GRADUATE STUDENT COMPETITION: ADSA-ASAS NORTHEAST SECTION ORAL: ADSA/ASAS NORTHEAST BRANCH GRADUATE STUDENT COMPETITION

0361 Glucose metabolism by bovine neutrophils characterized by mass spectrometry and [$^{13}C_6$] glucose.

Y. Qu*[1], B. J. Bequette[1], T. H. Elsasser[2] and K. M. Moyes[1], [1]*Department of Animal and Avian Sciences, University of Maryland, College Park,* [2]*USDA/ARS Growth Biology Lab, Beltsville, MD.*

At the present time, the metabolic priority for and subsequent pathway fate of glucose by bovine blood neutrophils (BBN) is unknown. During early lactation, glucose availability is low. Consequently, a resulting energy-compromised state may partly explain the lack of robust immune response observed at this time. The objective of this study was to characterize the metabolic utilization of glucose by BBN from dairy cows in early and mid-lactation by incubation of BBN with [$^{13}C_6$] glucose followed by gas chromatography–mass spectrometry (GC–MS). Jugular blood (~150 mL) was collected from 4 healthy multiparous dairy cows in early (n = 2; < 21 d in milk) and mid-lactation (n = 2; > 90 d in milk). The BBN were isolated and adjusted to a final concentration of 6×10^6 cells/mL using RPMI/5% fetal bovine serum containing 4 m*M* of a 50:50 mix of unlabeled and ^{13}C-labeled glucose. Cells were incubated for 2 h at 37°C, 95% humidity, and 5% CO_2. Nonessential AA in metabolic equilibrium with Krebs cycle intermediates and lactate in BBN were monitored by GC–MS. Data were analyzed by the student's t test for unpaired comparison. Significance was declared at $P \leq 0.05$. No stage of lactation effect was observed. Although 43% of the pyruvate pool derived from glucose, a small proportion of this pool entered the Krebs cycle. Our results suggest that the pyruvate carboxylase flux was minimal and only 5% of the acetyl-CoA pool was derived from the pyruvate pool. In conclusion, although BBN exhibit high rates of glycolysis, glucose is not the main substrate oxidized for energy in the Krebs cycle, and perhaps other extracellular substrates (e.g., fatty acids, glutamine, aspartate) are preferred. Future studies are needed to examine the metabolic utilization of other substrates and their influence on the function of BBN.

Key Words: cow, glucose, neutrophil

0362 Exploring the Molecular diversity and density of the rumen microbiome within the impala (*Aepyceros melampus melampus*) from Pongola, South Africa.

L. M. Cersosimo*[1], B. St-Pierre[1], W. van Hoven[2], and A. D. G. Wright[1], [1]*The University of Vermont, Burlington,* [2]*University of Pretoria, Pretoria, South Africa.*

The rumen microbiome of domesticated ruminants has been studied extensively, but few studies have explored the microbiome of wild ruminants and no studies have used a metagenomic approach to examine the rumen microbiota within the impala (*Aepyceros melampus melampus*). Impala, like all domesticated ruminants, have a consortium of anaerobic microorganisms inhabiting their foregut (i.e., the rumen) that contribute to the feed efficiency of the animal, as well as greenhouse gas emissions. The present investigation seeks to expand our knowledge about the rumen microbial community of the impala while drawing comparisons to their domesticated relatives that could lead to advances in both feed efficiency and methane mitigation strategies. The rumen contents from 4 male impala culled in the Kwazulu-Natal province of Pongola, South Africa, were collected postmortem. Once bacterial and archaeal amplicons were generated, next-generation sequencing techniques (i.e., Roche 454-pyrosequencing) were used to identify the diversity of bacterial and methanogenic archaeal (i.e., methanogens) 16S rRNA gene sequences. Real-time PCR amplification of the 16S rRNA gene was used to estimate the density of the bacterial population (R^2 = 0.997), while the mcrA (methyl coenzyme-M reductase) gene was used to estimate the density of the methanogen population (R^2 = 0.997). Using the bioinformatic platform, MOTHUR, a total of 20,124 methanogen sequence reads were assigned to 344 operational taxonomic units (OTUs) using a sequence identity cutoff of 3%. A high sampling efficiency was observed with OTU coverages > 99% for each individual impala, along with similar Shannon indices of 1.90 ± 0.003. The Ribosomal Database Project (RDP) Classifier classified 94.3% of the reads to the genus *Methanobrevibacter,* 4.2% of the reads to the genus *Methanosphaera*, with the remaining 1.5% of the reads being unclassified. Most notably was the abundance of *Methanobrevibacter thaueri*-related methanogen sequence reads in all 4 impala samples, representing > 30% of each animal's total sequences. This high abundance of *Methanobrevibacter thaueri*-related methanogen sequences has not been observed in previous studies of domesticated or wild ruminants, suggesting that either the diet and/or the rumen physiology of the impala may differ from previously studied ruminants. The diversity of the rumen bacteria will also be elucidated and discussed. Future studies to look at other factors that affect the rumen environment, like gender, should be performed to see if these findings are consistent.

