RUMINANT NUTRITION VIII

0675 Characterization of rumen microbial community composition of buffalo fed diets varying in forage:concentrate ratio. B. Lin[*1,2], C. Zou[1], F. Cox[2], G. Henderson[2], P. H. Janssen[2], X. Liang[1], and G. Attwood[2], [1]*Buffalo Research Institute, The Chinese Academy of Agricultural Sciences, Nanning, China,* [2]*AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand.*

Murrah and Nili-Ravi are two widely used dairy water buffalo breeds in Asian countries. In this study, we investigated the diversity of ruminal microbes in six Murrah and six Nili-Ravi water buffalos maintained in China. The buffalos were separated into two groups; each group contained three Murrah and three Nili-Ravi buffalos, and two different diets (with forage to concentrate ratios of 3.2 or 1.6) were fed to the two groups. After feeding the diets for 15 d, ruminal fluid was sampled by stomach tube before the morning feeding. The bacterial and archaeal 16S rRNA genes and the ciliate protozoal 18S rRNA genes from the 12 rumen samples were sequenced by multiplex 454 Titanium pyrosequencing, and the sequence data was analyzed using QIIME 2.0 software. Our results showed that at the phylum level, Bacteroidetes was the predominant bacterial group, accounting for 42% to 72% of total bacteria, followed by Firmicutes, Fibrobacter and Proteobacteria. At the genus level, *Prevotella* dominated, accounting for 22 to 58% of total bacteria, followed by *Fibrobacter*, *Paludibacter* and *Ruminococcus*. While there were differences between the bacterial community compositions of different animals, there was no obvious correlation of bacterial community composition, at the phylum or genus level, with the diets or with buffalo breeds. For the archaea, *Methanobrevibacter*-related organisms were the dominant group, accounting for around 80% of the total, followed by *Methanoplasmatales* (RCC, 15%) and *Methanosphaera* (3%). Similar to the bacterial community, there was no clear correlation of the archaea community profile with diet or buffalo breed. The ciliate protozoal communities differed between the samples analyzed, although *Entodinium* was the most abundant group of ciliates in every sample, accounting for more than 40% of total protozoa. The second largest ciliate group varied in different samples with *Isotricha*, *Polyplastron* or *Dasytricha* the dominant genera after *Entodinium*. In summary, the predominant genera observed in the bacterial, archaeal and ciliate protozoal communities in rumen samples of Murrah and Nili-Ravi buffalo were *Prevotella*, *Methanobrevibacter* and *Entodinium,*, respectively. Overall, the buffalo rumen microbial communities varied greatly between individual animals, regardless of the diet composition or the buffalo breed used in this study.

Key Words: buffalo, rumen, microbial community, forage to concentrate ratio

0676 Bacterial diversity associated with different primer pairs on different diets in the rumen microbiome of Kankrej cattle. D. W. Pitta[*1], N. Indugu[2], S. Kumar[1], K. B. Prajapathi[3], A. K. Patel[4], N. Parmar[4], A. B. Patel[4], B. Reddy[4], and C. Joshi[4], [1]*University of Pennsylvania, Kennett Square,* [2]*University of Pennsylvania, Kennett Square,* [3]*Sardharkrushinagar Dantiwada Agricultural University, India,* [4]*Anand Agriculture University, India.*

A comprehensive analysis of the bacterial diversity in the rumen of Kankrej cattle was investigated in this study. Two groups of four animals were assigned to two diets of Dry and Green roughage, respectively. In each dietary group, four animals (replicates) were group fed one of three dietary treatments for 6 wk each; dietary treatments were K1 (50% concentrate, 50% dry/green roughage), K2 (25% concentrate, 75% dry/green roughage), and K3 (100% dry/green roughage). The rumen samples were collected at the end of 6 wk period and separated to solid and liquid fractions. The genomic DNA was extracted from each sample and PCR-amplified for V1-V3, V4-V5, and V6-V8 hypervariable regions, sequenced on 454 Roche platform and analyzed for bacterial diversity using QIIME. A total of 600, 851 pyrotags were analyzed in this study. Differences in community composition were based on UniFrac distance metric calculated by primer, diet, treatment, fraction, and animal and analyzed by Permanova test. Different primers had a significant ($P < 0.001$) effect on community compositions. There was no difference between diets but the inclusion of concentrate had an effect on community composition ($P < 0.01$). Also community compositions between fiber and liquid fractions were different ($P < 0.01$). Phylogenetic analysis revealed significant differences in the rumen microbiome mediated by primer ($P < 0.001$). In primer pair 1 associated bacterial communities, the predominant phyla were *Bacteroidetes* and *Firmicutes* which together constituted > 90% of abundance. These two phyla were influenced by dietary treatment ($P < 0.001$) and fraction ($P < 0.05$). Among communities associated with primer pairs 2 and 3, *Firmicutes* was predominant and contributed up to 90% of the fiber fraction. Dietary treatment had an influence on the abundance of B*acteroidetes* ($P < 0.001$) and *Firmicutes* ($P < 0.001$) with K1 showing higher ($P < 0.001$) *Bacteroidetes* while K2 and K3 treatments had higher ($P < 0.001$) abundance of *Firmicutes*. Other bacterial phyla such as *Proteobacteria* and *Fibrobacter* together contributed up to 6% of the bacterial abundance that was influenced by dietary treatment ($P < 0.001$) and fraction ($P < 0.001$) across all three primer pairs. The identified repertoire of bacterial populations was dependent on the primer pair, as targeting the V1-V3 region resulted in greater diversity profiles of the rumen microbiome in this study. Within each primer pair, there were no differences between dry and green roughages. However, inclusion

