Influence of maternal factors on the rumen microbiome and subsequent host performance[1]

Hannah C. Cunningham,* Kathleen J. Austin,* and Kristi. M. Cammack†

*Department of Animal Science, University of Wyoming, Laramie, WY 82072; †West River Ag Center, South Dakota State University, Rapid City, SD 57702

Transl. Anim. Sci. 2018.2:S101–S105
doi: 10.1093/tas/txy058

INTRODUCTION

Increase in demand for quality meat and milk products coupled with limited quantity and quality of resources exacerbates the need for improved production efficiency in livestock. The rumen microbiome enables ruminant livestock to convert low-quality forages into high-quality end products and provides energy for the host (Church, 1988; Flint and Bayer, 2008). Differences in the microbiome have been reported between high and low feed efficient animals (Guan et al., 2008; Carberry et al., 2012).

The early microbiome is critical for host immunity and development of absorptive capacity of the rumen (Church, 1988; Taschuk and Griebel, 2012); factors influencing the colonization of the rumen microbiome may have lasting effects on host performance and alter feed efficiency. Evidence suggests a strong link between host genetics and the rumen microbiome (Hernandez-Sanabria et al., 2013; Roehe et al., 2016), indicating potential to select for a more desirable rumen microbiome based on maternal breed and/or crossbreeding strategies. Additionally, mode of delivery and the intrauterine environment can affect gut microbial colonization of offspring (Thum et al., 2012; Guzman et al., 2015; Aagaard et al., 2016; Chu et al., 2016). We hypothesized that maternal factors would alter the rumen microbiome in progeny and these changes would persist into adulthood impacting host feed efficiency and performance. Our objective was to determine the impacts of differing modes of delivery, rearing types, and maternal breeds on the calf microbiome and subsequent effects on feed efficiency.

MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee.

Cow Management and Diet

Mature Charolais (**Char**, n = 40) and Angus (**Ang**, n = 40) cows were used in this study. Cows were bred via natural service and their expected calving date was calculated as 250 d after the date the bull was introduced. Cows were fed ad libitum grass hay (6.8% CP, 40.2% ADF, 56.8% TDN, 1.2 NE_m MCal/kg, 0.64 NE_g MCal/kg) and 0.91 kg ×

[1]This project was supported by Agriculture and Food Research Initiative Competitive Grant no 2016-67016-24903 from the USDA National Institute of Food and Agriculture. The author would like to acknowledge University of Wyoming undergraduate students (Sierra Powell, Jaelyn Whaley, Hailey Barton, and McKenna Brinton), graduate students (Kelly Carpenter, Courtney Sutton, and Colby Hales), and Laramie Research and Extension Center (Dr. Doug Zalesky and Travis and Kaci Smith) for their contributions to this project including animal procurement, management, and sample collections.

[2]Corresponding author: hcunnin6@uwyo.edu

Received 1 May, 2018.
Accepted 2 May, 2018.

d^{-1} DDGS (29.9% CP, 12.3% ADF, 75.0 TDN%, 1.79 NE_m MCal/kg, 1.16 NE_g MCal/kg). Cows within each breed were randomly assigned to one of three treatment groups; 1) control group (**CON**; n = 12); 2) caesarean section group (**CSECT**; n = 12); and 3) bottle reared group (**BOT**; n = 12). The CSECT group was monitored closely for signs of parturition and a veterinarian performed the caesarean section using standard protocol including pain management and postsurgical care.

Calf Management and Calf Rumen Fluid Sample Collection

Cows in both CON and CSECT treatments reared their respective calf until weaning at approximately 180 d. The BOT cows were separated from their calves 24-h postparturition and calves were reared on artificial milk replacer until weaning. All calves had ad libitum access to hay and water.

At approximately 1.5 mo of age, all calves were fed Purina Stocker Grower (Purina Mills/Land O'Lakes, Inc; 15.3% CP, 10.0% ADF, 83.2% TDN, 2.12 NE_m MCal/kg, 1.44 NE_g MCal/kg on DM basis) at the rate of 0.91 kg × animal^{-1} × d^{-1} through weaning (~180 d of age). Postweaning calves were maintained on Purina Stocker Grower (average 4.54 kg × animal^{-1} × d^{-1}) and ad libitum hay for 73 ± 13.55 d prior to the start of the feed test. Calves were combined across the treatments to make a single cohort and acclimated to the GrowSafe system 2 wk prior to the start of the feed test. At this time, calves were transitioned to a forage-based complete pellet (13.3% CP, 22.3% ADF, 70.6% TDN, 1.65 NE_m MCal/kg, 1.04 NE_g MCal/kg on DM basis). Calves had ad libitum access to the forage pellet and water throughout the feed test.

