

Monday, July 12, 2010

SYMPOSIA AND ORAL PRESENTATIONS

Graduate Student Paper Competition: ADSA Southern Section

37 Biohydrogenation intermediates of ¹³C-labeled docosahexanoic acid in ruminal batch cultures. C. M. Klein* and T. C. Jenkins, *Clemson University, Clemson, SC.*

There are no reported pathways of docosahexanoic (DHA) biohydrogenation; however, DHA is metabolized by ruminal microorganisms. In this study a 0.5mg pulse dose of uniformly ¹³C labeled DHA in ethanol was injected at 0h as a metabolic tracer. This study did not examine every fatty acid metabolite, but rather identified several saturated and trans-18:1 fatty acid metabolites and set a foundation for future work. Ruminal microorganisms collected from a lactating Holstein cow were incubated in 10 mL batch cultures for 0, 6, 24 and 48 h. Treatment groups were 0.5% ¹³C DHA and 0.5% DHA. Duplicate cultures were methylated and then separated on a 100-m CP-Sil 88 column. Abundances of the quasimolecular (M) and M+n ions were determined by mass spectroscopy in chemical ionization mode. Enrichment was calculated as $\{M+n/[M+(M+n)]\} \times 100$ in labeled minus unlabeled cultures and tested for their difference from zero by *t*-test ($P < 0.01$). DHA decreased from 0.207mg to 0.041mg between 0 and 48h and had an enrichment of 98.1%. Oleic, linoleic, and linolenic acids decreased 0.431, 0.962 and 0.081mg in the same time period. Stearic acid increased 1.37mg and trans-18:1 monenes increased 0.415 mg. Palmitic, eicosanoic, and docosanoic acids increased 0.196, 0.008 and 0.005 mg respectively. Palmitic, stearic, all trans-18:1, eicosanoic, and docosanoic acids were examined for enrichment. Docosanoic acid was highly enriched at 24h and 48h to 20.2% and 16.3% (SE = 0.466; $P < 0.01$). Neither eicosanoic acid, nor any trans-18:1 monenes were enriched at any time ($P < 0.05$). Low levels of enrichment ($<0.1\%$; $P < 0.01$) were found in palmitic and stearic acids. This study clearly identifies docosanoic acid as an intermediate of DHA biohydrogenation. It also identifies that, in this system, eicosanoic acid and trans-18:1 fatty acids were not intermediates. Stearic acid had minor enrichments, but DHA only made up 3.13% of total fat or 10.3% of metabolized unsaturated fat.

Key Words: docosahexanoic acid, biohydrogenation

38 Cows genetically more susceptible to mastitis have altered neutrophil migration patterns. A. A. Elliott*, S. Minkin, J. Biggerstaff, J. Dunlap, and G. M. Pighetti, *University of Tennessee.*

The largest loss in profit for dairy farmers occurs with mastitis, an inflammation of the mammary gland. Our prior research identified a marker in the CXCR1 gene associated with mastitis and decreased neutrophil migration in vitro. Because neutrophil migration is critical for eliminating most infections, our ongoing research seeks to identify the specific mechanisms causing impaired migration. The first study evaluated actin polymerization, one of the first steps in neutrophil migration, in cows with different CXCR1+777 genotypes. Neutrophils from cows with GG (n = 11) and CC (n = 11) genotypes were isolated and stimulated with zymosan activated sera (ZAS). Cells were fixed and stained for F-actin and subsequently evaluated for F-actin content, distribution, and cell morphology. Neutrophils of the CC cows had significantly lower average F-actin polymerization than the GG cows (P

= 0.05). Because F-actin polymerization drives neutrophil movement, lower amounts could partly explain reduced migration. In contrast, cell morphology and F-actin distribution was similar between genotypes. Our second study focused on directed migration of neutrophils toward interleukin-8 (IL8). The migration of neutrophils from GG (n = 4) and CC (n = 4) genotypes was captured under a microscope and velocity, acceleration, distance of the path, distance from origin, largest X distance and largest Y distance were analyzed for each individual cell. Cells from GG genotype traveled further and more directly compared with CC genotype cells. Our findings suggest lower F-actin polymerization in combination with a lower ability to directly and efficiently move toward the site of infection could impair neutrophil response to infection in cows with the CC genetic background and may contribute to increased mastitis susceptibility.