of concentrate in the diet altered the community composition with noticeable shifts at the phylum level.

Key Words: rumen microbiome, Kankrej cattle, QIIME, concentrate, roughage

0677 Development of rumen microbiota in dairy calves: Impact of weaning and different weaning strategies.

S. C. Li*[1], M. A. Steele[2], P. Azevedo[1], M. Carson[2], J. C. Plaizier[1], H. Derakhshani[1], and E. Khafipour[1,3], *[1]Dep. of Animal Science, University of Manitoba, Winnipeg, Canada, [2]Nutreco Canada Agresearch, Guelph, ON, Canada, [3]Dep. of Medical Microbiology and Infectious Diseases, Winnipeg, MB, Canada.*

Weaning stress affects the establishment of rumen bacterial community in dairy calves, which is important for rumen development and future rumen physiology. This study examined the effects of different weaning strategies on the microbial composition of rumen in 24 male and 20 female Holstein dairy calves. Calves were blocked according to gender and birth weight, and randomly assigned to a step-down weaning (SW) or an abrupt weaning (AW) treatment. All calves had free access to water and starter intake throughout the experiment and 9 L/d of milk until d 36 of life and weaned on d 49 of life. Calves in SW group were weaned gradually by reducing milk intake from 9 to 4.5 L/d from d 37 to d 48 while the AW calves were abruptly weaned on d 49 by reducing milk intake from 9 to 0 L/d. Rumen fluid was sampled on d 36 (pre-weaning) and on d 54 (post-weaning) of life. DNA was extracted and V4 region of 16S rRNA gene was amplified and subjected to paired-end Illumina sequencing. The output paired-end reads were merged using PANDASeq assembler and analyzed using QIIME. The resulted operational taxonomic units were aligned to Greengenes database. Alpha-diversity of bacterial community was calculated using different richness estimators. Differences in β-diversity of microbiota across treatments and time points were tested using Permutational ANOVA. Alpha-diversity of microbiota declined post-weaning, indicating a compositional heterogeneity reduction in rumen population. Beta-diversity of microbiota differed ($P < 0.05$) between pre- and post-weaning. Different weaning strategies did not affect α and β-diversities. Predominant phyla before weaning included *Bacteroidetes* (66%), *Firmicutes* (19%) and *Proteobacteria* (11%). Another 18 phyla were present at low abundance; each below 1% of population. In post-weaned calves, *Bacteroidetes* was reduced (44%) while *Firmicutes* and *Proteobacteria* increased. However, a smaller increase in *Firmicutes* and a greater increase in *Proteobacteria* were observed after weaning in SW compared to AW calves (28% vs. 38%, and 22% vs.17%, respectively). Eighty-two out of 348 identified bacterial genera were different between pre- and post-weaned calves across AW and SW treatments, which accounted for 50% of sequences. The impact of weaning strategy was observed on several genera, including but not limited to, several members of *Alphaproteobacteria*, RF32, *Alcaligenaceae*, and *Helicobacter*. Data provided novel information on the profile of rumen microbiota associated with weaning and weaning strategy that can be used as biomarkers in future studies.

Key Words: calves, weaning, rumen microbiota

0678 The potential benefit of corn dried distillers' grain (co)products (DDG) in the mitigation of methane production in cattle: An in vitro analysis.

