Investigation of maternal breed and rearing type on the calf rumen microbiome from day 28 through weaning[1,2]

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INTRODUCTION

The livestock sector faces a disequilibrium in the financial balance of inputs and expected outputs, leading to a focus to improve feed efficiency. Ruminant livestock are able to convert low-quality forages into high-quality end products (meat, wool, fiber, etc.) largely due to the microbiome present in the rumen. Ruminal fermentation accounts for 70% of total dietary energy (Flint and Bayer, 2008), thus is critical to host performance and efficiency. Indeed, previous data indicate that more efficient animals have a less rich and diverse microbiome in both species and gene content (Shabat et al., 2016). Several differences in specific microbial abundances have also been associated with divergence in feed efficiency in both cattle (Guan et al., 2008; Myer et al., 2015) and sheep (Ellison et al., 2015; Ellison et al., 2017).

Although the rumen is not truly functional until approximately 4-weeks of age (Church, 1988), microbial fermentation and the end products are critical for the development of the rumen and immune system (Flatt et al., 1958; Suárez et al., 2006; Malmuthuge et al., 2012). A large body of evidence suggests that maternal factors including both pre and postpartum factors can influence offspring gastrointestinal tract microbiome in several species (Ley et al., 2005; Dominguez-Bello et al., 2010), including ruminants (Cannon et al., 2010). Data suggest that manipulations that occur early in life may persist into adulthood (Abecia et al., 2014; Yáñez-Ruiz et al., 2015), providing the potential for alterations of the rumen microbiome to influence feed efficiency later in life. Thus, we hypothesized that the rumen microbiome of calves would be altered by maternal factors and these changes would persist through weaning. Our objective was to determine if maternal breed and rearing type would affect the early calf microbiome through weaning.

MATERIALS AND METHODS

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

Cow Management and Diet

Mature, bred Charolais (**Char**; n = 24) and Angus (**Ang**; n = 24) cows were purchased and housed at the Laramie Research and Extension

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Center well in advance of calving. These breeds are recognized for differences in growth rates and yield, reflecting an evolutionary divergence in these biological types (Cundiff, 2005). Cows were bred via natural service and their expected calving date was calculated to be 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 2 lb d^{-1} DDGS (29.9% CP, 12.3% ADF, 75.0 TDN%, 1.79 NE_m MCal/kg, 1.16 NE_g MCal/kg). Prior to parturition, cows were moved into pens, separated by breed, and then randomly allocated to one of two treatment groups at parturition resulting in four treatment groups in total; Char control (**CharCON**, n = 12), Ang control (**AngCON**, n = 12), Char bottle (**CharBOT**, n = 12), and Ang bottle (**AngBOT**, n = 12). Calving was closely monitored to ensure delivery of a live calf and to determine time of calving. Both bottle and control groups were allowed to calve naturally and calves received colostrum from their respective dams. At 24-h postparturition, the AngBOT and CharBOT calves were removed from their dams and reared on artificial milk replacer (High energy Nurse Chow100; Purina Mills/Land O'Lakes, Inc.) until weaning. Each treatment group was housed in separate pens.

Calf Management and Calf Rumen Fluid Sample Collection

At parturition, calves were monitored to ensure survivability. Calves were allowed ad libitum access to their dam's colostrum (only for the first 24 h in AngBOT, CharBOT groups) as well as hay. At approximately 1.5 months of age, calves were fed Purina Stocker Grower at the rate of 2 lb h^{-1} d^{-1} through weaning (180 d of age). At days 28 and 180, rumen fluid was collected from calves via oral-lavage using methods described by Lodge-Ivey et al. (2009). Briefly, a flexible vinyl tube, 0.5-cm inner-diameter and 3 ft in length, was lubricated and passed through the mouth into the rumen; 20–30 mL rumen fluid was removed using suction via an attached syringe. The samples were aliquoted, flash frozen, and stored at −80 °C for subsequent analysis.

Rumen Microbial DNA Extraction

Rumen fluid samples were used for shotgun metagenomic sequencing. First, DNA was isolated from 8 calves per treatment group using methods described by Yu and Morrison (2004). Briefly, rumen fluid was thawed immediately prior to use and 0.25 g was added to sterilized zirconia (0.3 g of 0.1 mm) and silicon (0.1 g of 0.5 mm) beads along with 1 mL of lysis buffer (500 mM NaCl, 400 mM Tris-HCl, 50 mM EDTA, 4% SDS). Tubes were then homogenized using a Mini-Beadbeater-8 at maximum speed for 3 min, incubated at 70 °C for 15 min with frequent mixing and centrifuged at 16,000 × g at 4 °C for 5 min. The supernatant (~1 mL) was transferred to a new 2-mL flat cap tube and 300 µL fresh lysis buffer was added to the pelleted beads. The homogenization, incubation, and centrifugation steps described above were repeated, and the supernatants were pooled. Nucleic acid was precipitated and then further purified using the QIAamp DNA Stool Mini Kit (Qiagen, Santa Clarita, CA) and the manufacturer's suggested protocol, except that buffer EB was used for elution. Aliquots of 2 µg (80 ng/µL) were shipped to the University of Missouri DNA Core Facility, Columbia, MO, 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. Briefly, 1 µg of genomic DNA was sheared using standard Covaris methods to generate an average insert size of 350 bp. The 3′ and 5′ overhangs were converted to blunt ends by an end repair reaction with 3′ to 5′ exonuclease/polymerase activity. Using purification beads (AMPure XP), the desired size fragment was selected. Then a single adenosine nucleotide was attached to the 3′ ends of the blunt fragments followed by ligation of Illumina indexed paired-end adapters. The library was purified twice using sample purification beads. This purified library was then quantified by a Qubit assay, and the library fragment size was confirmed by using the Fragment Analyzer (Advanced Analytical Technologies, Inc.). The library was then diluted and sequenced according to Illumina's standard sequencing protocol for the HiSeq.

