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Analysis of the gut bacterial communities in beef cattle and their association with feed intake, growth, and efficiency[1,2,3]

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ABSTRACT: The impetus behind the global food security challenge is direct, with the necessity to feed almost 10 billion people by 2050. Developing a food-secure world, where people have access to a safe and sustainable food supply, is the principal goal of this challenge. To achieve this end, beef production enterprises must develop methods to produce more pounds of animal protein with less. Selection for feed-efficient beef cattle using genetic improvement technologies has helped to understand and improve the stayability and longevity of such traits within the herd. Yet genetic contributions to feed efficiency have been difficult to identify, and differing genetics, feed regimens, and environments among studies contribute to great variation and interpretation of results. With increasing evidence that hosts and their microbiomes interact in complex associations and networks, examining the gut microbial population variation in feed efficiency may lead to partially clarifying the considerable variation in the efficiency of feed utilization. The use of metagenomics and high-throughput sequencing has greatly impacted the study of the ruminant gut. The ability to interrogate these systems at great depth has permitted a greater understanding of the microbiological and molecular mechanisms involved in ruminant nutrition and health. Although the microbial communities of the reticulorumen have been well documented to date, our understanding of the populations within the gastrointestinal tract as a whole is limited. The composition and phylogenetic diversity of the gut microbial community are critical to the overall well-being of the host and must be determined to fully understand the relationship between the microbiomes within segments of the cattle gastrointestinal tract and feed efficiency, ADG, and ADFI. This review addresses recent research regarding the bacterial communities along the gastrointestinal tract of beef cattle; their association with ADG, ADFI, and feed efficiency; and the potential implications for beef production.

Key words: feed efficiency, gastrointestinal tract, operational taxonomic units, 16S ribosomal RNA

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INTRODUCTION

Historically, global beef production has been tasked with producing more pounds of protein with fewer resources. This is currently highlighted by the necessity to feed a global population estimated to be 9.7 billion by the year 2050 (UNDESA, 2015). Although selection traditionally has been the only method to consistently increase the food produced per animal, novel molecular and nutritional and tools have been examined for the improvement of nutrient utilization and efficiency in cattle. These include, but are not limited to, the analysis

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of genetic and environmental variation of genetic markers associated with feedlot residual feed intake and differentially expressed genes in the ruminal papillae associated with feed efficiency class (Saatchi et al., 2014; Kern et al., 2016). Current advances in nutritional, genetic, and transcriptomic beef research provide the opportunity to improve on traits such as ADG and DMI, reduce the associated variation, and optimize feed efficiency.

Previous studies of efficiency in ruminants have focused exclusively on the evaluation of the reticulorumen, because energy production and nutrient supply to the host as a function of ruminal fermentation and microbial community activity are paramount to production efficiency (Kim et al., 2011; Hernandez Sanabria et al., 2012; McCann et al., 2014). However, the functional and metabolic capacities of the gastrointestinal tract (**GIT**) segments are distinct and contribute to the health and nutritional status of the animal. Such distinctions are also reflected within the respective bacterial populations, which collectively have an impact on host nutrition and energy balance. Understanding and optimizing factors that contribute to variation in feed efficiency is contingent on the comprehensive analysis of the GIT and its microbiome. To begin to address these issues, this review summarizes the association between the GIT bacterial communities of steers and feed intake, growth, and feed efficiency.

