pH, ammonia, long chain fatty acids (LCFA) and VFA. Consecutive a.m. and p.m. milk samples were taken from each cow during last 2 wk of 21-d period and analyzed for fat, protein, LCFA and somatic cell count (SCC). Dietary DMI (kg/d) was lower during second period and higher for the 10% pigeon pea diet ($P < 0.05$). Milk protein was higher for cows fed the 20% pigeon pea diet compared with 10% diet ($P < 0.05$). Milk ECM was higher for cows fed control diet compared with 10% pigeon peas ($P < 0.05$). Treatment had no effect on milk yield ($P > 0.10$). Diets did not affect ruminal fluid pH ($P > 0.10$); however, pH was different for sampling periods ($P < 0.01$). Ruminal ammonia decreased until 8h post-feeding at which time it peaked consistent with changes in ammonia concentrations that usually peak 3-5h post-feeding on diets high in plant proteins. Dietary treatments altered ruminal fluid VFA with lower concentrations of acetate and higher concentrations of propionate for the control diet, resulting in lower acetate: propionate ratio ($P < 0.05$). The P10 diet resulted in higher ruminal isovalerate ($P < 0.05$). Ruminal cis 9, trans 11 and trans 10, cis 12 conjugated linoleic acid (CLA) isomers were not affected by dietary treatments ($P > 0.10$). The P10 diet had highest ruminal synthesis of c9, t11, but control cows had highest ruminal synthesis of t10, c12 ($P > 0.10$). Milk CLA isomers were similar ($P > 0.10$) among treatments. Trends were observed for greater ($P > 0.10$) c9, t11 and t10, c12 for P10 diet. Pigeon peas may be used as a protein supplement in dairy diets without affecting milk production, DMI or ruminal environment when they replace corn and soybean meal.

Key Words: dairy, forage, fatty acids

Ruminant Nutrition: Research Methods

801 Everting the omasum into the reticulum to identify the sensory receptors in the omasum of the sheep. W. L. Grovum*, *Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada*.

The purpose of this work was to expose the inner surface of the omasum to identify its sensory receptors. Such work has been precluded to date because there was no technique for displaying its epithelia while keeping their circulation and vagal innervation intact. The main problem has been the location of the omasum under the liver, caudal to the diaphragm between the cranial sac of the rumen and the ribs on the right. Also, the reticulo-omasal orifice is nearly closed whereas the bean-shaped omasum is approximately 16 cm in diameter. To evert the omasum, sheep were anesthesized with sodium pentobarbital, intubated and placed on a ventilation pump to first remove 7 ribs (6-12) along with associated tissues on the left and thus expose the reticulum. Then, it was incised from its apex upwards on its left and right sides between major blood vessels and opened like a clam-shell, care being taken to prevent contamination of the thoracic and abdominal cavities with digesta. The cut edges of the reticulum and the skin incision were clipped together to close the large opening in the animal. The reticulo-omasal orifice thus exposed was stretched to 4 - 5 cm in diameter to elute the omasal contents between the leaves into the reticulum by pumping warm normal saline through a 2 mm ID copper tube while dislodging the particles with 2 fingers. The much shrunken omasum was then gently pushed from behind and pulled into the reticulum. The omasal leaves now projected outwards from the omasal body and were easily manipulated to identify neuronal receptors sensitive to stretch and tactile stimulation.

Key Words: omasum, sensory function, sheep

802 Standardization of an in vitro method using *Streptomyces griseus* enzyme to predict rumen undegraded protein. I. Schadt*[1], P. J. Van Soest[2], and G. Licitra[1,3], [1]*CoRFiLaC, Regione Siciliana, Ragusa, Italy*, [2]*Cornell University, Ithaca, NY*, [3]*D.A.C.P.A. University of Catania, Italy*.

