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in group 3 was 0.72% higher than that in the control group ($P < 0.05$). The evaluation of whole-plant corn silage quality scores (CSQS) was: Group 3 (71.21) > Group 2 (66.82) > Group 2 (63.82) > control group (62.76). In summary, adding 40 mg/kg glucose oxidase to the whole-plant corn silage has the optimal effect on the quality.

Key Words: glucose oxidase, whole-plant corn silage, sensory score, whole-plant corn silage quality scores

2520V Using Fourier-transform infrared spectroscopy to predict urinary allantoin and creatinine from urine and milk samples. L. A. C. Ribeiro*, T. Bresolin, S. I. A. Apelo, and J. R. R. Dorea, *University of Wisconsin–Madison, Madison, WI.*

Microbial protein (MicP) accounts for most of the total amino acids flowing to the small intestine and is considered a high-quality protein for dairy cows. Quantifying MicP yield as well as factors influencing its supply is of major importance in dairy cattle nutrition. However, MicP is difficult to measure in research and commercial settings. For that reason, research studies have extensively used internal markers such purine derivatives and creatinine (for urinary volume) to estimate total MicP yield. Infrared spectroscopy has been widely used for milk and feed analyses as also to predict feed components and milk traits. This technology is fast, noninvasive, nondestructive, and has great potential to predict difficult-to-measure phenotypes of large-scale operations in a timely manner. The objective of this study was to evaluate if Near Infrared Spectroscopy (NIR) and Mid-IR obtained from urine and milk samples, respectively, could be used to predict urinary allantoin (ALN) and creatinine (CRE). We evaluated 3 covariate sets for each urinary compound: (1) urine NIR; (2) milk MIR; and the combination of urine and milk spectra. Samples were collected from 185 Holstein cows at the University of Wisconsin–Madison. Quality prediction was assessed used partial least squares (PLS) by randomly splitting data set into training and test set (75% and 25% of the data set, respectively). The number of components in each model was selected based on 5k-fold cross-validation. The best predictions for urinary ALN were observed when urine NIR was used as covariate set ($R^2 = 0.60$; RMSE = 3.65 mM/l). Combining milk MIR with urine NIR did not improve ALN prediction ($R^2 = 0.56$; RMSE = 3.60 mM/l). CRE was not accurately predicted by urine NIR ($R^2 = 0.18$; RMSE = 3.01 mM/l), milk MIR ($R^2 = 0.04$; RMSE = 3.40 mM/l), or combination of both ($R^2 = 0.21$; RMSE = 3.00 mM/l). Our results suggest that urine spectrum has important information related to allantoin concentration, which could be used as an additional source of data for predictions of complex traits such as intake, microbial synthesis, and nitrogen efficiency.

Key Words: infrared spectroscopy, purine derivates, allantoin

2521V Feeding amylolytic and proteolytic exogenous enzymes: Effects on ruminal fermentation of dairy cows. M. Bugoni[1], C. S. Takiya[1], P. C. Vittorazzi Junior[1], N. T. S. Grigoletto[1], G. Gomes da Silva[1], R. G. Chesini[1], L. V. B. de Alcantara[1], T. Durman[2], and F. P. Rennó*[1], [1]*University of São Paulo, Pirassununga, São Paulo, Brazil,* [2]*Alltech Brazil, Maringá, Paraná, Brazil.*