ANIMALS, DIET, AND DESIGN

The GIT bacterial community was determined from 2 contemporary groups of steers, as reported by Myer et al. (2015a,b,c, 2016). The steers selected for this study came from a population of cattle being developed to have a high percentage of the following breeds: Angus, Beefmaster, Brahman, Brangus, Braunvieh, Charolais, Chiangus, Gelbvieh, Hereford, Limousin, Maine Anjou, Red Angus, Salers, Santa Gertrudis, Shorthorn, Simmental, South Devon, and Tarentaise. Annually, heifers and cows were artificially inseminated with semen from prominent industry bulls of their dominant breed. This program resulted in offspring ranging from 50 to 75% of the same breed as their sire with the exception of Angus and Hereford, which ranged from 50 to 100% of the same breed as their sire. Individual feed intake was measured using an Insentec feeding system (Insentec B.V., Marknesse, the Netherlands). Steers were fed a ration (DM basis) of 57.35% dry-rolled corn, 30% wet distillers grain with solubles, 8% alfalfa hay, 4.25% supplement (containing 772 mg monensin/kg), and 0.4% urea. Feed intake and BW gain were measured over a 63-d period (Lindholm-Perry et al., 2013). Steers were selected from 2 contemporary groups. Group 1 (n = 148) comprised spring-born calves that were 371 ± 1 d of age and weighed 522 ± 4 kg at the start of the feed intake measurement. Group 2 (n = 197) comprised fall-born calves that were 343 ± 1 d of age and weighed 448 ± 4 kg at the start of the feed intake measurement. At the end of each feeding period, ADFI and ADG were calculated. Steers were then ranked based on their standardized distance from the bivariate mean (ADG and ADFI) assuming a bivariate normal distribution with a calculated correlation between ADG and ADFI. From these 2 groups, 4 steers with the greatest deviation within each Cartesian quadrant were sampled (n = 16 steers/contemporary group). In the event a sire breed was overrepresented within a quadrant, a steer with the next highest rank of a different breed was selected. Quadrant 1 comprised steers that had greater ADG (2.14 ± 0.08 kg/d) and greater ADFI (12.76 ± 0.37 kg/d), quadrant 2 comprised steers that had greater ADG (1.84 ± 0.08 kg/d) and less ADFI (8.36 ± 0.08 kg/d), quadrant 3 comprised steers that had less ADG (1.26 ± 0.08 kg/d) and less ADFI (7.86 ± 0.08 kg/d), and quadrant 4 comprised steers that had less ADG (1.38 ± 0.08 kg/d) and greater ADFI (11.64 ± 0.08 kg/d). The cattle were sampled as part of a factorial arrangement such that high and low feed intake was stratified with high and low BW gain. As the cattle were selected based on their phenotypic expression, no treatment was applied and all results are reported in the context of their observed phenotypes. Selected steers were harvested and digesta was collected from the reticulorumen, jejunum, cecum, and colon after completion of the feeding period. Immediately following sampling, samples were individually stored in buffered peptone water (pH 7.0) + 15% glycerol stock for processing and kept at −70°C for long-term storage after processing.

DIVERSITY OF THE GASTROINTESTINAL TRACT BACTERIAL COMMUNITIES

In these studies, targeted, deep 16S rRNA-based community profiling, via amplification of the V1–V3 hypervariable regions of the 16S rRNA gene, was used to determine the microbial communities. After sequencing (Illumina MiSeq platform; Illumina, Inc., San Diego, CA) and read processing, 5,565,909, 10,847,155, 17,757,018, and 20,593,775 cleaned reads from the sampled rumen, jejunum, cecum, and colon contents, respectively, of 32 steers were used for analyses of the GIT bacterial communities. Within the rumen, an average of 1,098 ± 382 operational taxonomic units (**OTU**; 0.03 dissimilarity) were captured, which were classified into 24 phyla, 48 classes, 89 orders, 173 families, and 317 genera. The jejunum yielded an average of 499 ± 159 OTU that were classified into 21 phyla, 51 classes, 94 orders, 198 families, and 397 genera. Analysis of the cecum content microbial communities indicated 5,572 ± 1,428 OTU that were classified to 18 phyla, 40

Table 1. Summary of diversity statistics among sequences from grouped samples within each gastrointestinal tract segment

	No. of OTU[2,3,4]				Shannon diversity index[3,4]			
Feed efficiency group[1]	Rumen	Jejunum	Cecum	Colon	Rumen	Jejunum	Cecum	Colon
$ADG_{Greater}$–$ADFI_{Greater}$	1,069 ± 309	424 ± 109	5,342 ± 1,446	5,764 ± 878	6.32 ± 1.21	3.65 ± 1.22	7.65 ± 1.20	7.85 ± 0.52
$ADG_{Greater}$–$ADFI_{Less}$	1,078 ± 479	504 ± 179	5,881 ± 1,086	6,098 ± 1,028	7.11 ± 0.93	4.61 ± 1.16	8.14 ± 0.94	8.15 ± 0.39
ADG_{Less}–$ADFI_{Less}$	1,172 ± 394	534 ± 194	6,820 ± 1,415	6,714 ± 1,148	7.06 ± 0.96	3.85 ± 1.17	8.40 ± 0.94	8.27 ± 0.49
ADG_{Less}–$ADFI_{Greater}$	1,072 ± 345	533 ± 145	4,746 ± 1,450	6,063 ± 1,129	6.89 ± 0.28	3.53 ± 1.09	7.37 ± 1.54	7.92 ± 0.74