Key Words: mastitis, chemotaxis, neutrophil

39 Effects of different levels of cottonseed hulls on rumen development and growth in dairy calves. R. M. Doescher*, C. C. Williams, C. F. Hutchison, B. F. Jenny, and A. H. Dolejsiova, *LSU AgCenter, Baton Rouge.*

A study was conducted to determine the effects of varying levels of cottonseed hulls on growth and metabolic indications of rumen development of dairy calves. Sixty-four Holstein calves (Heifers, n = 40; Bulls, n = 24) were randomly assigned to one of 4 dietary treatments which included calf starters containing no cottonseed hulls (control; C), 10% cottonseed hulls (10% CSH), 15% cottonseed hulls (15% CSH), or 20% cottonseed hulls (20% CSH). Calves were fed their respective treatments beginning on d 8 until d 56 of age. Body weights were measured at birth and biweekly thereafter until d 56 of age. Withers and hip heights were measured beginning on d 14 and biweekly thereafter until d 56 of age. Feed intake and fecal scores were recorded twice daily through d 56. On d 14, 28, 42, and 56, rumen fluid was collected for analysis of pH, volatile fatty acids (VFA), and ammonia (NH₃), and blood was collected for analysis of plasma urea nitrogen (PUN) and β-hydroxybutyrate (BHBA). There was no treatment effect ($P > 0.1$) on average daily starter intake, fecal scores, body weight, wither height, and hip height. Calves consuming CSH had higher ($P < 0.05$) rumen pH than C. Rumen pH also decreased ($P < 0.01$) as calves aged. There was no treatment effect ($P > 0.1$) on rumen acetate, propionate, butyrate, and total VFA concentrations. There was a trend for a treatment by week interaction ($P < 0.1$) and a treatment effect ($P < 0.1$) on NH₃ production, with calves consuming 10% CSH having higher concentrations. There was no significant treatment effect ($P > 0.1$) on BHBA, but a main effect of sex ($P < 0.05$) was observed in which males had higher BHBA concentrations. There was no significant treatment effect ($P > 0.1$) on PUN concentrations, but a main effect of sex ($P < 0.05$) was observed with females having greater concentrations. Overall, incorporating cottonseed hulls into a calf starter showed no significant effect on growth and rumen development in Holstein dairy calves.

Key Words: cottonseed hulls, dairy calves, rumen development

Graduate Student Paper Competition: ADSA-ASAS Northeast Section

40 Effects of herbs and essential oils on in vitro batch culture ruminal fermentation. J. A. Tekippe^{*}, A. N. Hristov¹, K. S. Heyler¹, V. D. Zheljzkov², J. Ferreira³, and G. A. Varga¹, ¹*Pennsylvania State University, University Park*, ²*Mississippi State University, NMREC, Verona*, ³*USDA-ARS, Beaver, WV*.

Medicinal herbs and essential oils were evaluated in a batch culture in vitro screening experiment as potential anti-methanogenic additives for ruminant diets. A total of 88 essential oils and 14 herbs were tested. Rumen inoculum enriched with particle-associated microorganisms was collected from a lactating dairy cow, 2 h before feeding. Incubation was conducted in serum bottles containing 1 g of a feed mixture (0.7 g alfalfa hay, 0.2 g corn starch, and 0.1 g solvent-extracted soybean meal), 1 mL of essential oils (10, 50, and 100 mg/L, final medium concentration), 19 mL of McDougall's buffer with 5 g/L glucose and 2.5 g/L acid-hydrolyzed casein, and 20 mL of ruminal inoculum. Bottles were then flushed with CO₂ and incubated at 39°C for 6 h. Corresponding 50 mL tubes were incubated for 24 h for NDF degradability analysis. Herbs were tested using the same procedure, except a portion of the alfalfa hay was replaced with 12.5, 50, 100, and 200 mg of plant material (air-dry basis), and NDF was tested using the DAISY apparatus. Blanks and monensin (5 mg/L, final medium concentration) were also incubated. At the end of the incubations, total gas and methane production, VFA and ammonia concentration, and NDF degradability were measured. Treatment by application level interactions were not significant for any of the essential oil treatments. Two of the essential oils increased acetate production, 12 increased propionate production, 10 increased butyrate production, 3 reduced methane production (by 20 to 30%), and 2 decreased ammonia production. With the herbs, 1 increased acetate production, 2 increased propionate production, 1 decreased methane production (by 30%), and 1 decreased ammonia production. Eight of the herbs increased NDF degradability at various inclusion levels. Overall, these results indicated that some essential oils and medicinal herbs may have a significant effect on ruminal fermentation in vivo.