Adding exogenous enzymes (ENZ) to diets may increase rumen degradability and energy available for milk yield. A study was conducted to evaluate the effects of amylolytic (Amaize, Alltech, USA) and proteolytic (Allzyme Vegpro, Alltech) ENZ on nutrient intake, milk yield and composition, and ruminal fermentation. Four Holstein cows with ruminal cannulas (185 ± 63.8 DIM and 35.8 ± 9.89 kg/d milk yield) were used in a 4 × 4 Latin square experiment with four 21-d periods. Treatments included: Control (CON), without ENZ; Amylolytic ENZ (A5), 0.5 g/kg diet DM; A5 + proteolytic ENZ 1X (A5P2), 0.2 g/kg; and A5 + proteolytic ENZ 2X (A5P4), 0.4 g/kg. Cows were milked twice daily and samples were collected for 3 consecutive days of each period. Ruminal digesta samples were collected on the last day of each period before feeding and every 2 h until 16 h after feeding and analyzed for pH, VFA, ammonia. Ruminal fermentation data were analyzed as repeated measures using the MIXED procedure of SAS modeling the fixed effects of treatment, period, time, and their interactions besides the random effect of animal. Differences between treatments were analyzed by orthogonal contrasts: CON *vs* ENZ; A5 *vs* A5P2+A5P4; and A5P2 vs. A5P4. No differences were detected for DMI or milk yield and composition. Cows fed ENZ had greater ($P = 0.012$) feed efficiency (FCM ÷ DMI) than CON cows (1.31, 1.40, 1.37, and 1.34 for CON, A5, A5P2, and A5P4, respectively). A trend for greater ($P = 0.053$) feed efficiency was observed for cows in A5 group than those in A5P2 and A5P4. No differences were detected for ruminal pH or NH_3-N concentration. Ruminal propionate molar percentage tended to be greater ($P = 0.083$) in cows fed ENZ than CON (18.3, 19.2, 18.2, and 18.8% for CON, A5, A5P2, and A5P4, respectively). Propionate molar percentage was greater ($P = 0.039$) in cows fed A5 than those in A5P2 and A5P4. Cows fed A5P4 tended to have greater ($P = 0.098$) propionate molar percentage than cows in A5P2. No other significant contrast effects were detected for VFA. Exogenous amylolytic and proteolytic ENZ resulted in greater feed efficiency and modulated ruminal fermentation of dairy cows.

Key Words: amylase, feed additive, protease

2522V Feeding amylolytic and proteolytic exogenous enzymes: Effects on nutrient digestibility, milk yield and composition of dairy cows. M. Bugoni[1], C. S. Takiya[1], P. C. Vittorazzi Junior[1], N. T. S. Grigoletto[1], G. Gomes da Silva[1], R. G. Chesini[1], F. M. dos Santos[1], L. F. Costa e Silva[2], and F. P. Rennó*[1], [1]*University of São Paulo, Pirassununga, São Paulo, Brazil,* [2]*Alltech Brazil, Maringá, Paraná, Brazil.*

Exogenous enzymes (ENZ) are added to diets to increase total enzymatic activity within the rumen and to improve nutrient utilization. A study was conducted to evaluate the effects of amylolytic (Amaize; Alltech, Nicholasville, KY) and proteolytic (Allzyme Vegpro; Alltech) ENZ on intake and apparent nutrient digestibility, and milk yield and composition of dairy cows. Twenty-eight Holstein cows (161 ± 87.7 DIM and 35.2 ± 5.19 kg/d milk yield) were blocked according to milk yield and DIM and used in a 4 × 4 Latin square experiment with four 21-d periods. Treatments included: Control (CON), with no ENZ; Amylolytic ENZ (A5), 0.5 g/kg diet DM; A5 + proteolytic ENZ 1X (A5P2), 0.2 g/kg; and A5 + proteolytic ENZ 2X (A5P4), 0.4 g/kg. Enzymes were provided mixed into the concentrate. Feed offered and orts were recorded daily. Cows were milked twice daily, and samples were collected for 3 consecutive days of each period and analyzed for total solids using mid-infrared method. Fecal samples were collected for 3 consecutive days of each period on 9-h intervals. Fecal excretion was calculated based on indigestible NDF content in feeds, orts, and feces. Data were analyzed using the MIXED procedure of SAS modeling the fixed effects of treatment, period, Latin square, and interaction between treatment and Latin square. Animal within square was considered a random effect. Differences between treatments were analyzed by orthogonal contrasts: CON *vs* ENZ; A5 *vs* A5P2+A5P4; and A5P2 vs. A5P4. No differences were detected in DMI or digestibility of DM, CP, or EE. Digestibility of NDF was greater ($P = 0.021$) in cows fed A5P4 than those fed A5P2 (58.1 and 55.3%, respectively). The 3.5% Fat-corrected milk was greater ($P \leq 0.032$) in cows fed ENZ than CON (33.3, 34.2, 34.5, and 34.6 kg/d