[1]$ADG_{Greater}$–$ADFI_{Greater}$ = greater ADG and greater ADFI; $ADG_{Greater}$–$ADFI_{Less}$ = greater ADG and less ADFI; ADG_{Less}–$ADFI_{Less}$ = less ADG and less ADFI; ADG_{Less}–$ADFI_{Greater}$ = less ADG and greater ADFI. $n = 8$ among groups.

[2]OTU = operational taxonomic units.

[3]Within a column, means for the individual subsamples did not differ ($P > 0.05$).

[4]Means among the feed efficiency groups within each gastrointestinal tract segment and metric were compared using ANOVA and the Tukey's test.

classes, 75 orders, 148 families, and 225 genera. Finally, an average of 6,025 ± 1,225 OTU were identified in the colon and classified to 20 phyla, 46 classes, 83 orders, 152 families, and 231 genera. Estimates of ruminal species–equivalent bacterial OTU using in silico methods have been reported as large as 5,271, representing 19 phyla (0.03 phylogenetic distance; Kim et al., 2011), but are similar among studies using next-generation sequencing technologies (Mao et al., 2015). However, it must be noted that host effects, as well as different methodologies, animals numbers, breed, age, and diet, likely are the source of the variation in OTU reported among similar studies (Weimer et al., 2010; Hernandez-Sanabria et al., 2012; Petri et al., 2013).

For comparison among samples within the same GIT segment, the individual samples were normalized based on sample rarefaction curves, of which 25,000 sequences per sample were used for rumen and jejunum analyses, 75,000 for the cecum, and 100,000 for the colon. The α-diversity metrics determined from these samples, consisting of bacterial diversity (Shannon diversity index) and OTU captured, are abbreviated and summarized in Table 1. Although the rumen has a great abundance of microorganisms, their functionality is more specialized and considerable redundancy exists among niches, contributing to community resilience. The rumenal microbiome is distinctively characterized by low phylogenetic diversity at higher taxonomic levels but overall great abundance of microbes, which is a result of the selective pressures exerted by the available substrates in the rumen. This distinct environment supports a multitude of specialized microorganisms, such as those involved in the degradation of complex carbohydrates, which can be categorized into a core microbiome (Petri et al., 2013). These relationships and diverse roles (e.g., cellulolytic, amylolytic, proteolytic) in the rumen are supported by the lower diversity index and overall OTU abundance, compared with that of the large intestine (Myer et al., 2015a,b,c).

The role of postruminal degradation of cellulose and starch, as well as the importance of microbial interaction and the maturation of the mucosal immune system, highlights the changes in abundance and diversity of bacterial populations observed in the large intestine. Additionally, whereas the greatest phylogenetic diversity and OTU count exist in the cecum and colon, the jejunum was greatly reduced in species abundance and diversity, with an average of 499 ± 159 OTU and a diversity index of 3.91 ± 0.48. The greater sequencing depth and number of animals per group permitted greater observed OTU than previous studies (Jami and Mizrahi, 2012; McCann et al., 2014), which was imperative to accurately determine the microbial communities within the GIT.

GASTROINTESTINAL TRACT BACTERIAL COMMUNITY COMPOSITION

The taxonomic groups represented in the GIT predominantly were of the phyla Firmicutes and Bacteroidetes, which varied greatly dependent on the region of the GIT (Fig. 1). Bacteroidetes populations dominated the rumen, whereas Firmicutes was the phylum in greatest relative sequence abundance within the more distal segments of the GIT. Within the jejunum, Bacteroidetes was greatly reduced (0.4 to 1.1 ± 0.26%), and Firmicutes populations accounted for more than 80% of the bacterial phyla.

