,\* *University of Ibadan, Ibadan, Oyo, Nigeria.* 

Fifteen days before parturition, studies was conducted using 16 West African Dwarf (WAD) ewes weighing between 22.8 and 26.0kg on a basal diet of Panicum maximum were allotted into 4 treatment groups A, B, C and D of 4 replicates each. The Mexican sunflower leaf meal (MSLM) replaced wheat bran (WB) gravimetrically at 0, 15, 30 and 45%. Treatment A served as control, while animals in treatments B, C and D received MSLM at 15, 30 and 45% respectively. The experiment lasted for one week. Digestibility was determined using a 6-d total fecal collection. Ewes were given ad libitum access to feed and water and routine vaccination and medication followed standard procedures. The 16 ewes were previously brought to heat (estrus) by synchronization and served by 2 rams (1:8). Parameters measured were voluntary dry matter intake (VDMI), which comprised concentrate dry matter intake (CDMI) and grass dry matter intake (GDMI), apparent digestibility coefficients of dry matter(DM), crude protein(CP), ether extract (EE), nitrogen-free extract (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), organic matter (OM), energy and feed conversion ratio (FCR). Data were analyzed using descriptive statistics and ANOVA. The VDMI (g/d) varied from 392.3 - 695.0 for ewes during digestibility. CDMI (g/d) varied from 191.4 - 565.0 and GDMI (g/d) varied from 137.5 - 227.3for ewes and this was significant (P < 0.05) with animals on treatment

B having the highest CDMI while animals on treatment C having the highest GDMI respectively. Apparent digestibility coefficients of EE, NDF, ADF, ADL, OM, energy and FCR were similar. Approximately 75.5  $\pm$  1.1% of the VDMI came from the supplement. Diets containing 15% MSLM was superior to others for CDMI (191.4 - 565.0 g/d), C P absorbed (42.1 - 99.5 g/d), CP apparent digestibility (86.5 - 91.5%), digestible DMI (52.3 - 85.2 g/d/kgW $^{0.75}$ ) and digestible CPI (14.3 - 28.4 g/d/kgW $^{0.75}$ ) while GDMI (137.5 - 227.3 g/d) for diets containing 30% and 45% MSLM were significant (P < 0.05). Therefore, MSLM could suitably replace wheat bran in the diets of pregnant ewe up to 30% level of inclusion without eliciting any adverse effect.

**Key Words:** Mexican sunflower, nutrient digestibility, pregnant West African dwarf ewe

M371 Rumen bacteria growth and pH of culture medium on different substrate concentrations. C. P. Ghedini<sup>1</sup>, R. P. Lana<sup>1</sup>, A. S. Oliveira<sup>2</sup>, D. C. Abreu\*<sup>1</sup>, R. M. Paula<sup>1</sup>, G. A. Freitas<sup>1</sup>, and M. G. Camilo<sup>3</sup>, <sup>1</sup>Universidade Federal de Viçosa, Viçosa, MG, Brazil, <sup>2</sup>Universidade Federal do Mato Grosso, Sinop, MT, Brazil, <sup>3</sup>Instituto Federal do Sudeste de Minas Gerais, Rio Pomba, MG, Brazil.

The objective was to evaluate the response on rumen bacteria growth rate as a function of increasing substrate. Incubations were performed in glass jars saturated with carbon dioxide gas and hermetically sealed, containing 6 mL of rumen liquid, 24 mL of artificial saliva, and substrate weights of 1.2; 0.6; 0.3; 0.1; 0.05 and 0 g, being half of each weight corresponding to sucrose and half soybean meal. The tubes were incubated in triplicate for 72 h at 39°C and the pH and optical density were evaluated at 0, 6, 12, 18, 24, 42 and 72 h of incubation. The experiment was analyzed as completely randomized design as a function of substrate concentration, time of incubation and the respective interaction. Bacterial growth reached high values after 6 h (P < 0.01), but with strong depression at 48 and 72 h, associated with the low pH of culture medium and microbial lyses. It was observed increase in microbial growth (P < 0.01) with increase in the level of substrate. There was substrate \* time interaction in microbial growth (P < 0.01), in which there was decrease in microbial growth with increase in time in low substrate levels, due to microbial lyses. Linear regression analyses of the reciprocal of the rate of microbial growth versus reciprocal supply of nutrients were made for the 48 times and 72 h of incubation, which had already reached the maximum growth, technique called Lineweaver-Burk equation. The theoretical maximum growth rate was 1.08 and substrate concentration required to achieve half of the maximum response was 0.128 g. The low observed K<sub>s</sub> shows that bacteria affinity for sucrose is high, leading to rapid fermentation of substrate, lowering the pH values below the level tolerable by the predominant microbial population in rumen. This effect takes, consequently the microbial lyses and reduction of optical density. In this experiment it was found that the curvilinear response of saturation kinetics is due to first order kinetics in low levels of nutrients (excess enzymes and/or low substrate) and because of the zero order kinetics in high levels of nutrients (saturated microbial enzyme activity by fermentation products). Supported by FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais).

**Key Words:** growth rate, saturation kinetics, sucrose