for CON, A5, A5P2, and A5P4, respectively). Feeding ENZ resulted in greater ($P = 0.008$) milk yield and tendencies for higher protein yield and feed efficiency. Cows fed with amylolytic and proteolytic ENZ were more productive, with minor impacts on apparent nutrient digestibilities.

Key Words: amylase, protease, starch

2523V The effect of posttreatment curing times of a fungal enzyme cocktail on in vitro NDF digestibility. J. H. C. van Zyl, Z. Skippers, and C. W. Cruywagen*, *Stellenbosch University, Stellenbosch, South Africa.*

The extracellular enzyme supernatant of a fungal strain, ABO374, isolated from South African soil and developed at Stellenbosch University, was used in the trial. The objective was to determine the effect of posttreatment curing times on in vitro NDF digestibility of alfalfa hay, wheat straw, and a typical dairy cow TMR. A negative control was not included because our previous research showed that ABO374 improved NDF digestibility in these forages. Substrates were treated with a 2.5% dilution of the ABO374 supernatant. After application, samples were weighed out into ANKOM F57 filter bags and allowed to cure for 0.5 h, 3 h, or 12 h at room temperature. These times were referred to as posttreatment curing times (PCT). After each PCT, a set of samples was transferred to an ANKOM Daisy incubator for in vitro incubation of 12 h. Rumen fluid of 2 cannulated dairy cows was used separately in different jars with a buffered Van Soest medium. The trial was done in 3 separate runs, one week apart, to yield 6 replications. Data were analyzed according to a main effects ANOVA with PCT and substrate as main effects. Across substrates, a PCT of 0.5 h and 3 h tended ($P = 0.08$) to result in higher NDF disappearance values than a PCT of 12 h (29.3%, 29.8% and 26.9%, respectively). Regarding different substrates, PCT had an effect ($P < 0.05$) on alfalfa NDF disappearance which was higher at 0.5 h and 3 h (30.1%, and 30.8%, respectively) compared with 12 h (26.2%). Increasing PCT had no significant effect on NDF disappearance in wheat straw (30.8%, 31.2% and 27.3%) at 0.5 h, 3 h and 12 h, respectively, or in the TMR (27.1%, 27.5% and 27.5%) at the respective posttreatment curing times. It was concluded that extended curing times (longer than 3 h) would not increase in vitro NDF digestibility in alfalfa hay, wheat straw or the TMR.

Key Words: fibrolytic enzymes, rumen fiber digestibility, in vitro incubation

2524V Palmitic acid supply and rumen unsaturated fatty acid load on rumen fermentation in a continuous culture system. F. Hentz*[1], L. Padilla[2], and F. Batistel[1], [1]*University of Florida, Gainesville, FL,* [2]*Utah State University, Logan, UT.*