In the rumen, the genus *Prevotella* made up greater than 90% of the Bacteroidetes population (Myer et al., 2015a). Additionally, *Prevotella* spp. were present in abundance from 45 to 57% of the total 16S rRNA sequences, in agreement with other reports specifying the dominant abundance of *Prevotella* species within the rumen (Stevenson and Weimer, 2007; Jami and Mizrahi, 2012). These data also highlight the specialized function of the reticulorumen and the aforementioned phylogenetic diversity differences among the sections of the GIT. Other bacterial genera that

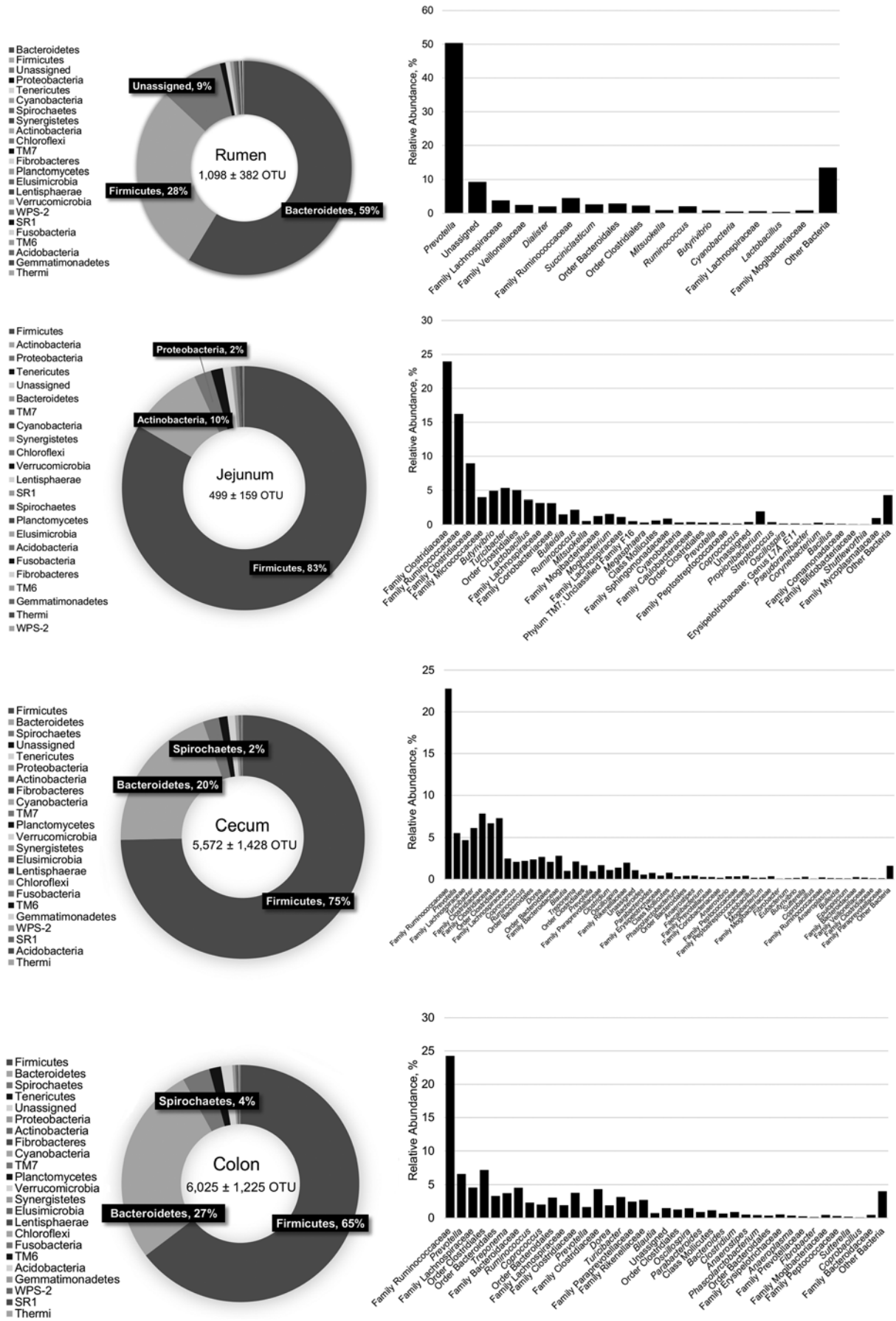

Figure 1. Summary of the taxonomic profiles for the relative phylum-level (donut chart; left) and genus-level (bar chart; right) abundance of each gastrointestinal tract (GIT) segment classified by representation at ≥0.001% of total sequences. Taxonomic composition of the GIT microbiota was compared based on the relative sequence abundance (sequences of a taxon/total sequences in a sample).