Key Words: essential oil, medicinal herb, rumen fermentation

42 Use of environmental protection best management practices by Maryland horse farm operators. N. M. Fiorellino^{*}, K. M. Wilson, and A. O. Burk, *University of Maryland, College Park*.

A survey study was conducted to identify use of environmental protection best management practices (BMPs) by horse farm operators in Maryland (MD). A 35-question survey was mailed to 1,000 MD horse farm operators using a multiple wave mailing strategy. Data were collected from February to March 2009. Survey response rate was 43.9%, with responses received from 91.3% of counties. Respondents housed 16.8 ± 1.6 horses on 11.6 ± 1.6 ha of turnout with a mean stocking density of 0.81 ± 0.04 ha horse⁻¹. Primary use of horse farms was recreational (32.8%), boarding (30.7%), and breeding (14.7%). On a scale from 1 (low) to 5 (high), respondents reported they had the least knowledge of farm cost-share programs (2.0 ± 0.06), grass and weed identification (2.8 ± 0.05), and soil conservation plans (3.0 ± 0.05) and the most knowledge of recommended stocking density (3.6 ± 0.06), rotational grazing (3.6 ± 0.05), and manure management (3.5 ± 0.05). Most respondents assessed their pasture vegetative cover at >70% (78.5%); however, respondents operating a farm with a high stocking density (<0.4 ha horse⁻¹) reported lower rates. The majority of respondents used a recommended stocking density of ≥ 0.61 ha horse⁻¹ (53.9%). Only 15.4% of respondents did not maintain a proper vegetative buffer between horses and water sources.

However, 71.4% of respondents stored their manure on an impermeable or permeable surface uncovered, 30.7% allowed horses unlimited access to surface water, 39.4% did not utilize sacrifice lots to rest pastures, and more than half (55.4%) of respondents reported soil erosion in their pastures. Soil erosion was noted less by respondents operating a farm for recreation and those with a low stocking density (>0.81 ha horse⁻¹). Only 3 of 10 recommended BMPs were being used correctly by the majority ($\geq 51\%$) of respondents, with proper manure storage, use of sacrifice lots, and rotational grazing being used by the lowest percentage of respondents. BMPs with low adoption rates should be the focus of future education programs for horse farm operators in MD to minimize environmental impact to the Chesapeake Bay Watershed.

Key Words: best management practices, horse, survey

43 Sources of variation and importance of the quantification of the in vitro NDF digestibility for estimating rates of NDF digestion. E. Raffrenato^{*} and M. E. Van Amburgh, *Cornell University, Ithaca, NY*.