Rumen fluid was collected at the conclusion of the feed test from Char CON (n = 8; three steers and five heifers); Char CSECT (n = 7; two steers and five heifers); Char BOT (n = 8; four steers and four heifers); Ang CON (n = 5; two steers and three heifers); and Ang BOT (n = 7; three steers and four heifers) calves. Samples were collected via oral-lavage using methods described by Lodge-Ivey et al. (2009). Due to unforeseen complications, no samples were able to be collected from Ang CSECT calves.

Cow and Calf Performance and Feed Intake Data Analyses

Calf 2-d BW was collected at the start and end of the feed test and every 2 wk throughout the duration of the test. Individual feed intake was recorded with the GrowSafe system to calculate ADG and residual feed intake (**RFI**). Residual feed intake was calculated as the difference between actual and expected feed intake of an individual animal, where expected intake was determined by regressing ADG and metabolic midweight on actual intake (Cammack et al., 2005). Low RFI was assigned to calves with a negative RFI (calves consumed less than expected; better feed efficiency), and high RFI was assigned to calves with a positive RFI (calves consumed more than expected; poorer feed efficiency). These data were analyzed using PROC MIXED of SAS (version 9.2; SAS Inst., Cary, NC). The final model for analyses of performance data included fixed effects of treatment, RFI class, and breed, and a random effect of sex. The LSMEANS were separated using LSD. Significance was determined at a $P \leq 0.05$ and a tendency for $0.05 < P \leq 0.10$.

Rumen Microbial DNA Extraction

Metagenomic shotgun sequencing was done using DNA extracted from rumen fluid samples according to methods described by Yu and Morrison (2004). The DNA was precipitated in ethanol and resuspended in Qiagen buffer EB to 80 ng/µL (2 µg aliquots) and shipped to the University of Missouri DNA Core Facility (Columbia) for sequencing.

Library Preparation and Metagenomic Sequencing

Libraries were constructed using manufacturer's (Illumina) protocol with reagents supplied in Illumina's TruSeq DNA PCR-free sample preparation kit. The library was then diluted and sequenced according to Illumina's standard sequencing protocol for HiSeq.

Metagenomic Sequencing Analysis and Identification of 16S rDNA Genes

Metagenomic sequences were quality filtered before 16S rDNA genes were identified using Metaxa2. Briefly, hidden Markov models using HMMER identified the conserved regions of the small subunit by aligning to the SILVA database which were subjected to a BLAST search. Taxonomic classification occurred by taking each rRNA entry and comparing the top five BLAST matches until a reliability score of 80 was achieved; this resulted in accurate taxonomic assignment (Bengtsson-Palme et al., 2015). These taxonomic profiles were further analyzed to assess diversity among and between samples using QIIME 1

(Caporaso et al., 2010). Factors of breed, sex, treatment, and RFI class were considered in these comparisons.

RESULTS

Calf Performance

Feed intake and ADG were not affected ($P > 0.05$) by breed or treatment. Both initial and final BW were greatest ($P < 0.008$) for CON calves, lowest for BOT, and CSECT intermediate. Feed efficiency (RFI) was different ($P = 0.02$) between Char and Ang calves, with Char having the more negative (more feed efficient) RFI compared to Ang (less feed efficient).

Rumen Microbiome

The effects of breed, sex, treatment, and RFI class all contributed ($P < 0.05$) to variation in taxa abundance. There were 82 taxa differentially abundant ($P < 0.05$) between Char and Ang; 39 differentially abundant ($P < 0.05$) between steers and heifers; 43 differentially abundant ($P < 0.05$) across treatments; and 41 differentially abundant ($P < 0.05$) across RFI classes.

Microbial richness (alpha-diversity) did not differ ($P \geq 0.27$) in respect to breed, sex, treatment, or RFI class. Microbial diversity between groups (beta diversity) differed across breeds ($P = 0.01$), in which the microbial diversity of the Ang rumen microbiome was more similar to other Ang microbiomes than to the Char microbiome. Additionally, calf microbial diversity was more similar ($P < 0.08$) within treatments than across the different treatments. Finally, differences in microbial diversity were also associated ($P < 0.01$) with the differing RFI classes.