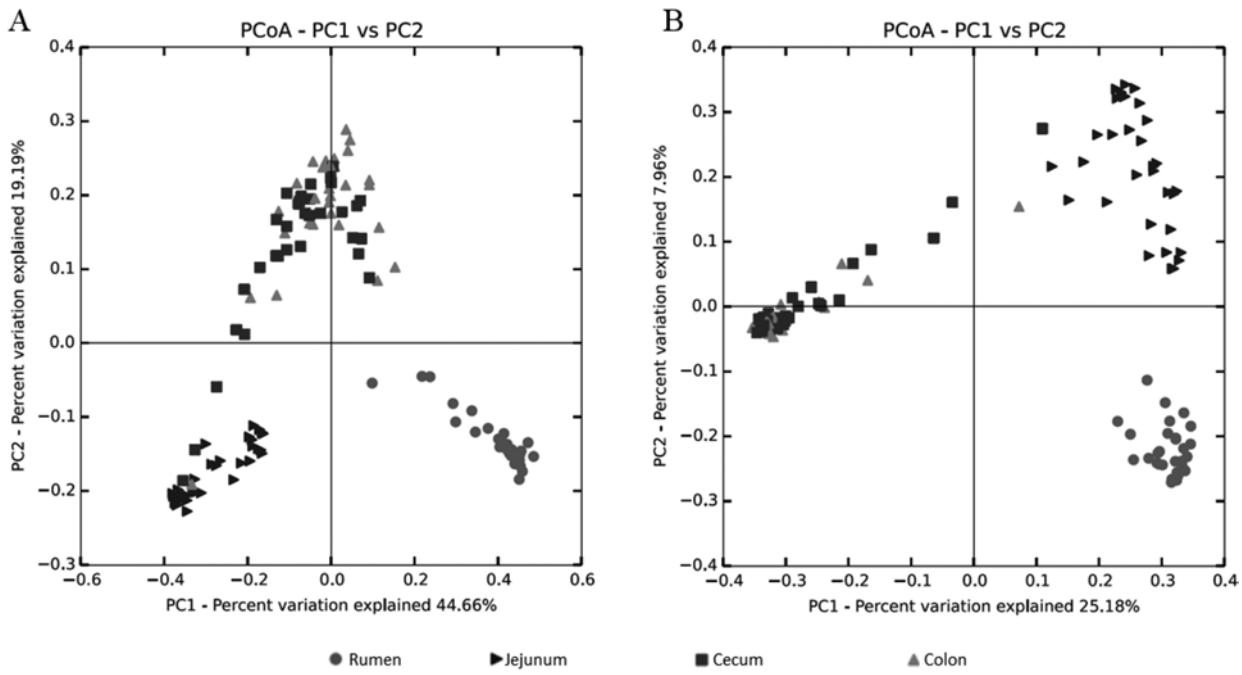

Figure 2. UniFrac (Lozupone and Knight, 2005) principal coordinate analysis (PCoA) displaying correlations among the bacterial communities of the gastrointestinal tract segments. A) Weighted PCoA analyzed from rarefied subsets of 25,000, 25,000, 75,000, and 100,000 sequences from each sample within the rumen, jejunum, cecum, and colon, respectively. B) Unweighted PCoA lyzed from rarefied subsets of 25,000, 25,000, 75,000, and 100,000 sequences from each sample within the rumen, jejunum, cecum, and colon, respectively. n = 32, represented by differing symbols.

dominated the rumen and were present at relative sequence abundances ≥ 1% of the total sequences included *Dialister* (2.6 to 4.1%), *Succiniclasticum* (2.0 to 4.0%), *Ruminococcus* (1.0 to 2.5%), *Butyrivibrio* (0.5 to 1.1%), and *Mitsuokella* (0.6 to 1.2%).