Accurate and precise estimations of intrinsic NDF digestibility and digestion kinetic parameters have been shown to be important for the evaluation of forages. Methods for measuring NDFD are more variable than those for NDF and there is no standard method. Within laboratories, the standard deviation (SD) of repeatability for single determinations of NDFD is 1.5 to 2 times that of the NDF assay. The SD of reproducibility among laboratories for single in vitro determination is 3 to 5 times that of NDF and can greatly impact the calculations for rate of digestion. Recently a method has been proposed to standardize the inoculum (Goesser et al., 2009) and although the CV of multiple runs was reduced, the method appeared to result in lower NDFD values. Our objective was to evaluate the sources of and reduce the variability of both NDF and NDFD assays. Several hundred forages were analyzed for aNDFom (Mertens, 2002), ADL (Van Soest et al., 1991) and NDFD using Goering and Van Soest buffer in 125 mL Erlenmeyer flasks under CO₂ using inoculum from multiple lactating cows within 20 min of collection and filtered in crucibles. One of the factors impacting the outcome of NDF, ADL and NDFD was the loss of soluble materials through the Gooch crucible. The use of glass filters (Whatman, 934-AH, 1.5 μ m) improved recoveries from 0 and 4% ($P < 0.05$) and 0 and 8% ($P < 0.05$) for NDF and NDFD, respectively, and reduced the CV below 2% for intra-run and below 3% for inter-runs, among all forages. In vitro (240 h) and in situ (360 h) NDF recovery was improved up to 15% with the use of the filter and through the use of bags with porosity of 15 μ m, respectively. Further, the use of the filter increased ($P < 0.05$) recovery of ADL from 3 to 36%. In conclusion improved recovery and inoculum handling allowed better estimations of NDF, NDFD, ADL and indigestible NDF and result in a more precise and accurate estimations of the rate of digestion (Van Amburgh et al., 2004). This data will serve to provide recommendations for the standardization of the NDFD procedure.

Key Words: NDF, NDFD, ADL

44 Effect of capsicum oil on feeding behavior and milk production in lactating dairy cattle. L. R. Tager^{*} and K. M. Krause, *West Virginia University, Morgantown*.

A preliminary study was conducted to study the effect of capsicum oil on feeding behavior and milk production in lactating dairy cows. Twenty lactating dairy cows (16 Holstein and 4 Ayrshire) were used in a crossover design using the Growsafe monitoring system (Growsafe

Systems Ltd., Airdrie, AB, Canada). After balancing for milk yield and DIM, 10 cows were randomly assigned to each of 2 pens (5 Growsafe bins/pen). Cows were fed a TMR with a 54:46 forage:concentrate ratio (DM basis) ad-libitum twice daily. Experimental treatments included: 2 g/cow/d capsicum oil (CAP; XT 6933; Pancosma S.A., France) or no oil (CON). Data was collected until 20 d of sound data were accumulated for each experimental period. There was no difference in DMI (23.05 kg/d vs. 23.17 kg/d; $P = 0.91$), number of meals/d (12 vs. 12; $P = 0.34$), total h spent eating/d (3.22 h vs. 3.16 h; $P = 0.89$), mean meal length (17.24 min vs. 17.23; $P = 0.96$), length of first meal after feeding (43.3 min vs. 46.2 min; $P = 0.21$), or feeding rate (7.49 kg/h vs. 7.78 kg/h; $P = 0.80$) between CAP and CON. Milk yield did not differ between CAP and CON (27.6 kg/d vs. 28.1 kg/d; $P = 0.72$). Based on this preliminary study, feeding CAP at 2 g/cow/d does not change feeding behavior or milk production. To add more experimental units and confirm the results of the preliminary study, this project is currently being replicated.

Key Words: capsicum, feeding behavior

45 Digestive fate of free ferulic acid in lactating dairy cows. M. A. Soberon*, D. J. R. Cherney, and D. A. Ross, *Cornell University, Ithaca, NY*.

Ferulic acid (FA), a phenolic compound with antioxidant and anti-cancer activities, naturally occurs in plants as one of the building blocks of lignin. Many veins of research have been devoted to releasing FA from the lignin complex to improve digestibility of plants fed to livestock. Thus, it is of interest to study the fate of a given dosage of FA in the dairy cow to determine the likelihood of its transfer from ingested feed to milk when available in free form. Six mid to late lactation Holstein cows at the Cornell Research Farm were given 14 d adaptation to diet and stall. Ad libitum access to a total mixed ration based on haylage and corn silage (31.1% NDF, 5.52 mg/g FA) was provided during the study. A cross over design was used so that each cow alternated weekly between Treatment (Trt) and Control. On d 1, jugular cannulas and urine catheters were placed in all cows. On d 2, Trt cows received a single dosage of 150 g pure FA powder via their fistula ($n = 4$) or a balling gun for nonfistulated cows ($n = 2$). Plasma, urine, feces, feed, orts, milk and rumen fluid were sampled intensively for the next 36 h. On d 8, the cows crossed over and the experiment was repeated. All samples were analyzed for FA concentration. FA administration did not have an effect on DMI ($P = 0.593$), milk yield ($P = 0.501$), milk fat yield ($P = 0.457$), milk protein yield ($P = 0.959$), SCC ($P = 0.495$), NDF content of orts ($P = 0.462$) or NDF content of feces ($P = 0.393$). The level of FA in the feces did not change as a result of Trt ($P = 0.399$). As expected, [FA] increased dramatically upon FA dosage and decreased over time until returning to basal levels in rumen fluid (detectable until 4.25 h post-dosage), plasma (detectable until 5.5 h post-dosage), and urine (detectable until 23 h post-dosage). Baseline values for FA in urine and rumen fluid were variable among cows and had an effect on [FA] in Trt cows. FA was detected in the milking following FA dosage (6.5 h post-dosage) at significantly higher concentrations in Trt cows. From this study, it is observed that orally ingested FA can be transported into the milk and that the physiological transfer of FA occurs from rumen to urine within 1 h and milk within 6.5 h.