Key Words: methanogens, bacteria, impala, rumen, *Methanobrevibacter*, operational taxonomic units

0363 Effects of ground flaxseed on milk production, milk composition, and methane emissions in organically-managed Jersey cows during the grazing season.

B. J. Isenberg*[1], A. F. Brito[1], A. B. D. Pereira[1], N. L. Whitehouse[1], R. B. Standish[1] and K. J. Soder[2], [1]*University of New Hampshire, Durham,* [2]*USDA-Agricultural Research Service, University Park, PA*

Our previous research feeding incremental levels of ground flaxseed (GFLAX; 0, 5, 10, or 15% of diet DM) to organically-managed Jersey cows linearly reduced yields of milk and milk components, DMI, and enteric CH_4 emissions. While milk yield did not differ significantly between 0 and 10%, 10% GFLAX significantly increased n-3 fatty acids and conjugated linoleic acid. Flaxseed is rich in n-3 fatty acids and energy; energy is a limiting factor to milk production in pasture-based diets. This study evaluated impacts of supplementing pasture with 10% of diet DM as GFLAX to Jersey cows on milk production and composition, and enteric CH_4 emissions. Twenty multiparous lactating organically managed Jersey cows, averaging 408 kg BW and 112 d in milk (DIM), were blocked by milk production and DIM and randomly assigned to 1 of 2 treatments: (1) control (soybean meal and ground corn grain mix as 10% of total DMI) or (2) ground flaxseed as 10% of total DMI. Treatments were top-dressed onto a 25% mixed grass-legume baleage, 23% grain meal, and 2% liquid molasses TMR (% of diet DM); pasture composed the remaining 40% DMI. The study extended from June 8 to September 27, 2013 with four 28-d periods, with the last 7 d used for data and sample collection. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with repeated measures over time; no treatment × period interaction was observed. Cows had access to mixed grass-legume pastures for about 16 h daily in a rotational strip grazing system. Cows were milked twice daily with milk production recorded each time. Milk samples were collected for 2 consecutive days and analyzed for fat and protein. A portable automated head chamber system was used for assessing CH_4 fluxes. Milk production did not differ significantly in cows supplemented (17.6 kg/d) or not (18.2 kg/d) with GFLAX. Similarly, concentration and yield of milk components did not differ significantly, averaging 4.20% and 0.76 kg/d for milk fat and 3.45% and 0.61 kg/d for milk protein. Intake of TMR was lower ($P < 0.01$) in cows with (10.5 kg/d) or without (10.8 kg/d) GFLAX supplementation. Methane emissions did not differ significantly, averaging 299 g cow^{-1} d^{-1} (control diet) and 295 g cow^{-1} d^{-1} (GFLAX diet). Feeding 10% of total DMI as GFLAX to grazing dairy cows had no negative impact on milk production and composition, but did not mitigate CH_4 emissions under conditions of the current experiment.