The jejunum is specialized for the absorption of proteins and carbohydrates, and, as expected, the bacterial populations of the jejunum were radically different compared with the rumen. The functional differences associated between the tissues were especially highlighted by the shift of Bacteroidetes populations dominating in the rumen to Firmicutes within the jejunum, constituting up to 90% of the phyla. Bacteroidetes populations were present only at lower abundances of 0.4 to 1.1%. Of the remaining phyla, Actinobacteria (6 to 13%), Proteobacteria (0.8 to 5.8%), and Tenericutes (0.4 to 4%) made up populations present at ≥1%. *Ruminococcus* (12.2 to 19.6%), *Butyrivibrio* (2.6 to 7.7%), *Lactobacillus* (2.8 to 4.2%), *Bulleidia* (0.8 to 1.9%), *Mogibacterium* (1.1 to 1.7%), *Mitsuokella* (0.05 to 1.27%), and *Propionibacterium* (0.07 to 7%) represented genera and OTU that were present at ≥1% within the jejunal content (Myer et al., 2016).

Due to function and proximity, the cecum and colorectal segments of the GIT were remarkably similar when comparing predominant bacterial taxa within their respective digesta. TThe postruminal degradation of cellulose and starch within the cecum may influence particular bacterial similarities to the rumen, where Bacteroidetes populations increased to 18 to 26% and 21 to 33% within the cecum and colon, respectively. However, Firmicutes populations were still the most abundant phylum within the large intestine, constituting up to 81% of the total sequences. Within the cecum, the phyla Spirochaetes (1.4 to 3.19%), Tenericutes (0.7 to 1.2%), and Actinobacteria (0.2 to 0.4%) were greatest in abundance of remaining phyla of substantial relative sequence abundance (≥0.1% of the sequences), and Spirochaetes (2.5 to 4.5%), Tenericutes (1.2 to 1.9%), Proteobacteria (0.3 to 0.5%), Actinobacteria (0.23 to 0.33%), and Fibrobacteres (0.02 to 0.29%) were present in greatest abundance among remaining phyla at ≥0.1% of the sequences within the colon. The abundant genera within the cecum and colon were vastly different compared with the rumen and jejunum. The bacterial populations from the cecal content primarily comprised the genera *Prevotella* (2.1 to 7.3%), *Turicibacter* (4.6 to 6.7%), *Coprococcus* (1.2 to 2.8%), *Ruminococcus* (1.5 to 2.7%), *Dorea* (2.2 to 3.3%), *Blautia* (0.5 to 2.0%), *Clostridium* (1.0 to 1.2%), and *Oscillospira* (1.1 to 1.6%; Myer et al., 2015b), whereas the colon largely comprised *Prevotella* (3.0 to 11.1%), *Ruminococcus* (1.7 to 2.9%), *Coprococcus* (1.0 to 2.9%), *Dorea* (1.7 to 2.2%), *Turicibacter* (1.9 to 4.4%), *Blautia* (0.3 to 1.3%), *Oscillospira* (1.1 to 1.6%), and *Parabacteroides* (0.4 to 1.4%; Myer et al., 2015c).