Metagenomic Sequencing Analysis and Identification of 16S rDNA Genes

Metagenomic sequences were quality filtered before 16S rDNA genes were identified using Metaxa2 (Bengtsson-Palme et al., 2015). Briefly, hidden Markov models using HMMER were used to identify the conserved regions of the small subunit by aligning to the SILVA database and then 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 classification but not necessarily specific classification (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).

RESULTS

There were 148, 780, 370, 754, and 676 taxa with significantly ($P < 0.05$) different abundances across treatment group, days, breed, days within treatment group, and days within breed, respectively. Alpha-diversity was not ($P = 0.21$) different between rearing type; however, d 28 had increased ($P = 0.001$) richness compared with samples at weaning. The Char cows had increased ($P = 0.05$) richness compared with Ang. The days within treatment group indicated that the CON animals (both Char and Ang) at d 28 had increased ($P < 0.012$) richness compared with CON at weaning, BOT d 28, and BOT at weaning. The BOT d 28 had increased ($P = 0.006$) richness compared with both BOT and CON at weaning. Alpha-diversity of days within breed indicated increased ($P = 0.006$) richness in Char d 28 compared with Ang at weaning and increased ($P = 0.006$) richness in Ang d 28 compared with Ang at weaning. Beta-diversity was not affected ($P > 0.1$) by rearing type, d 28 samples were more ($P = 0.001$) similar to each other than at weaning, and Char were more similar to Char than to Ang. The BOT d 28 were more similar ($P < 0.001$) within their treatment group than all other comparisons except CON d 28. Beta-diversity was significantly ($P = 0.001$) different between BOT at weaning and BOT d 28 and CON d 28, yet tended ($P = 0.06$) to be different from CON at weaning. The CON at weaning were more similar ($P < 0.05$) to other CON weaning samples than to BOT d 28, BOT at weaning, and CON d 28. Finally, CON d 28 were more similar ($P = 0.001$) to each other than to BOT d 28, and both CON and BOT at weaning. The days within breed beta-diversity indicated that Char d 28 samples were more similar ($P < 0.05$) to each other than to Ang and Char at weaning and Ang d 28. The Ang at weaning samples were more similar ($P = 0.001$) to each other than to Char and Ang on d 28 but were similar ($P = 0.82$) to Char at weaning. Finally, Ang d 28 samples were different ($P = 0.001$) in terms of beta-diversity from Char d 28 and both Char and Ang at weaning.

DISCUSSION

The rumen microbial profiles compared across rearing type did not differ in richness; however, differences in richness were evident with breed, days, days within treatment, and days within breed. Compositional differences were not evident for rearing type but were evident for all other comparisons. Several taxa had differential abundances across all comparisons, indicating that these microbial taxa may be key contributors.

Maternal breed has been indicated to alter the rumen microbiome and has a strong host-genetic relationship with the microbiome of the rumen (Roehe et al., 2016). Specific sire breed influences on the offspring rumen microbiome have been reported (Hernandez-Sanabria et al., 2013); and in dairy cows, variation in the rumen microbiome between Holstein and Jersey breeds exists (Paz et al., 2016). Our data reflect differences in Char and Ang rumen microbial profiles, suggesting selection potential as well as divergence in microbiome potentially caused by divergence in feed efficiency as seen between Ang (low efficiency) and Char (High efficiency; Cundiff, 2005).

Many studies investigate weaning strategies and the impact on the rumen microbiome, predominantly to evaluate optimal feeding strategy for improved growth and development (Rey et al., 2012; Jami et al., 2013; Dias et al., 2017). In beef cattle operations, this type of rearing is generally not an option; however, an understanding of the impact that maternal milk has on the calf microbiome is critical. The effect of the stage of development on the microbiome is consistent (Benson et al., 2010; Bath et al., 2013; Jami et al., 2013) indicating a stabilization of the rumen microbiome around weaning. The d 28 samples are critical as they reflect the period of transition from a pre-ruminant to a functional ruminant (Church, 1988). Our data agree with the literature in that the d 28 samples have increased richness compared with samples at weaning, suggesting a stabilization of the microbiome in more mature animals.