DISCUSSION

Charolais and Ang cattle are two biologically different breeds that have been reported to be divergent for feed efficiency. Savietto et al. (2014) reported a negative RFI for Char (−0.124) and positive RFI for Ang (0.332). The date generated in this study were in agreement with the literature; Char calves had a lower RFI (−0.78 kg/d) on average compared to Ang calves (1.21 kg/d). Microbial phylotype frequencies differences have also been reported between Ang and Char (Hernandez-Sanabria et al., 2013). In our study, 82 taxa were differentially abundant between Ang and Char. Of these 82 taxa, 70 were more abundant in Ang calves than Char calves. The two taxa with the greatest abundance differences across breeds were an unclassified *Prevotella* which was more abundant in Char compared to Ang, and unclassified *Gammaproteobacteria* which was more abundant in Ang compared to Char. Both of these genera belong to phyla that are most abundant in the rumen (Bacteroidetes and Proteobacteria, respectively; Jami et al., 2014). Beta diversity also differed across breeds; the Ang microbiome was more similar to other Ang compared to Char microbiome. This suggests that in general, microbial composition differs between these two breeds but the number of different species is similar (alpha-diversity). The lack of difference in alpha-diversity across all comparisons in this study could be explained by the age of the calves. All calves were weaned 73 ± 13.6 d prior to the feed test and data suggests a stabilization of the rumen microbiome at weaning (Jami et al., 2013) which may contribute to the lack of differences in number of microbial taxa identified.

In total, 39 taxa were differentially abundant between steers and heifers. The taxa more abundant in heifers were predominantly from the phylum Proteobacteria, whereas those more abundant in steers belonged largely to Bacteroidetes and Firmicutes phyla. Paz et al. (2018) recently reported differences in key microbial families between heifer and steer models associated with divergence in feed efficiency, suggesting that sex may be another contributing factor for variation in the rumen microbiome, including profiles associated with feed efficiency variation.

The treatments for this study were selected in an effort to identify and quantify the effect of maternal factors such as parturition and rearing on the calf microbiome. The effect of mode of delivery has been studied primarily in humans and differences in microbial abundances and diversity between vaginal- and caesarean-delivered infants has been reported (Biasucci et al., 2008, 2010; Neu and Rushing, 2011). Although not statistically significant, *Bifidobacteria* and *Bacteroides* were more abundant in CON calves compared to CSECT calves, which is in agreement with Biasucci et al. (2008) who reported similar abundance differences in vaginally vs. caesarian delivered infants. Biasucci et al. (2008) also reported a less diverse bacterial microbiome in the caesarian infants, which is also in agreement with our study.

The birthing process is critical to the establishment of the offspring microbiome, but maternal colostrum and rearing can also impact early microbial establishment. Data from this study indicate

microbial diversity differences between CON and BOT calves; several taxa also differed in abundance across these two treatments. While BOT calves did receive maternal colostrum for 24 h, they were not reared with their dam and instead reared on artificial milk replacer until weaning. The separation from mature animals can alter the rumen microbiome (De Paula Vieira et al., 2012) and in humans, infants raised on formula have a more diverse microbiome compared to those that were breastfed (Bezirtzoglou et al., 2011). Both rearing environment and nutrition may be potential reasons for the differences in taxa abundance and beta diversity between CON and BOT calves.

Lastly, calves were housed as a single cohort for feed-efficiency testing and classification (high RFI vs. low RFI) to determine rumen microbial differences associated with feed-efficiency divergence. Lesser numbers of microbial taxa and gene-content richness have been associated with higher efficiency animals (i.e., low RFI; Shabat et al., 2016). While not statistically significant ($P = 0.27$), species richness was numerically lower in low RFI (high efficiency) compared to high RFI (low efficiency) calves, in agreement with the findings of Shabat et al. (2016). Overall, microbial composition differed between high and low RFI calves according to the beta-diversity assessment. A total of 41 microbial taxa were differentially abundant across RFI classifications. Only two of these taxa were Archaea, with the remaining 39 taxa being bacteria. Interestingly, one of these Archaea was an unclassified species of the *Methanobrevibacter* genus that was more abundant in high RFI calves. This is in agreement with data from Zhou et al. (2009) but differs from Ellison et al. (2017), who reported an increased abundance of *Methanobrevibacter smithii* in low RFI lambs. An increase in methanogenic pathways has been associated with lowly efficient animals compared to highly efficient animals (Shabat et al., 2016) and reduced methane emissions have been associated with selection based on improved RFI (Basarab et al., 2013). These could be explained by the loss of energy associated with production of methane (Church, 1988; Millen et al., 2016) which may in turn decrease efficiency. Differences in rumen taxa and gene composition were predictive of RFI phenotypes with high accuracy (91%; Shabat et al., 2016).