In situ rumen protein degradation might change relative to feed intake and production. Incubation times of an in vitro method to achieve equal extents should be adapted, consequently. However, a continuous change of enzyme time is not practical for laboratory use. The purpose of the present study was the development and validation of a calibration curve to predict undegraded protein (UP) at any necessary enzyme time from one measurement at a single incubation time of 18 hours (1T). UP of 32 feeds was determined using *S. griseus* enzyme (40.6 U per gram true protein) at 4, 8, 12, 18, 24, 36 and 48 hours. The feeds were divided into 18 calibration and 14 validation feeds. UP of calibration feeds at incubation times was subtracted from UP at 18 hours and mean deviations at individual times were calculated. Deviations were plotted upon the natural logarithm of respective times to obtain the calibration curve. UP of validation feeds at 4, 8, 12, 24, 36 and 48 hours was predicted from the measured residue at 18 hours. The 1T method was validated against methods using two incubation times of 18 and 36 hours (2T) and all seven incubation times (7T). Individual calibration curves for all 32 feeds were generated by plotting UP either after 18 and 36 hours (2T) or after all seven incubation times (7T) upon the natural logarithms of the respective times. All three methods were examined for predictive capacity by plotting measured values upon predicted values and by calculating proportional deviations (PD) of calculated from measured values. Calculated and measured UP was generally highly correlated with $R^2 = 0.96$ ($n = 98$), $R^2 = 0.96$ ($n = 224$), and $R^2 = 0.99$ ($n = 224$), at 1T, 2T, and 7T, respectively. PD was decreasing ($p < 0.01$) with values of $0.066 > 0.049 > 0.030$ at 1T, 2T, and 7T, respectively. PD was highest at 48 hours using 1T and 7T. Elimination of times longer than 36 hours decreases PD at 1T to 0.054 being not different from PD at 2T.

Key Words: in vitro method, rumen protein degradation

803 Methodology to improve the sensitivity and repeatability of in vitro gas production. D. R. Mertens*, *US Dairy Forage Research Center, Madison, WI.*

The goal of this research was to improve the measurement of in vitro gas production during early fermentation and reduce variability among runs. Eleven forages (immature and mature grasses and legumes) were used in four in vitro runs over a six week period. Ruminal contents were collected from four lactating cows, and inoculum preparation was initiated within 20 min after collection. Ruminal fluid was separated from solids by squeezing through two layers of cheesecloth. Solids weighing one-half of the required ruminal fluid was blended for 60 s with twice their weight of CO_2-purged, reduced and warmed media. Ruminal fluid and blended solids were combined and squeezed through four layers of cheesecloth. Inoculum was kept warm using a jacketed beaker with circulating water at 39°C and purged with CO_2. Bottles (50 ml) containing samples and 6 ml of buffered media were purged with CO_2, reduced and warmed in a waterbath. Each bottle was inoculated with 4 ml of blended inoculum, closed with a septum and crimp

cap, and immediately placed in an incubator and attached to pressure transducer via needle puncture. Inoculum weight was recorded for each bottle. Blended inoculum actively fermented during inoculation and gas pressure increased within .01 hr of inoculation. Pressure was measured every .01 h during inoculation to identify zero times, then every .1 h for 3 to 6 h of fermentation and every .5 h thereafter (44 to 48 h). Blended inoculum generated significant gas in blanks. A flexible sigmoid function was used to fit blank data and calculate a blank-correction for each bottle based on weigh of inoculum. Asymptotic gas production of blanks varied from 2.7 to 3.2 mls per gram of inoculum and was different among runs using PROC GLM (P<.05). Rapid fermentation was indicated by 28% of total gas produced within 3 h of fermentation. Coefficients of variation among-runs were 8.1, 6.5, 6.0, 5.5, and 5.2% at 3, 6, 12, 24 and 36 h, respectively. It was concluded that this method has the sensitivity and repeatability necessary to detect small differences in digestion kinetics.

Key Words: gas production, digestion kinetics, in vitro methods

804 Effect of lignin linkages with other plant cell wall components on in vitro and in vivo NDF digestibility of forages. E. Raffrenato*[1], R. Fievisohn[2], K. W. Cotanch[2], R. J. Grant[2], L. E. Chase[1], and M. E. Van Amburgh[1], [1]*Cornell University, Ithaca, NY*, [2]*W. H. Miner Agricultural Research Institute, Chazy, NY*.