In conclusion, our data suggest that maternal breed and stage of development can affect the rumen microbiome of calves. Although distinct differences in rearing type were not evident in terms of richness, several taxa abundances, beta-diversity, and interactions with d indicate that rearing type does play a role. The rumen microbiome is critical to host performance and the elucidation of factors contributing to variation in the microbial profiles may lead to intervention strategies towards improving host performance.

LITERATURE CITED

Abecia, L., K. E. Waddams, G. Martínez-Fernandez, A. I. Martín-García, E. Ramos-Morales, C. J. Newbold, and D. R. Yáñez-Ruiz. 2014. An antimethanogenic nutritional

intervention in early life of ruminants modifies ruminal colonization by archaea. Archaea. 2014:841463. doi:10.1155/2014/841463

Bath, C., M. Morrison, E. M. Ross, B. J. Hayes, and B. G. Cocks. 2013. The symbiotic rumen microbiome and cattle performance: a brief review. Anim. Prod. Sci. 53:876–881. doi:10.1071/AN12369

Bengtsson-Palme, J., M. Hartmann, K. M. Eriksson, C. Pal, K. Thorell, D. G. Larsson, and R. H. Nilsson. 2015. metaxa2: 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

Benson, A. K., S. A. Kelly, R. Legge, F. Ma, S. J. Low, J. Kim, M. Zhang, P. L. Oh, D. Nehrenberg, K. Hua, et al. 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci. USA. 107:18933–18938. doi:10.1073/pnas.1007028107

Cannon, S. J., G. C. Fahey, Jr, L. L. Pope, L. L. Bauer, R. L. Wallace, B. L. Miller, and J. K. Drackley. 2010. Inclusion of psyllium in milk replacer for neonatal calves. 2. Effects on volatile fatty acid concentrations, microbial populations, and gastrointestinal tract size. J. Dairy Sci. 93:4744–4758. doi:10.3168/jds.2010-3077

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

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

Cundiff, L. V. 2005. Beef cattle: Breeds and genetics. In: Ullrey, D. E., C. K. Baer, and W. G. Pond, editors. 2011 Encyclopedia of animal science. 2nd ed. CRC Press, Boca Raton, FL; p. 74.

Dias, J., M. I. Marcondes, M. F. Noronha, R. T. Resende, F. S. Machado, H. C. Mantovani, K. A. Dill-McFarland, and G. Suen. 2017. Effect of pre-weaning diet on the ruminal archaeal, bacterial, and fungal communities of dairy calves. Front. Microbiol. 8:1553. doi:10.3389/fmicb.2017.01553

Dominguez-Bello, M. G., E. K. Costello, M. Contreras, M. Magris, G. Hidalgo, N. Fierer, and R. Knight. 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc. Natl. Acad. Sci. USA. 107:11971–11975. doi:10.1073/pnas.1002601107

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

Ellison, M. J., G. C. Conant, W. R. Lamberson, T. M. Taxis, H. C. Cunningham, K. J. Austin, and K. M. Cammack. 2015. The effect of residual feed intake status on rumen microbial profiles in ewe lambs. J Anim Sci Technol. 66:133–137.

Flatt, W. P., R. G. Warner, and J. K. Loosli. 1958. Influence of purified materials on the development of the ruminant stomach. J. Dairy Sci. 41:1593–1600. doi:10.3168/jds.S0022-0302(58)91138-X

Flint, H. J., and E. A. Bayer. 2008. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. Ann. NY 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

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. Z. Zhou, editor. 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

Ley, R. E., F. Bäckhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA. 102:11070–11075. doi:10.1073/pnas.0504978102

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

Malmuthuge, N., M. Li, P. Fries, P. J. Griebel, and L. L. Guan. 2012. Regional and age dependent changes in gene expression of toll-like receptors and key antimicrobial defence molecules throughout the gastrointestinal tract of dairy calves. Vet. Immunol. Immunopathol. 146:18–26. doi:10.1016/j.vetimm.2012.01.010

Myer, P. R., T. P. L. Smith, J. E. Wells, L. A. Kuehn, and H. C. Freetly. 2015. Rumen microbiome from steers differing in feed efficiency. PLOS ONE. 10:e0129174. doi:10.1371/journal.pone.0129174

Paz, H. A., C. L. Anderson, M. J. Muller, P. J. Kononoff, and S. C. Fernando. 2016. Rumen bacterial community composition in holstein and jersey cows is different under same dietary condition and is not affected by sampling method. Front. Microbiol. 7:1206. doi:10.3389/fmicb.2016.01206

Rey, M., F. Enjalbert, and V. Monteils. 2012. Establishment of ruminal enzyme activities and fermentation capacity in dairy calves from birth through weaning. J. Dairy Sci. 95:1500–1512. doi:10.3168/jds.2011-4902

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, and R. J. Wallace. 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

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

Suárez, B. J., C. G. Van Reenen, W. J. Gerrits, N. Stockhofe, A. M. van Vuuren, and J. Dijkstra. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen

development. J. Dairy Sci. 89:4376–4386. doi:10.3168/jds.S0022-0302(06)72484-5

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.