[1]Average pen DM intake of group-fed animals. [2]Nonfiber carbohydrates = 100-(ash + NDF + CP + fat). [3]Somatic cell count.

Key Words: Dairy cow, Fibrolytic enzymes, Digestion and milk yield

595 Effect of pH and enzyme supplementation to a total mixed ration on microbial fermentation in continuous culture. D. Colombatto*[1,2], G. Hervas[3], W. Yang[1], and K. Beauchemin[1], [1]*Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada,* [2]*Facultad de Agronomia, Universidad de Buenos Aires, Argentina,* [3]*Estacion Agricola Experimental (CSIC), Leon, Spain.*

The effects of pH and enzyme addition were examined in continuous culture using a 4 x 4 Latin square design, with four 9-d periods consisting of 6 d for adaptation and 3 d for measurements. The buffer pH was adjusted to 100% (high) or 60% (low) of the normal concentration of artificial saliva. Fermenters were fed twice daily a diet consisting of 30% alfalfa hay, 30% corn silage, and 40% rolled corn (DM basis). The silage was milled fresh and the TMR was fed fresh to the fermenters (64% DM). The EM was a protease containing no other major activities, and was applied daily to the TMR, at least 12 h before feeding. Treated feed was stored at 4°C until fed. Ranges of pH were 6.0-6.6, and 5.4-6.0 for high and low, respectively. Degradability of OM, NDF, ADF, and cellulose were reduced ($P < 0.05$) by low pH, but hemicellulose and protein degradation were not affected. EM addition increased ($P < 0.01$) NDF degradability (by 43% and 25% at high and low pH, respectively), largely due to an increase in hemicellulose degradation (by 79% and 51%, respectively). Total volatile fatty acids (VFA) and its molar proportions were decreased ($P < 0.05$) by low pH, but were not affected by EM. Protein degradation was only numerically ($P = 0.17$) increased by EM. Total N flow tended ($P = 0.07$) to be reduced with EM, but neither bacterial nor dietary N flow was affected by the treatments. Microbial protein synthesis was not affected by either pH ($P = 0.29$) or EM ($P = 0.86$). Methane production, expressed as a proportion of total gases, was decreased ($P < 0.001$) at low pH, but was not affected by EM. It is concluded that it is possible to adapt the CC system to use fresh feeds instead of dried feeds. Overall, the results indicate that the EM used in this study has significant potential to increase fiber degradability without increasing methane production.

Key Words: Continuous culture, Digestion, Enzymes

596 Effect of the sequence of fat and antibiotic-ionophores on ruminal fermentation and microbial lipids. M. G. Daves* and V. Fellner, *North Carolina State University, Raleigh, NC.*

Rumen inoculum was obtained from a fistulated cow, filtered, and incubated in 8 dual-flow fermentors. The basal diet consisted of 100 % alfalfa pellets and was offered twice daily (14g DM/d). Cultures were allowed two days of adaptation and then used to test the sequence effects of fat, monensin, and bacitracin addition. On day 3, two fermentors received monensin, two received bacitracin, and the other four received fat. On day 6, fat was added to the cultures receiving monensin and bacitracin, and two of the four fermentors fed fat were offered monensin and the other two bacitracin for an additional three days. A total of four replications were conducted. Data were analyzed using the Mixed procedure of SAS for repeated measurements. Monensin reduced methane 15% and 23% when compared to fat and bacitracin, respectively. Adding monensin prior to fat lowered methane by 22% compared to the addition of fat prior to monensin. Monensin increased ($P < 0.01$) propionate compared to bacitracin but increased valerate ($P < 0.03$) and iso-valerate ($P < 0.0001$) compared to fat. Addition of monensin prior to fat increased ($P < 0.03$) valerate when compared to cultures that received fat prior to monensin. Fat increased ($P < 0.003$) isobutyrate compared to monensin. Isobutyrate was higher in cultures that received bacitracin prior to fat than those that received fat first. Monensin increased ($P < 0.01$) C16:0 compared to fat. Levels of C18:0 ranged between 23mg/g of total FA (monensin) to 30 mg/g of total FA (fat), but the difference was not significant. Cultures receiving monensin had lower concentrations of C18:0 than those receiving bacitracin, but the sequence of fat addition had no effect. The addition of additives and their sequences did not alter total or trans-C18:1 levels. Monensin increased ($P <0.05$) cis-C18:1 compared to bacitracin or fat. When monensin was added prior to fat cis-C18:1 was higher ($P <0.05$) than when fat was added prior to monensin. Monensin, either alone or when added prior to fat, was more effective in altering fermentation compared to bacitracin. Inclusion of fat prior to monensin or bacitracin altered the response to the ionophore-antibiotics.