Oversupply of rumen unsaturated fatty acids (RUFAL) can disrupt the cell membrane function and permeability of rumen microorganisms. Nonrumen bacteria incorporate palmitic acid as a mechanism to reduce membrane permeability. We hypothesized that rumen bacteria use a similar mechanism, thereafter, providing saturated fatty acids in the diet could support bacterial metabolism under RUFAL. The objective of this study was to evaluate the effects of dietary palmitic acid associated with RUFAL level on rumen fermentation. The study was conducted as a 2 × 2 factorial treatment arrangement in a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were a) a control diet without supplemental fatty acids (FA), b) the control diet plus 1.5% palmitic acid, c) the control diet with palmitic acid plus Low RUFAL (2.0% of diet DM), d) the control diet with palmitic acid plus High RUFAL (4.0% of diet DM). Soybean oil and soyhulls were used to create variation in RUFAL levels. The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture. Daily fermenter effluent was collected over 24-h post-feeding and a 30% subsample was pooled by fermenter within period. Data were analyzed using a mixed model including the fixed effect of FA, RUFAL, and its interaction, and the random effects of period and fermenter. The differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. No interaction between FA and RUFAL was observed. Treatments with High RUFAL decreased fiber digestibility (, total VFA concentration, and acetate concentration ($P \leq 0.04$). Palmitic acid increased fiber digestibility and total VFA concentration ($P \leq 0.03$). Butyrate concentration was not affected by treatment. Our preliminary results indicate that RUFAL negatively impacts rumen fermentation and palmitic acid is not able to overcome its negative effect.

Key Words: palmitic acid, soybean oil

2525V Effect of lysine and methionine on mRNA expression of transcription factors by primary bovine mammary epithelial cells. B. Li*[1], C. Reyes[1], J. Kim[1], A. Edick[2], M. Fox[1], S. Ahmady[1], J. Doelman[3], S. Burgos[2], and J. Cant[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*McGill University, Montreal, Quebec, Cananda,* [3]*Trouw Nutrition, Amersfoort, the Netherlands.*

Lys and Met are often considered limiting to milk protein synthesis in the mammary glands of lactating dairy cows. Essential amino acids (EAA) affect the activity of mRNA translation factors to regulate protein synthesis within cells, but the transcriptional response has not been investigated. Transcription factors (TF) that have been identified as mediators of transcriptional responses to extracellular EAA in animal cells are encoded by ATF4, ATF6, FOS, JUN, EGR1, MYC, SREBF1, and HIF1. To explore which of these TFs are affected in mammary epithelial cells by Lys and Met, their mRNA expression in vitro was evaluated over the first 48 h after changing extracellular Lys and Met concentrations. Epithelial cells were harvested from one rear mammary gland of 3 dairy cows and cultured in DMEM/F12 and Medium 170. Cells were passaged 3 times and, when plates reached confluence, they were differentiated by the inclusion of prolactin in the medium for 5 d. Treatments were designed to provide Lys or Met at concentrations below and above normal physiological concentrations in plasma of lactating dairy cows while maintaining all other EAA at normal levels. Treatments were: 1) 20 μM Lys (LY), 2) 320 μM Lys (HY), 3) 5 μM Met (LM), 4) 80 μM Met (HM) and were cultured in duplicate. Cells were harvested for RNA isolation and qRT-PCR analysis at 0, 8, 16, 24, 32, 40, and 48 h after initiation of EAA treatment. Linear contrasts were estimated by ANOVA assuming fixed effects of treatment and time. Expression of ATF4 was 3 times higher on LY compared with HY ($P < 0.01$) between 24 and 48 h of incubation. There were no significant effects of Lys on the expression of the other 7 TF. Expression of ATF4 was also elevated on LM compared with HM ($P < 0.01$) between 24 h and 48 h but JUN and MYC were both 2 times higher on HM compared with LM ($P < 0.01$) at 48 h. Findings indicate that ATF4 was upregulated by deficiencies of either Lys or Met, JUN and FOS were upregulated by Met only, while other canonical AA response TFs were affected little during the first 48 h after changing extracellular Lys or Met concentration.

Key Words: essential amino acid, transcription factors, primary cell

2526V Effects of leucine on rumen microbial community structure during in vitro ruminal fermentation. L. Sun[1], T. Brenna[2], L. Ma[1], Z. Wu[1], J. Xu[3], and D. Bu*[1,4], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Dell Pediatric Research*