Lignin limits forage digestibility and imparts this effect by decreasing digestible energy and limiting dry matter intake. Measurements of acid detergent lignin (ADL) do not always account for variability in digestibility, especially at fermentation times representing rumen residence times of forages. The chemistry of ADL cross-linkages with cell wall polysaccharides rather than amount of ADL has been suggested as a better predictor of NDF digestibility (NDFD). The objective of our work was to evaluate the effects of ester and ether linked p-coumaric (PCA) and ferulic acid (FA) on in-vitro and in-vivo NDFD. Twenty-four corn silages (CS) were incubated in-vitro for 24hr NDFD. Digested residues were analyzed for NDF, ADF and ADL and ester and ether linked PCA and FA were determined in those fractions. Three of the CS were fed to 6 fistulated cows for 3 wks in three iso-NDF diets. Diet, rumen and feces samples were taken every 3hr for three days, 10d from the start of the study. Intact samples, NDF and ADF residues were analyzed for ester and ether linked PCA and FA. Extraction of phenolic acids was by 2N and 4N NaOH treatment, followed by HPLC equipped with a diode array detector using a reverse-phase column. Ether-linked PCA and FA were calculated as the difference between total and ester-linked phenolic acids. The content of ester and ether linked PCA and FA in both NDF and ADF residues showed negative correlations with 24hr NDFD, -0.16 to -0.87 for NDF and -0.71 to -0.95 for ADF. In particular, the ester linked PCA content of ADF explained 92% of the variation in 24hr NDFD. Correlations decreased for longer fermentation lengths, demonstrating that PCA and FA limit only rate and not extent of in-vitro digestion. The analyses from the in-vivo study confirmed the in-vitro results, demonstrating the highest total tract NDFD (70%) for the CS with the lowest phenolic acid and ester linked PCA content in the ADF fraction.

Key Words: digestibility, lignin, phenolic acids

805 Do the time of access to food, the supplementation with additives and the graze affect ruminal inocula used for *in vitro* gas production trials? A. Pérez-Ruchel[1], A. Britos[1], E. Almanza[1], J. L. Repetto[2], N. Pomiés[1], and C. Cajarville*[1], [1]*Departamento de Nutrición Animal, Facultad de Veterinaria, Montevideo, Uruguay*, [2]*Departamento de Bovinos, Facultad de Veterinaria, Montevideo, Uruguay*.

The objective of this study was to evaluate if the time of access to food (TAF), the supplementation or not with buffer or yeasts, and the graze affect rumen inocula of ruminants consuming pastures. Twenty cannulated wethers consuming forage from a temperate pasture (*Lotus corniculatus*, CP 14%) were allocated into 5 groups: AD had forage available all day; R, RB, RS, RG had forage available 6h/d. AD, R, RB, RS were housed in metabolic cages; RG was grazing. RB ate buffers (2% DMI, 75% NaHCO3-25% MgO); RS yeasts (6.2×10^9 UFC/d of *Saccharomyces cerevisiae*). Rumen inocula was collected at hour 2 after the beginning of the ingestion from each animal and mixed within grups. 0.5 g of 3 substrates (*Avena sativa*, *Lotus corniculatus* and wheat straw) were introduced in flasks by triplicate for each inoculum and incubated at 39.5°C. Gas pressure was measured during 96 h. Cumulative gas production data were fitted to the model: gas= a+b{1-exp[-kd(t-L)]} (a+b (mL): potential gas production; kd (h^{-1}): rate of gas production; L (h): lag time). Parameters were analysed by GLM considering 'substrate', 'inocula' and its interaction. Means for 'inocula' were separated by orthogonal contrasts studying the effect of TAF (AD *vs* R), use of additive (R + RG *vs* RB + RS), additive used (RB *vs* RS), and graze (R *vs* RG). Non interactions 'substrate' × 'inocula' were detected. Animals fed during all day presented higher kd (P=0.046) and lower L (P<0.001), which should indicate a more stable microbial population. Instead offered food and TAF were the same, grazing animals presented lower a+b (P=0.024) but with a higher kd (P=0.002), suggesting different ruminal environments. The use of additives had no effect, instead RS tended to have higher kd and lower L. We concluded that TAF and grazing affected the inocula characteristics. *Acknowledgements: PDT-DICyT (78/12 and S/PSP/02/48), CSIC, and CODENOR S.A.*

Key Words: in vitro fermentation, temperate pastures, additives

806 *In vitro* assessment of effects of microalgae type, protection of microalgae, and dilution rate on dry matter disappearance and methane emission in a rumen simulation system. R. Kinley*[1], K. Glover[1], R. Teather[2], S. Iverson[3], and A. Fredeen[1], [1]*Nova Scotia Agricultural College, Truro, Nova Scotia, Canada*, [2]*Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada*, [3]*Dalhousie University, Halifax, Nova Scotia, Canada*.

