

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 and reflects high in plant proteins. Dietary treatments affected ruminal fluid

VFA with lower concentrations of acetate and higher concentrations of propionate for the control diet, resulting in higher 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*, *i11*, but control cows had highest ruminal synthesis of *i10*, *c12* ($P > 0.10$). Milk CLA isomers were similar ($P > 0.10$) among treatments. Trends were observed for greater ($P > 0.10$) *c9*, *i11* and *i10*, *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. Grovum¹, 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 1.6 cm in diameter. To evert the omasum, sheep were anaesthetized with sodium pentobarbital, intubated and placed on a ventilator pump to first remove 7 ml/hr (8–12) a long with associated tissues on the left and then 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 evert 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 probed from behind and pulled into the reticulum. The omasal leaves were 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. Schaefer¹, P. J. Van Soest², and G. Licitra^{1,3}, ¹CoRR/LaC, Regione Siciliana, Ragusa, Italy, ²Cornell University, Ithaca, NY, ³D.A.C.P.A. University of Catania, Italy.

In vitro 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 5 different enzyme (406 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 logarithm 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 $R^2 = 0.96$ ($n = 98$), $R^2 = 0.94$ ($n = 224$), 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. Mertens¹, 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 CO₂-purged, reduced and warmed media. Ruminal fluid and blended solids were combined and squeezed thru two layers of cheesecloth. Inoculum was kept warm using a jacketed beaker with circulating water at 39°C and purged with CO₂. Bottles (50 ml) containing samples and 6 ml of buffered media were purged with CO₂, reduced and warmed in a waterbath. Each bottle was inoculated with 4 ml of blended inoculum, closed with a septum and crimp

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