M. A. Fonseca*[1], D. K. A. Silva[2], H. D. Naumann[3], T. R. Callaway[4], and L. O. Tedeschi[1], *[1]Texas A&M University, College Station, [2]Federal Rural University of Pernambuco, Garanhuns, Brazil, [3]University of Missouri, Columbia, [4]USDA-ARS, College Station, TX.*

The objective of this work was to determine the impact of different levels of DDG on the digestibility of OM and the production of methane (CH_4) using an in vitro technique. Nine diets were formulated with Bermuda grass hay, ground corn, and alfalfa hay mixed with DDG (0, 10, 20, 30, 40, 50, 60, 80, and 100% of diet DM). These nine diets and alfalfa hay alone (laboratory control) were ground (1 mm), and incubated with rumen fluid (from grazing cows) and media. Blanks (rumen fluid and media only) were used to adjust the CH_4 production. Three replicates and four incubation times (3, 6, 24, and 48 h) were investigated in the bottle's headspace using a syringe for collection. Because these diets had different amounts of potentially fermentable substrate, adjusted CH_4 was computed as CH_4 concentration divided by the amounts of NDF (CH_4NDF, mM/g NDF), NFC (CH_4NFC, mM/g NFC), fermentable carbohydrate (FCHO = CHOA + CHOB1 + CHOB2) (CH_4FCHO, mM/g FCHO), OM (OM = 100– Ash) (CH_4OM, mM/g OM), and fermentable OM (FOM = OM– Ash– EE) (CH_4FOM, mM/g FOM) in the diet sample. The PROC MIXED of SAS (SAS Inst., Cary, NC) was used to analyze the data assuming a repeated measure design. Diets and incubation times were assumed as fixed factors and replicate within diet as random factor. Except for CH_4FCHO and CH_4NFC, diet effect was significant ($P < 0.001$) for all other adjusted CH_4 concentrations, suggesting that FCHO and NFC may not explain differences in CH_4 concentration. Regarding incubation time and its interaction with diet, all adjusted CH_4 concentration were highly significant ($P < 0.001$). There was a tendency to decrease CH_4 concentration as the DDG level increased, but the level of DDG in the diet had to be greater than 50% to yield significant reduction in the adjusted CH_4 concentration, CH_4FOM and CH_4OM (CH_4 per unit of OM) when the fat content was greater than 7.39% DM. Significant linear regressions for adjusted CH_4 concentration ($CH_4 = 14.5 - 0.667 \times$ Fat; $r^2 = 0.64$, RMSE = 1.48 mM, $n = 46$) and CH_4FOM (CH_4FOM $= 83.8 - 3.35 \times$ Fat; $r^2 = 0.53$, RMSE = 9.30 mM/% FOM, $n = 46$) on fat content of the diets were observed. These in vitro results suggested that under normal ru-

minal fermentation CH_4concentration will decrease as dietary fat increases if ruminal retention time is between 24 and 48 h.

Key Words: abatement, greenhouse gas, ruminant

0679 Use of avian antibodies against lipopolysaccharides to improve gastrointestinal function in early lactation dairy cows. L. Ibarbia[*1], F. Cunha[1], K. N. Galvão[1], F. Maunsell[1], A. Donovan[1], and N. DiLorenzo[2], *[1]Dep. of Large Animal Clinical Sciences; University of Florida, Gainesville, [2]University of Florida, Marianna.*

Subacute ruminal acidosis (SARA) has been associated with decreased milk production in early lactation dairy cows. During SARA, increased lipopolysaccharides (LPS) are released in the rumen from the breakdown of gram negative bacteria, increasing LPS concentrations and potential translocation to blood. Presence of LPS in the blood stream leads to activation of the acute phase response in peripheral blood, which in turn leads to a reduced DMI and milk production. An avian-derived anti LPS polyclonal antibody preparation (PAP-LPS) was developed (Camas Inc., LeCenter, MN) to use as a feed additive in dairy cattle. Our objective was to evaluate the effectiveness of PAP-LPS in decreasing the intensity of the inflammatory response and increasing milk yield on early lactation dairy cows. Primiparous (Pr; n = 174) and multiparous (Mu, n = 226) Holstein cows from one herd were randomly allocated to one of three treatments: 1) PAP-LPS (n = 55 Pr and n = 70 Mu), 2) Placebo (n = 66 Pr and 77 Mu), and 3) Control (n = 53 Pr and 79 Mu). Cows on PAP-LPS received a dose of 3 g/d of PAP-LPS via oral drench in a water suspension, while Placebo received the same dose of a PAP-LPS inactivated by an acid-shock treatment. Control cows did not receive any treatment. The PAP-LPS and Placebo treatments were administered from d 0 (calving) until 14 DIM. Milk production was recorded from d 0 to d 98 and weekly averages were used in a repeated measures analysis using the PROC MIXED of SAS and a first-order autoregressive covariate structure. The model included the fixed effects of treatment, parity and treatment x parity interactions. Feeding PAP-LPS increased (P = 0.04) total milk production on the first 98 DIM when compared with Control (3881 and 3769 kg, respectively), while only tended to increase it (P = 0.07) when compared to placebo (3785 kg). A week x treatment interaction was observed (P = 0.088) for weekly average milk production. Weekly average milk production was greater (P < 0.05) for PAP-LPS vs. Control on weeks 3, 4, and 10. On week 6, cows fed PAP-LPS had greater (P < 0.05) milk production (44.9 kg/d) when compared with both Control (43.1 kg/d) and Placebo (43.1 kg/d). In conclusion, feeding PAP-LPS increased milk production in early lactation dairy cows, likely due to a reduced inflammatory response during diet transition.