The impact of microalgae type, microalgae protection against microbial degradation, and fluid turnover rate was evaluated *in vitro* using five continuous culture fermentors in a 3 times replicated factorial of control + 3 alga × 3 flow rates × 3 protection levels, completed over 18 periods. Mixed inoculums from 5 lactating cows were maintained at pH 6.8 and 39°C, with rotary mixing (13 rpm), in 1200 mL (working capacity) Versatec fermentors. Thirty grams of a dry 50/50 forage/concentrate mix (2 mm screen) and 1.2 g of microalgae were fed daily in equal 12 h increments. Each period consisted of 12 d of stabilization followed by repeated measurements over the final 4 d. The production of CH_4 was determined by gas chromatography by measuring its concentration in the head space with constant gas flow. A 3–way interaction (alga × flow rate × protection) effect on methane emission was significant (P<0.001). Compared to the control all 3 algae reduced overall DM disappearance (DMD; P<0.001) but not NH_4–N concentration (P=0.285). Production of NH_4–N was significantly reduced by increasing flow rate (P=0.002), while both overall DMD and CH_4 emissions were increased (P<0.001). Algae–1 significantly reduced CH_4 production at lower levels of pro-

tection and flow rate combined ($P<0.001$). The level of protection of algae had no significant effect on DMD, CH_4 or NH_4–N ($P>0.374$), but lower CH_4 emissions were generally observed with increasing protection level. Slower turnover rate and lower protection level may serve to promote toxic effects of the algae on rumen microbes, as indicated by the observed onset of floating digesta mat instability, which may reflect reduced production of fermentation gases (CH_4 and CO_2).

Key Words: methane, microalgae, rumen

807 Comparing real-time PCR to purine analysis in regard to estimation of bacterial crude protein. E. Castillo-Lopez*, J. Miner, and P. Kononoff, *University of Nebraska*, *Lincoln*.

The objectives were to evaluate the estimation of rumen bacterial crude protein (BCP) based on two methods, real-time PCR (RTPCR) and purine analysis, and, secondly, to determine the impact of yeast on estimation of BCP. In the first trial, an *in vitro* fermentation was carried out. Treatments were I) Control: grass hay and corn, II) Added distillers dried grains (DDG) and III) Added DDG with added solubles (DDG+S). After 48 h of fermentation, the amount of BCP was estimated based on purine analysis and RTPCR. The RTPCR probe targeted DNA encoding the bacterial 16S rRNA to establish the ratio of bacterial DNA:BCP. Data were analyzed as a completely randomized design arranged in a 2X3 factorial. Estimates of BCP production were 0.383 and 0.285 ±0.013 g BCP/L of fermentation fluid based on purine analysis and RTPCR, respectively, and were different ($P < 0.01$). There was ($P < 0.01$) an effect of treatment on BCP production estimates, 0.39, 0.35 and 0.26 ±0.016 g BCP/L for the control, DDG, and DDG+S respectively. The method by treatment interaction tended to be different ($P = 0.06$). Specifically, BCP of the control was 0.41 and 0.38 ±0.023 g BCP/L for purine analysis and RTPCR respectively, and were not different ($P = 0.34$). The BCP estimated for DDG were 0.43 and 0.26 ±0.023 g BCP/L for purine analysis and RTPCR respectively, and were different ($P < 0.01$). The BCP estimated for DDG+S were 0.30 and 0.22 ±0.023 g BCP/L for purine analysis and RTPCR and were different ($P = 0.04$). In the second trial, bacteria were isolated from ruminal fluid from one steer and split into six samples. Yeast was added to three samples at one third of total DM, DNA was extracted from all samples, and BCP was estimated using RTPCR. Data were analyzed as a completely randomized design. Estimates for BCP were 0.29 and 0.27 ±0.013 g/L digesta for samples with and without yeast, respectively, and were not different ($P = 0.38$). Results suggest purine analysis may overestimate BCP due to the presence of yeast cells, while RTPCR is unaffected by the presence of yeast. Thus, RTPCR may be a more accurate method to measure BCP.