Key Words: Ionophore-antibiotics, Fat, Continuous cultures

597 Comparison of different starch analysis methods for feedstuffs. K.-H. Suedekum*[1], M. B. Hall[2], and M. Paschke-Beese[1], [1]*University of Kiel, Germany,* [2]*University of Florida, Gainesville.*

The official EU method for starch analysis on feedstuffs is a polarimetric procedure utilizing the optical activity of dispersed starch. For pure starches and high-starch commodities, this method provides reliable and accurate results. When applied to feedstuffs low in starch and high in fiber or protein, reliability is considerably reduced and values often overestimate true starch values. The objective of this work was to compare the polarimetric method with different enzymatic procedures. All enzymatic procedures involved a preliminary digestion step with a heat-stable α-amylase (Termamyl 120 L), dissolved either in a Na-citrate buffer at pH 5.8 or in water, and release of glucose by action of amyloglucosidase in a Na-acetate buffer at pH 4.6. Glucose was quantified subsequently using either phosphorylation or oxidation to gluconic acid. Free glucose was determined in a separate assay to yield unbiased estimates of glucose from starch. Eleven test samples were utilized comprising low-starch (distillers' grains, soybean meal, citrus pulp, total mixed ration with 25% citrus pulp) and high-starch (corn starch, potato starch, hominy feed) commoditites. As a general observation, starch values determined polarimetrically (mean, 48.9% of dry matter) were higher ($P < 0.0001$) than those determined enzymatically (mean, 43.1% of dry matter). Comparisons among enzymatic procedures showed that Na-citrate buffer as an incubation medium for the α-amylase yielded higher starch values than water (44.4 versus 40.5% of dry matter; $P < 0.0001$). Type of quantification of glucose (phosphorylation versus oxidation to gluconic acid) gave the same average starch concentration (43.1% of dry matter; $P = 0.7846$). Results from this study indicate that Na-citrate buffer was better than water as an incubation medium for α-amylase, and that glucose released by α-amylase plus amyloglucosidase action can be quantified by phosphorylation or oxidation. Differences between polarimetric and enzymatic methods, and variation among enzymatic procedures require further investigation.

Key Words: Starch, Feed analysis, Methods

598 A novel technique to assess particle distribution of rations and forages using digital imaging. A. Bach*[1], A. Anglada[1], X. Puigvert[2], and L. Bosch[2], [1]*ICREA-IRTA Dairy Systems, Spain,* [2]*Universitat de Girona, Spain.*

The objective of this study was to develop a simple and efficient technique to determine particle size distribution of forages and total mixed rations (TMR) for dairy cattle using digital imaging. The particle size distribution of different rations from different dairy farms was evaluated using the Penn State Separator and the new digital image technique. The new technique is based on a digital picture from a small sample (about 500 g) of forage or TMR on a black surface. Afterwards, the picture is analyzed with software for digital measurements to determine the length and the area of most of the particles in the picture. Following the analysis, a distribution chart is constructed. The preliminary results indicate that both, the average area of the particles determined digitally and the average particle size determined with the Penn State Separator were correlated with milk fat percentage. However, the correlation was stronger when the particles were analyzed using digital measures ($R^2 = 0.26$; $P = 0.09$) than when using the Pen State Separator ($R^2 = 0.07$; $P = 0.43$). Therefore, digital imaging is a more precise method to estimate the consequences of particle size distributions of TMR on milk fat percentage. The advantage of using digital imaging vs the Penn State Separator is that the former provides reliable results even with wet TMR. In several occasions, the Penn State Separator failed with wet TMR because a fraction of the small particles remained attached to larger particles with greater water content. Therefore, to obtain reliable results with the Penn State Separator with high-silage TMR, the determination should be conducted on a dry sample. The use of digital imaging does not require drying the sample prior to particle evaluation. Also, digital imaging provides an entire distribution pattern of almost all the particles in the TMR, instead of only the three break points (1.18, 8, 19 mm) that the Penn State Separator yields. This technique