Key Words: pasture, ground flaxseed, methane

0364 Farm-level evaluation of implementing feeding best management practices (BMP) on Pennsylvania dairy farms.

H. L. Weeks*, T. W. Frederick, L. M. Hagan, K. S. Heyler, and A. N. Hristov, *Department of Animal Science, The Pennsylvania State University, University Park, PA*

Feeding best management practices (BMP) can have a significant impact on the environmental footprint of dairy farms. The objective of this study was to evaluate the environmental and productive effects of implementing feeding BMP on commercial dairy farms in Pennsylvania. Fifteen farms (124.8 ± 20.5 ha, 169 ± 39 cows, and 31.4 ± 0.2 kg cow^{-1} d^{-1} milk production) in central and southeast Pennsylvania participated in the study. A set of 4 background total mixed ration (TMR), forage, milk, feces, urine samples, as well as feed intake and production data, were collected from each cooperator farm biweekly between January and March of 2013 (preBMP period). Following the implementation of applicable feeding BMP, chosen by the producer, including reduction of dietary crude protein (CP; $n = 9$) and P ($n = 5$), adjusting rations for changes in forage dry matter ($n = 10$), and group feeding of the lactating herd ($n = 2$), another set of 4 sampling and data collection events took place between June and August of 2013 (postBMP period). Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with farm as a random effect. On farms in which dietary CP was reduced (from 17.2 to 15.9%; $P < 0.01$), fecal N (FN; 2.78 vs. 2.63%; $P = 0.01$), total urinary N (UN; 0.071 vs. 0.055%; $P < 0.01$), urinary urea N (UUN; 52.2 vs. 44.4mg/dL; $P < 0.01$), and milk urea N (MUN; 16.9 vs. 13.9 mg/dL; $P < 0.01$) decreased (pre- vs. postBMP, respectively). Only 3 farms successfully decreased dietary P (from 0.42 to 0.40; $P < 0.01$), which resulted in decreased fecal P concentration (0.85 vs. 0.69; $P < 0.01$). Group feeding was implemented on 2 farms. The average CP of the rations fed on these farms decreased from 15.7 to 14.4% ($P = 0.03$), which resulted in decreased, UN (0.077 vs. 0.048%; $P < 0.01$), UUN (56.1 vs. 36.9 mg/dL; $P < 0.01$), and MUN (17.4 vs. 13.7 mg/dL; $P = 0.03$). Milk production and DMI were not affected by BMP implementation. Bulk tank milk fat (3.91 vs. 3.56%; $P < 0.01$) and milk protein (3.13 vs. 2.98%; $P < 0.01$) decreased from pre- to postBMP on all farms, perhaps due to seasonal effects. In conclusion, reduced dietary CP decreased N concentrations in urine and feces and reduced dietary P decreased fecal P concentration on commercial dairy farms.

Key Words: dairy farm, dietary protein, dietary phosphorus

0365 The impact of dairy advisory teams on farm improvement in Pennsylvania dairies.

M. H. Buza*, L. Holden, and R. C. Goodling, *The Pennsylvania State University, University Park, PA*

Dairy producers continuously seek ways to improve their farm, and many choose to form a dairy advisory team (DAT) to improve management. The objectives were: (1) to compare key measures before and after the team to determine if the use of a DAT was effective, and (2) to compare a group of 20 herds with a DAT to Pennsylvania (PA) averages for key measures. Teams were formed between May 2008 and January 2013. The range for herd size was 42 to 694 ± 139 cows. Herd size, milk yield, somatic cell score (SCS), peak milk yield, age at first calving (AFC), days in milk (DIM), pregnancy rate, and cull and mortality rate were key measures analyzed. The changes in key measures, after using DAT for at least 1 yr, were analyzed using a paired t test. After DAT use, herds had higher ($P < 0.01$) herd sizes (187 vs. 177 cows) and higher ($P = 0.066$) milk yields of 31.3 vs. 32.1 kg, as well as lower ($P = 0.077$) AFC of 24.9 vs. 25.4 mo and lower ($P = 0.054$) percent of herd with SCS $\geq$ 4 of 21.7 vs. 23.9%. The DAT herds' January Dairy Herd Improvement Association (DHIA) test data were compared with Dairy Metric's PA average for January 2014 using a 1-sample t test. Farms with DAT had significantly ($P < 0.05$) higher milk yield of 33.9 vs. 31.9 kg and peak milk yield for lactation 1 with 36.1 vs. 33.9 kg. There was no significant difference between the averages for DAT herds and PA herds for peak milk yield in older cows, but DAT herds had numerically higher peaks (44.3 vs. 42.7 kg and 47.4 vs. 45.9 kg for second and third lactation, respectively) in older cows. Herds with DAT had significantly ($P < 0.001$) lower AFC of 24.4 vs. 25.6 mo and lower percentage of herd with SCS $\geq$ 4 with 20.15 vs. 26.6%. There were no differences for pregnancy rate, DIM, or cull or mortality rate. Use of DAT led to larger herd size, greater milk yield, lower AFC, and better SCS. Herds with DAT had higher milk yield, lower AFC, and better SCS compared with PA averages. Use of a DAT was beneficial to dairy farms.