Key Words: dairy cows, LPS, avian antibodies

0680 Large-subunit rDNA based differentiation of anaerobic rumen fungi using restriction fragment length polymorphism. D. Sumit[1,2,3], S. Kumar[*2,4], D. W. Pitta[4], J. Edwards[1], T. Callaghan[1], G. Grifith[1], P. Mudgil[2], and A. Puniya[2], *[1]Aberystwyth University, Aberystwyth, UK, [2]National Dairy Research Institute, Karnal, India, [3]Agharkar Research Institute, Pune, India, [4]University of Pennsylvania, Kennett Square.*

The contribution of rumen anaerobic fungi to animal production systems is substantial despite their lower numbers in the rumen. Utilizing both cultivation-based and advanced genomic tools, several reports have described the repertoire of ligno-cellulolytic enzymes secreted by these fungi. However, characterization of anaerobic rumen fungi is a tedious process with difficulties in isolation methods, their pleomorphic nature and lack of appropriate targeted genetic markers to understand fungal diversity. Previously, we have identified a new marker, D1/D2 domain of 28S rDNA of LSU (large-subunit; LSU) region which provided a better resolution in species differentiation of genus *Orpinomyces* than internal transcribed spacer (ITS) region, a preferred genetic marker for most fungal diversity studies. In the current study, we applied the same D1/D2 marker to assess species level distribution of several fungal lineages. For this, nearly 100 isolates of known lineages (*Orpinomyces*, *Neocallimastix*, *Anaeromyces*, *Piromyces*, *Caecomyces*, *Cyllamyces*) and unknown anaerobic fungi were cultivated from the rumen of different herbivorous animals. The isolates were extracted for their genomic DNA using modified method of Cetyl tri-methyl ammonium bromide (CTAB) and also MoBio genomic DNA extraction kit. The genomic DNA samples were PCR-amplified for D1/D2 regions of LSU (≈ 780 bp) using NL1 and NL4 primers. The amplified PCR products were purified, sequenced and characterized for phylogeny. Based on the phylogenetic analysis, representative sequences from identified fungal lineages were further subjected to in silico restriction digestion that led to the identification of two restriction enzymes, *Alu*I (recognition sequence, AG▼CT) and *Hinf*I (recognition sequence, G▼ANT C). About 34 selected sequences were co-digested by these two restriction enzymes using Restriction Fragment Length Polymorphism (RFLP) assay. The RFLP analysis of electrophoretic runs revealed distinct riboprints for individual fungal lineages that were identified in this study. Sequences annotated to similar fungal lineages showed comparable riboprints, which confirmed that targeting D1/D2 region of the LSU gave repeatable results. Our study summarized that RFLP-based differentiation of fungal lineages was better accomplished when D1/D2 region of the LSU was used as a genetic marker.

Key Words: rumen, anaerobic fungi, LSU, PCR-RFLP, co-digestion

0681 Responses in rumen microbiomes of *Bos taurus* and *Bos indicus* steers fed rice straw and supplemented protein. E. A. Latham[*1], J. C. McCann[2], K. Weldon[1], T. A. Wickersham[1], J. Coverdale[1], and W. E. Pinchak[3], *[1]Texas A&M University, College Station, [2]University of Illinois, Urbana, [3]Texas A&M AgriLife Research, Vernon.*