Key Words: bacterial crude protein, distillers grains, purine analysis

808 Evaluation of supplementation or controlled-release capsule (CRC) to supply *n*-alkane as an intake marker in steers fed switchgrass or alfalfa hay. S. Chavez*, C. Lane, M. Braxton, A. Bruner, E. Leonard, J. Burns, and G. Huntington, *North Carolina State University*, *Raleigh*.

The objective was to evaluate *n*-alkanes as intake markers provided either in supplement or CRC. Seven ruminally fistulated beef steers (BW = 422 ± 36 kg) were fed 1 kg of soyhulls supplement once daily and alfalfa (n =3) or switchgrass (n=4) twice daily (18 g/kg BW). Each steer received dotriacontane (C32) in supplement or intraruminal CRC in a balanced changeover of 16-d periods. Delivery from the CRC was periodically measured with calipers. Fecal grab samples and total feces were collected during the last 5 d of each period. Fecal grab samples were divided into aliquots that were freeze-dried (FD) or oven-dried (OD) to a constant weight at 60°C. Forage and fecal alkanes were saponified, extracted with heptane, and analyzed by gas chromatography. The two forages were used to evaluate different levels of forage hentriacontane (C31) to predict intake. Overall, predicted intake (6.21 ± 1.26 kg DM/d) did not differ ($P = 0.12$) from measured intake (5.88 ± 1.27 kg DM/d). Fecal C31 (1,037 ± 55 mg/kg DM) and C32 (141 ± 13 mg/kg DM) did not differ ($P < 0.26$) between OD and FD in alfalfa. Fecal C31 (124 ± 18 mg/kg DM) and C32 (112 ± 10 mg/kg DM) did not differ ($P < 0.75$) between OD and FD in switchgrass. Intake predicted by fecal grab samples (6.21 ± 0.34 kg DM/d) did not differ ($P < 0.89$) from intake predicted by 5-d total fecal collection (6.28 ± 0.34 kg DM/d). Intake predicted by supplementation (6.29 ± 0.34 kg DM/d) did not differ ($P < 0.82$) from intake predicted by measured release of C32 from the CRC (6.13 ± 0.34 kg DM/d). Measured CRC delivery was appreciably less than manufacturer's specifications. Use of manufacturer's specifications would increase predicted intake from 1.18 to 1.35 times among the steers. In conclusion, alkanes can be used as markers to predict intake by using fecal grab samples rather than collecting total feces. Hay intake can be predicted accurately with once daily alkane supplementation, and samples can be OD or FD with no difference in alkane concentrations.

Key Words: alkanes, intake, steer

Swine Species: Symposium: Environmental Concerns Based on Swine Production

809 Research and extension needs in air and water quality. D. J. Meisinger*, *US Pork Center of Excellence*, *Ames, IA*.

The US Pork Center of Excellence in cooperation with the National Pork Board′s Environmental Committee hosted two invitational workshops to develop research and extension needs, one for air quality and one for water quality. These workshops had perfect attendance of the invitation list with the result being an exhaustive set of research and outreach needs. The air quality group organized themselves into breakout groups by gases, odors, and particulate matter. The water quality group did the same with the topics of nutrients and sediments, pharmaceuticals and hormones, and pathogens. Each breakout group answered the following questions:

- Identify research that is underway or has already been completed.
- Identify what is known and can be developed into extension materials and programs.
- Identify what still needs to be answered by further research efforts.
- Work to form a consensus targeting future research investments and efforts.
- Work to identify opportunities for soliciting research funding from external sources.

The deliverables from these two meetings were as follows:

- Identification of producer materials that should be developed based on available research.
- A recommended priority list for development of producer educational and informational materials based on available research.