Key Words: dairy advisory team, dairy herd improvement, dairy herd key measures

0366 Plant-derived compounds, trans-cinnamaldehyde and eugenol, reduce adhesion and invasion of *Staphylococcus aureus* in bovine mammary epithelial cells in vitro.

D. Jaganathan*[1], A. Kollanoor-Johny[1], K. Vekitanarayanan[1], G. W. Kazmer[1], L. Kuo[2], Y. B. Wang[2], and K. E. Govoni[1], [1]*Department of Animal Science, University of Connecticut, Storrs,* [2]*Department of Statistics, University of Connecticut, Storrs.*

Staphylococcus aureus is 1 of the major bacterial pathogens involved in ruminant mastitis globally. The disease has major impacts on animal health and milk quality leading to severe economic losses to the dairy industry. *S. aureus* adhesion to and invasion of mammary epithelial cells (MEC) is critical for the establishment of the disease. We hypothesized that 2 plant-derived antimicrobials, *trans*-cinnamaldehyde (TC) and eugenol (EG), would reduce *S. aureus* adhesion to and invasion of primary bovine MEC in vitro. Mammary epithelial cells were isolated from mammary glands of lactating cows postslaughter and were characterized using multicytokeratin immunostaining. Confluent MEC monolayers were inoculated with 4 *S. aureus* strains (Strain ST 35, Thorn 17, 15, and M9175) at mid-log phase separately (multiplicity of infection– 100:1) either in the presence or absence (control) of subinhibitory concentrations (SICs: greatest concentration that did not inhibit bacterial growth) of TC (0.006%) and EG (0.03%), and incubated for 2 h for bacterial adhesion. Infected cells were treated with gentamicin (100 µg/mL) for 1 h at 37°C for enumerating invaded bacteria. All experiments included duplicate samples and were repeated 3 times. Data were analyzed using PROC ANOVA and significance determined at $P < 0.05$. For strain Thorn 17, pre-exposure of *S. aureus* (5 h) and MEC (12 h) to EG reduced *S. aureus* adhesion to and invasion of MEC by ~1.6 ± 0.01 $\log_{10}$ CFU/mL (control = 6.67 ± 0.01 $\log_{10}$ CFU/mL; $P < 0.0001$) and 2.8 ± 0.11 $\log_{10}$ CFU/mL (control = 3.9 ± 0.02 $\log_{10}$ CFU/mL; $P < 0.0001$), respectively. Preexposure of *S. aureus* and MEC to TC reduced *S. aureus* adhesion to and invasion of MEC by ~2.2 ± 0.02 $\log_{10}$ CFU/mL (control = 6.7 ± 0.02 $\log_{10}$ CFU/mL; $P < 0.0001$) and 2.85 ± 0.08 $\log_{10}$ CFU/mL (control = 3.94 ± 0.01 $\log_{10}$ CFU/mL $P < 0.0001$), respectively. Similar results were observed with strains ST 35, M9175 and Thorn 15. In conclusion, SICs of TC and EG reduced *S. aureus* adhesion to and invasion of MEC. In vivo studies using a mammalian model to validate these results are warranted.

Key Words: mammary epithelial cells, plant compound, *Staphylococcus aureus*

0367 Effect of dietary supplementation of *Capsicum* extract on feed intake, milk production and composition, rumen fermentation, and rumen microbial populations in dairy cows.