Bos indicus typically perform better than *Bos taurus* when consuming a low-quality diet; however, the response to supplementation is generally greater in *Bos taurus*. The underlying mechanisms supporting these responses have not been elucidated. Characterization of differences in rumen bacterial populations and their role in the two subspecies may provide insight. Five cannulated Angus and Brahman steers were used in concurrent 5 × 5 Latin squares with repeated measures. Animals were offered ad libitum access to rice straw (4.7% CP). Treatments consisted of an unsupplemented control and two levels (50 and 120 mg N/kg BW) of isonitrogenous supplements (30% CP), either high (H; 74%) or low (L, 26%) in UIP. Rumen sample were collected at 0 and 4 h post-feeding, separated into liquid and solid fractions and frozen immediately in liquid N. Rumen bacterial taxa were sequenced utilizing a Roche 454 platform based on the 16s rRNA gene. At 97% sequence similarity, 97,826 OTUs were identified, which included 21 phyla, 108 families, and 255 genera. Each taxa was analyzed using PROC MIXED with period and animal as random effects. The top seven phyla accounted for > 98% of observed abundance. Six phyla differed as a function of fraction, time, and fraction × time interaction ($P < 0.05$). *Bacteroidetes* (65%) and *Firmiucutes* (28%) were dominant phyla, both tended to differ as a function of subspecies × treatment and treatment ($P < 0.06$). The top 14 families accounted for > 93% observed abundance. Unlike phyla, families were influenced more by subspecies and treatments than fraction ($P < 0.05$). *Prevotellaceae* (> 42%), *Ruminococcaceae* (> 13%), *Sphingobacteriaceae* (> 8%), and *Lachnospiraceae* (> 7%) were the dominant families. Genus abundance also varied as a complex function of subspecies, time and various associated interactions with treatment and fraction ($P < 0.05$). Twenty genera were present at > 0.8% average abundance and accounted for over 85% of total observed abundance. Dominant genera were *Prevotella* (29.5%), unknown *Prevotellaceae* (11%), unknown *Ruminococcaceae* (8.5%) and unknown *Sphingobacteriaceae* (7%). Overall bacterial community diversity was greater than expected because rice hay is recalcitrant to bacterial digestion. Similarly, consistent differences in family and genus taxa between *B. indicus* and *B. taurus* suggest important roles the symbiotic rumen microbiome may have in the capacity of *B. indicus* to utilize low-quality forage over a range of supplement types and levels.

Key Words: cattle, microbiome, supplementation

0682 Effects of dietary fat source and monensin on methane to carbon dioxide ratio, VFA profile, and performance of finishing steers. A. C. Pesta[*1], A. K. Watson[1], R. G. Bondurant[1], S. C. Fernando[2], and G. E. Erickson[1], *[1]University of Nebraska-Lincoln, Lincoln, [2]University of Nebraska, Lincoln.*

A finishing study was conducted to evaluate effects of supplemental fat type and presence or absence of monensin on methane to carbon dioxide ratio (CH_4:CO_2), ruminal VFA profile, and performance of finishing steers. Steers ($n = 60$, initial BW = 414 ± 16 kg) were individually fed for 125 d using Calan gates. Steers were stratified by initial BW and assigned randomly to one of six treatments. Four diets were designed to compare fat source: CON (corn-based diet with no added fat), MDGS (50% modified distillers grains plus solubles added), OIL (3% corn oil added), and TAL (3% tallow added). Added fat diets were formulated to provide 6.5% total dietary fat. An additional two treatments were added to factorialize presence or absence of monensin (375 mg daily) with CON or MDGS diets. At time of feeding, exhaled breath samples were collected from each animal at weekly intervals throughout the study using a custom built automated gas collection system and were analyzed for CH_4 and CO_2, using gas chromatography. Carbon dioxide was used as an internal marker and CH_4:CO_2 was used to quantify the effects of diet on methane emission. Rumen fluid collected via esophageal tubing on d 55, before feeding, was analyzed for VFA profile. Treatment differences were evaluated using pre-planned contrasts. No diet × monensin interaction was observed ($P = 0.19$). The CH_4:CO_2 for cattle fed MDGS was greater ($P = 0.03$) than CON, 0.057 compared to 0.050, respectively. No effect ($P = 0.56$) of monensin inclusion on CH_4:CO_2 was observed. Differences in CH_4:CO_2 observed may be due to composition of dietary fat, as steers fed MDGS had greater CH_4:CO_2 than those fed TAL or CON ($P < 0.03$), while those consuming OIL were intermediate. No differences were observed ($P = 0.45$) for ruminal acetate to propionate ratio (A:P) due to fat type or presence of monensin, as A:P of all diets fell between 1.08 and 1.40. Finishing performance was also unaffected as no differences in DMI ($P = 0.48$), ADG ($P = 0.37$), or G:F ($P = 0.78$) were observed. Composition of the finishing diet, particularly source of added fat does impact CH_4:CO_2.

Key Words: distillers grains, fat, methane