Key Words: ferulic acid, cow

46 The effect of form of trace mineral supplementation on lactation, neutrophil function, and vaccination response in Holstein cows. L. M. Nemeč*¹, J. D. Richards², C. Atwell², D. E. Diaz², and T. F. Gressley¹, ¹*University of Delaware, Newark*, ²*Novus International Inc., St. Charles, MO*.

The aim of this study was to compare inorganic versus chelated forms of supplemental Cu, Mn, and Zn on production, neutrophil activity, antibody titer, and plasma and milk mineral concentrations. Holstein cows ($n = 25$) averaging 63 d in milk were assigned to a 12-week completely randomized design study in 2 groups. Diets were supplemented at 100% of NRC requirements either by inorganic trace minerals (ITM) in sulfate and oxide forms, or by chelated trace minerals (Mintrex; Zn, Cu and Mn as Mintrex Zn [Zn(HMTBa)₂]; Mintrex Cu [Cu(HMTBa)₂]; Mintrex Mn [Mn(HMTBa)₂]; Novus International Inc., St. Charles, MO). Intake and milk production were recorded daily. Milk composition was measured weekly, and milk Cu, Mn, and Zn was determined at wk 0 and 8. Blood Cu and Zn concentrations and neutrophil activity were measured at wk 0, 4, 8, and 12. Neutrophil activity was measured in vitro assays of phagocytosis, reactive oxygen species (ROS) production, and chemotaxis. A rabies vaccination was administered at wk 8, and vaccine titer response at wk 12 was measured by both Rapid Fluorescent Focus Inhibition Test (RFFIT) and ELISA. Wet chemistry analysis of the total mixed rations indicated dietary Cu was 17 and 24 ppm, Mn was 40 and 62 ppm, and Zn was 84 and 138 ppm for the ITM and Mintrex diets, respectively. There was no effect of treatment on milk production (42 kg/d), milk composition, plasma minerals, neutrophil ROS production, or neutrophil chemotaxis. Dry matter intake tended to be lower for Mintrex than ITM cows (24.1 vs. 25.3 kg/d, $P = 0.06$). Milk Cu concentration was greater for Mintrex than ITM cows (76 vs 57 μ g/L, $P < 0.01$). Neutrophil phagocytosis tended to increase for the Mintrex treatment ($P = 0.09$). Rabies antibody titer was approximately 2.5 fold higher for Mintrex than ITM cows, regardless of assay ($P = 0.08$ for RFFIT; $P = 0.009$ for ELISA). In summary, the Mintrex treatment increased bioavailability as indicated by increased milk Cu and enhanced both innate and adaptive immune responses as indicated by increased neutrophil phagocytosis and vaccine titer response, respectively.

Key Words: trace minerals, neutrophils, parity

47 The effects of length of storage on the composition and nutritive value of corn silage. M. C. Der Bedrosian*¹, L. Kung Jr.¹, and K. E. Nestor Jr.², ¹*University of Delaware, Newark*, ²*Mycogen Seeds, Indianapolis, IN*.