J. Oh*[1], F. Giallongo[1], H. L. Weeks[1], T. W. Frederick[1], A. N. Hristov[1], and E. H. Wall[2], [1]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [2]*Pancosma, Geneva, Switzerland.*

Dietary supplementation of *Capsicum* extract (CE) has been reported to increase feed intake and modify ruminal fermentation in cattle. The objective of this experiment was to investigate the effects of CE on feed intake, digestibility, N utilization, milk performance, ruminal fermentation, and ruminal microbial diversity in lactating dairy cows. Eight multiparous Holstein cows (days in milk, 50 ± 9.6 d; BW, 591 ± 32.6 kg), including 3 ruminally-cannulated, were used in a replicated 4

× 4 incomplete Latin square design with 25-d periods. Treatments were 0 (CON), 250, 500, and 1000 mg CE cow^{-1} d^{-1}. The CE was mixed with a small portion of the TMR and top-dressed. Apparent total tract digestibility was not affected by treatment. Treatments also had no effect on urinary-N, urea-N, urinary purine derivatives, and fecal-N excretions. Milk yield tended to quadratically increase ($P = 0.08$, SEM = 0.86) with CE: 50.5, 51.9, 51.5, and 50.3 kg/d, respectively. Dry matter intake was not affected by CE (27.0 ± 0.64 kg/d). Feed efficiency tended to increase quadratically ($P = 0.08$; SEM = 0.047) with CE supplementation: 1.90, 1.93, 2.02, and 1.96 kg/kg, respectively. Milk fat yield increased ($P = 0.05$) for CE treatments compared with CON (1.58 vs. 1.46 kg/d, respectively). Relative to CON, CE increased ($P = 0.04$) 4% FCM and tended to increase ($P = 0.06$) energy corrected milk yields. Concentration of milk lactose was decreased ($P = 0.01$) by CE. Compared with CON, concentration of β-hydroxybutyrate (BHBA) in blood plasma tended to be higher ($P = 0.07$) for CE and quadratically responded ($P = 0.02$) to CE supplementation level. Plasma NEFA was not affected by CE. Ruminal pH tended to be decreased ($P = 0.06$) by CE compared with CON. Ruminal ammonia and VFA concentrations were not affected by treatment, although acetate tended to linearly decrease ($P = 0.11$) with CE. Predominant bacteria in rumen contents were *Ruminococcaceae* spp. and *Prevotella* spp. (10.8 to 22.6% of the total population). Compared with CON, *Prevotella* spp., *Butyrivibrio* spp., and *Roseburia* spp. were decreased ($P \leq 0.05$) by CE. In conclusion, feed intake, total tract digestibility of nutrients, and N utilization were not affected by dietary CE supplementation. However, CE increased milk production, milk fat yield, and plasma BHBA concentration. Collectively, these results suggest that CE may increase lipid mobilization for milk fat synthesis in dairy cows.