The realm of the rumen microbiome has grown exponentially over the past decade. With continued research efforts, the potential to improve livestock production by manipulating the rumen microbiome is possible. Maintaining desired shifts in the rumen microbiome is difficult due to microbial resiliency; however, intervention early in life appears promising for achieving lasting effects (Abecia et al., 2014; Yáñez-Ruiz et al., 2015). Data from our study suggest that maternal factors can influence the rumen microbiome beyond weaning and may have implications for divergence in feed efficiency.

LITERATURE CITED

Aagaard, K., C. J. Stewart, and D. Chu. 2016. Una destinatio, viae diversae: does exposure to the vaginal microbiota confer health benefits to the infant, and does lack of exposure confer disease risk? EMBO Rep. 17:1679–1684. doi:10.15252/embr.201643483

Abecia, L., E. Ramos-Morales, G. Martínez-Fernandez, A. Arco, A. I. Martin-Garcia, C. J. Newbold, and D. R. Yáñez-Ruiz. 2014. Feeding management in early life influences microbial colonisation and fermentation in the rumen of newborn goat kids. Anim. Prod. Sci. 54:1449–1454. doi:10.1071/AN14337

Basarab, J. A., K. A. Beauchemin, V. S. Baron, K. H. Ominski, L. L. Guan, S. P. Miller, and J. J. Crowley. 2013. Reducing GHG emissions through genetic improvement for feed efficiency: effects on economically important traits and enteric methane production. Animal 7 (Suppl 2):303–315. doi:10.1017/S1751731113000888

Bengtsson-Palme, J., M. Hartmann, K. M. Eriksson, C. Pal, K. Thorell, D. G. Larsson, and R. H. Nilsson. 2015. METAX2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. Mol. Ecol. Resour. 15:1403–1414. doi:10.1111/1755-0998.12399

Bezirtzoglou, E., A. Tsiotsias, and G. W. Welling. 2011. Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). Anaerobe. 17:478–482. doi:10.1016/j.anaerobe.2011.03.009

Biasucci, G., B. Benenati, L. Morelli, E. Bessi, and G. Boehm. 2008. Cesarean delivery may affect the early biodiversity of intestinal bacteria. J. Nutr. 138:1796S–1800S. doi:10.1093/jn/138.9.1796S

Biasucci, G., M. Rubini, S. Riboni, L. Morelli, E. Bessi, and C. Retetangos. 2010. Mode of delivery affects the bacterial community in the newborn gut. Early Hum. Dev. 86 Suppl 1:13–15. doi:10.1016/j.earlhumdev.2010.01.004

Cammack, K. M., K. A. Leymaster, T. G. Jenkins, and M. K. Nielsen. 2005. Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. J. Anim. Sci. 83:777–785. doi:10.2527/2005.834777x

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7:335–336. doi:10.1038/nmeth.f.303

Carberry, C. A., D. A. Kenny, S. Han, M. S. McCabe, and S. M. Waters. 2012. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Appl. Environ. Microbiol. 78:4949–4958. doi:10.1128/AEM.07759-11

Chu, D. M., K. M. Meyer, A. L. Prince, and K. M. Aagaard. 2016. Impact of maternal nutrition in pregnancy and lactation on offspring gut microbial composition and

function. Gut Microbes 7:459–470. doi:10.1080/19490976.2016.1241357

Church, D. C. 1988. The ruminant animal: digestive physiology and nutrition. Prentice-Hall, Inc, Upper Saddle River, NJ.