The objective of this study was to evaluate the effect of length of storage on the composition and nutritive value of corn silage. The primary treatments were a brown midrib (BMR) and a non-BMR silage hybrid (nBMR) harvested at a normal DM (32%) and a high DM (41%). Forages were vacuumed and heat-sealed in quintuplet storage bags and ensiled for various periods of time between 0 to 360 d before analyses. Nutrient analysis, fermentation end products and in vitro NDF (NDF-D; 30 h) and starch digestion (starch-D; 7 h) techniques were used to evaluate the samples. Over both maturities and time of ensiling, compared with nBMR, BMR silage was lower ($P < 0.01$) in lignin, CP and ADF but higher in starch content. The NDF content of silages was not affected by treatment. Of the fermentation end products, the concentration of acetic acid increased substantially with length of storage for all treatments specifically increasing as much as 140% between d 45 to d 365 for normal DM BMR silage. The NDF-D was greater overall all times for BMR (68% DMB) than nBMR (52%) but there was an interaction ($P < 0.01$) between hybrid and maturity. Increasing maturity did not affect NDF-D of nBMR but it decreased for BMR by approximately 5

percentage units. Fiber digestion did not change substantially between 45 and 365 d regardless of hybrid or DM content. Starch digestibility was highest ($P < 0.01$) in normal DM nBMR samples at d 0 (about 80%) and lower (65 to 68%) for other treatments. This was most likely due to the fact that normal DM nBMR had a low starch content (21%) compared with other treatments ($31 \pm 3\%$). Starch-D increased ($P < 0.01$) about 6 to 8 percentage units for nBMR silages but increased ($P < 0.01$) approximately 15 percentage units for BMR silages over the ensiling period. Relative to the 2 corn silage hybrids used in this study, the results show that time of ensiling does not affect NDF-D but it results in marked increases in starch digestion. The NDF-D of BMR but not the nBMR hybrid was affected by harvesting at a higher DM. Allowing corn silage to ensile for prolonged periods of time can improve its nutritive value.

Key Words: Silage, BMR

48 Effect of forage particle length on rumen fermentation and chewing activity of late lactating and dry dairy cows. F. X. Suarez-Mena*, G. I. Zanton, and A. J. Heinrichs, *The Pennsylvania State University, University Park.*

The objective of this study was to determine forage particle size effects on chewing activity, rumen pH, and rumen fill in dairy cattle fed rations with similar physically effective NDF but different mean particle length. Two experiments were conducted to determine chewing behavior, rumen

pH, and rumen fill. Treatments consisted of 3 diets differing only in forage length (geometric mean length, mm): hay (5.40, 8.96 and 77.90, for short (S), medium (M) and long (L) diets, respectively) for Experiment 1 (E1), and straw (10.16 S, 24.68 M and 80.37 L) for Experiment 2 (E2). Hay or straw comprised the sole source of forage (50% for E1 and 75% for E2). Both experiments used 3 rumen cannulated, non-pregnant Holstein dairy cows, late lactating in E1 and dry in E2, with 3×3 Latin square designs. Chewing behavior was visually monitored for 48 h at 2-min intervals. In E1, DMI (18.3 ± 2.1 kg/d), pH (6.4 ± 0.1), time spent eating (280 ± 22.5 min/d), time spent ruminating (487 ± 17 min/d), and total time spent chewing (767 ± 34 min/d) were not different, while min/kg of DMI and min/kg of NDF intake tended to increase linearly ($P < 0.1$) as forage length increased. Rumen digesta volume (L; 113.3 S, 117.8 M, and 114.4 ± 17.1 L) had a quadratic response ($P < 0.05$) and rumen digesta weight tended to respond quadratically ($P < 0.1$); however, differences were numerically small. In E2, DMI (8.3 ± 1.3 kg/d), pH (6.7 ± 0.1), time spent eating (236 ± 23.5 min/d), time spent ruminating (468 ± 45.2 min/d), total time spent chewing (704 ± 67.7 min/d) and min/kg NDF intake were not different, while min/kg of DMI had a trend for a quadratic effect ($P < 0.1$). Rumen digesta volume (111 ± 18.8 L) and weight (103 ± 17.4 kg) were not different. Changes in rumen characteristics and chewing in cows in late lactation and dry cows were not detected or were small when the particle size of the forage was changed.

Key Words: particle size, chewing, rumen pH