Key Words: capsicum extract, feed intake, milk production

0368 The effects of CO_2 and HEPES buffer on in-vitro chemotaxis assays of bovine neutrophils.

A. M. Barnard*, R. Nebenhaus, R. M. Dyer, and
T. F. Gressley, *University of Delaware, Newark.*

Neutrophil (PMN) chemotaxis can be measured in vitro with or without 5% CO_2. The production of carbonic acid during incubation with 5% CO_2 may reduce pH, possibly affecting cellular migratory function. The goals of this study were to determine whether incubation with 5% CO_2 impacts PMN chemotactic function, and if the addition of HEPES buffer affects response to CO_2. Neutrophils were isolated from 12 lactating cows over 5 different test dates and cell concentration adjusted to 2×10^6 cells/mL. Media consisted of HBSS containing 5% FBS with or without the addition of 10 mM HEPES. The bottom wells of a 48-well chemotaxis chamber (Neuro Probe Inc., Gaithersburg, MD) contained 28 μL of media supplemented with 50 ng/mL of complement component 5a (C5a) or 100 ng/mL of Interleukin-8 (IL-8). A 5 μm polycarbonate membrane separated the top and bottom wells. Neutrophil suspension (50 μL) was added to the top wells, and the chambers were incubated at 37°C for 1 h, in the presence or absence of 5% CO_2. Negative controls did not contain chemoattractant in either well while positive controls contained 50 ng/mL C5a in both top and bottom wells. Raw adherence (RawAd) was determined by counting PMN adhered to the bottom of the membrane in 5 microscope fields per well. Relative adherence (RelAd) was calculated as (RawAd test well)/(RawAd negative control well) × 100%. Data were analyzed using the Glimmix procedure of SAS (SAS Inst. Inc., Cary, NC) with the fixed effects of HEPES, CO_2, chemoattractant and all interactions and random effects of date and cow within date. RawAd and RelAd were both affected by CO_2 ($P = 0.03$; 0.002, respectively) and chemoattractant ($P = < 0.0001$ and 0.0002, respectively). The presence of CO_2 caused a decrease in both RawAd and RelAd. For both RawAd and RelAd, chemotaxis towards C5a or IL-8 did not differ but both were greater than chemotaxis to controls. RawAd was also affected by CO_2 × chemoattractant ($P = 0.02$) and HEPES × CO_2 ($P = 0.0001$). RawAd for C5a or IL-8 wells was approximately twice that of control wells in the absence of CO_2, but was not significantly different from controls in the presence of CO_2. RawAd decreased in the presence of CO_2 when HEPES was not present, but was unaffected by CO_2 in the presence of HEPES. These results suggest that incubating PMN with CO_2 reduces the chemotactic response and that HEPES partially ameliorated this effect.

Key Words: neutrophils, chemotaxis, bovine

0369 The 2001 Dairy NRC Ration Evaluation Software effectively predicts dietary strong ion and DCAD concentrations in lactating dairy cow diets.

M. E. Iwaniuk* and R. A. Erdman,
University of Maryland, College Park.

Recent research suggests that increasing DCAD concentration using buffers in lactating dairy cow diets results in increased milk yield and milk fat percentage. Dietary buffers, such as $NaHCO_3$ and K_2CO_3, alter DCAD concentration and have been used in lactating dairy cow diets for decades. However, most of the published work on buffer supplementation was conducted before the development of the DCAD concept. Thus, these data have not been included in previous metaanalyses of DCAD effects on dairy cow performance. One problem with the use of these data is the lack of measured concentrations for each of the minerals required for calculation of DCAD (Na + K– Cl, meq/kg DM). To overcome this obstacle, a study was conducted to determine if the 2001 Dairy NRC Ration Evaluation Software could be used to estimate missing dietary ion concentrations. Data from 44 journal articles on the effects of buffers and DCAD that were published from 1965 to 2011 were used in the study. Experimental diets where Na, K, and Cl concentrations were analyzed were used as test observations. Ingredient information for each diet was extracted from each article and entered into the 2001 NRC software to estimate dietary mineral concentrations. The NRC predicted Na, K, Cl, and DCAD were regressed on the respective measured concentrations and the regression statistics are reported. The root mean prediction square error (RMPSE) expressed as a fraction of the mean concentration was small. Chloride data were clustered in 2 distinct populations (0.25 to 0.5 and > 1.0%) which may have resulted in slope confidence intervals different from 1. However, the regression slopes for Na, K, and DCAD were not different from 1 ($P > 0.05$). Intercept values when significant (Na and Cl) were small compared with the mean concentration for each mineral. Residual plots indicated no mean or linear bias. In conclusion, the NRC software accurately estimated mineral and DCAD concentrations and could be used to estimate missing ion concentrations in future metaanalyses of DCAD experiments.

Key Words: DCAD, NRC Software, dairy cows

Table 0369.

Item	Obs	Mean	SD	Slope	95% CI	Int	$P <$	R^2	RMPSE
Na, %	129	0.39	0.18	0.98	± 0.065	0.04	0.003	0.88	0.06
K, %	137	1.45	0.52	0.96	± 0.072	0.04	0.511	0.84	0.21
Cl, %	84	0.71	0.40	0.86	± 0.091	0.12	0.002	0.81	0.17
DCAD, meq/kg	60	343.9	143.9	0.96	± 0.102	-23.4	0.266	0.86	54.7