De Paula Vieira, A., M. A. G. von Keyserlingk, and D. M. Weary. 2012. Presence of an older weaned companion influences feeding behavior and improves performance of dairy calves before and after weaning from milk. J. Dairy Sci. 95:3218–3224. doi:10.3168/jds.2011–4821

Ellison, M. J., G. C. Conant, W. R. Lamberson, R. R. Cockrum, K. J. Austin, D. C. Rule, and K. M. Cammack. 2017. Diet and feed efficiency status affect rumen microbial profiles of sheep. Small Rumin. Res. 156:12–19. doi:10.1016/j.smallrumres.2017.08.009

Flint, H. J., and E. A. Bayer. 2008. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. Ann. N. Y. Acad. Sci. 1125:280–288. doi:10.1196/annals.1419.022

Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore. 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol. Lett. 288:85–91. doi:10.1111/j.1574-6968.2008.01343.x

Guzman, C. E., L. T. Bereza-Malcolm, B. De Groef, and A. E. Franks. 2015. Presence of selected methanogens, fibrolytic bacteria, and proteobacteria in the gastrointestinal tract of neonatal dairy calves from birth to 72 hours. Plos One 10:e0133048. doi:10.1371/journal.pone.0133048

Hernandez-Sanabria, E., L. A. Goonewardene, Z. Wang, M. Zhou, S. S. Moore, and L. L. Guan. 2013. Influence of sire breed on the interplay among rumen microbial populations inhabiting the rumen liquid of the progeny in beef cattle. Plos One 8:e58461. doi:10.1371/journal.pone.0058461

Jami, E., A. Israel, A. Kotser, and I. Mizrahi. 2013. Exploring the bovine rumen bacterial community from birth to adulthood. ISME J. 7:1069–1079. doi:10.1038/ismej.2013.2

Jami, E., B. A. White, and I. Mizrahi. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. Plos One 9:e85423. doi:10.1371/journal.pone.0085423

Lodge-Ivey, S. L., J. Browne-Silva, and M. B. Horvath. 2009. Technical note: bacterial diversity and fermentation end products in rumen fluid samples collected via oral lavage or rumen cannula. J. Anim. Sci. 87:2333–2337. doi:10.2527/jas.2008-1472

Millen, D. D., M. De Beni Arrigoni, and R. D. Lauritano Pacheco, eds. 2016. Rumenology. Springer International Publishing, Cham, Switzerland.

Neu, J., and J. Rushing. 2011. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin. Perinatol. 38:321–331. doi:10.1016/j.clp.2011.03.008

Paz, H. A., K. E. Hales, J. E. Wells, L. A. Kuehn, H. C. Freetly, E. D. Berry, M. D. Flythe, M. L. Spangler, and S. C. Fernando. 2018. Rumen bacterial community structure impacts feed efficiency in beef cattle. J. Anim. Sci. 96:1045–1058. doi:10.1093/jas/skx081

Roehe, R., R. J. Dewhurst, C. A. Duthie, J. A. Rooke, N. McKain, D. W. Ross, J. J. Hyslop, A. Waterhouse, T. C. Freeman, M. Watson, et al. 2016. Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance. Plos Genet. 12:e1005846. doi:10.1371/journal.pgen.1005846

Savietto, D., D. P. Berry, and N. C. Friggens. 2014. Towards an improved estimation of the biological components of residual feed intake in growing cattle. J. Anim. Sci. 92:467–476. doi:10.2527/jas.2013-6894

Shabat, S. K., G. Sasson, A. Doron-Faigenboim, T. Durman, S. Yaacoby, M. E. Berg Miller, B. A. White, N. Shterzer, and I. Mizrahi. 2016. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J. 10:2958–2972. doi:10.1038/ismej.2016.62

Taschuk, R., and P. J. Griebel. 2012. Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. Anim. Health Res. Rev. 13:129–141. doi:10.1017/S1466252312000096

Thum, C., A. L. Cookson, D. E. Otter, W. C. McNabb, A. J. Hodgkinson, J. Dyer, and N. C. Roy. 2012. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? J. Nutr. 142:1921–1928. doi:10.3945/jn.112.166231

Yáñez-Ruiz, D. R., L. Abecia, and C. J. Newbold. 2015. Manipulating rumen microbiome and fermentation through interventions during early life: a review. Front. Microbiol. 6:1133. doi:10.3389/fmicb.2015.01133

Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 36:808–812.

Zhou, M., E. Hernandez-Sanabria, and L. L. Guan. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microbiol. 75:6524–6533. doi:10.1128/AEM.02815-08