Phylogenetic data was reanalyzed from the GIT bacterial community studies (Myer et al., 2015a,b,c, 2016) to discern differences and/or similarities among the

segments of the GIT. The principal coordinate analysis (**PCoA**) of overall diversity, based on the weighted and unweighted UniFrac metric, also demonstrated the distinct microbial communities of the rumen compared with those from other segments of the GIT (Fig. 2). Additionally, although the small and large intestinal bacterial communities are more similar to each other than to those of the rumen, most of the similarity validated by the PCoA clustering occurred between the cecum and colon. These data demonstrate the shift in bacterial phylogenetic diversity as digesta travels to more distal segments of the GIT, which is highlighted by segment function and physiology. Although the differences in microbial community composition among GIT segments in cattle are well reported (Frey et al., 2010; de Oliveira et al., 2013; Myer et al., 2015a,b,c, 2016), these studies likewise indicated differences among microbial comminutes of adjacent GIT sections, specifically the cecum and colon. These findings are less likely due to physical or chemical properties of the segments and tissues but due to either host tissue–associated microbes or, more likely, host factors affecting microbial colonization. These factors, such as Toll-like receptors (**TLR**), which can trigger an immune response via recognition of host epimural bacteria and their products, have been demonstrated to associate with changes in the relative abundance of ruminal epithelial bacteria due to increases in TLR expression (Liu et al., 2015). An increase in endotoxic lipopolysaccharide (**LPS**) in rumen fluid, cecal digesta, and feces due to subacute ruminal acidosis (**SARA**) and symptomatic perturbation of microbial fermentation have also been reported in Holsteins (Li et al., 2016). Moreover, TLR that interact with LPS, such as TLR-4, are found on intestinal epithelium and, in cattle, have been suggested to affect LPS–TLR signaling, which, in turn, may affect the microbial ecology of the gut (Iwami et al., 2000; Ma et al., 2011). Altered composition of the microbiota in the GIT is inevitably a combination of these factors, modulating gut microbiota and affecting systemic metabolism.

As a byproduct of microbial fermentation, reducing the amount of methane (CH_4) gas produced in the reticulorumen and cecum has the potential of improving feed efficiency through the reduction of feed energy lost as CH_4 gas. Although the studies in the current review were limited to bacterial communities, Freetly et al. (2015) analyzed methanogen relative sequence abundances using a similar model in which total BW gain was regressed on total DMI. When examining cattle that differed in residual gain (**RG**) at the same DMI, enteric methane production, in vitro rumen and cecum methane production, total methanogens, and predominant methanogen groups did not differ between positive and negative RG groups. The lack of differences in the study did not support the hypothesis that differences in RG at an average intake are a consequence of methanogen relative sequence abundance or methane production.

Aside from the influence of variation in feed intake on methane production (Hegarty et al., 2007), differences in CH_4 production may be associated more with the physical characteristics or genetics of the animal. Researchers recently determined genomic heritabilities for CH_4 emissions from beef cattle, examining 747 Angus cattle phenotyped for CH_4 traits and genotyped for 630,000 SNP, subsequently deriving genomic expected breeding values for CH_4 traits (Hayes et al., 2016). These results are indicative of the ability to more accurately reduce methane emissions via genetic selection. Many factors contribute to the variation in feed efficiency, and increasing our understanding of the ME lost as CH_4, selection for reduced CH_4 emissions in cattle, or shifts in archaea due to and/or causing shifts in residual feed intake and RG will aid in maximizing the productivity and sustainability of beef production enterprises.

ASSOCIATIONS WITH FEED EFFICIENCY

As the use of omics technologies in livestock continues to mature, increasing numbers of studies have begun to interrogate the composition of rumen and lower GIT microbiomes and their associations with production phenotypes. Understanding the entire microbial community among GIT segments, including those taxa that are not core members, will aid and reshape how researchers address the optimization of beef production. The goal of such research is to use deep omics-based methods and analyses to identify microbial indicators for variation in ADG, ADFI, and feed efficiency in cattle that can be used for selection of greater feed efficient animals.

In general terms, the Firmicutes-to-Bacteroidetes ratio is regarded to be of significant relevance in gut microbial composition. The ratio is often implicated in obesity research, and a decreased Firmicutes-to-Bacteroidetes ratio has been directly related to weight loss, and increases in the ratio of these phyla resulted in an increased capacity for energy harvest from food (Ley et al., 2006). Interestingly, in the rumen, significantly increased abundances of Firmicutes were observed within the greater ADG and less ADFI ($\mathbf{ADG_{Greater}}$–$\mathbf{ADFI_{Less}}$) group ($P = 0.0364$), representing the feed efficient group, supporting the relationship between gain and the Firmicutes-to-Bacteroidetes ratio. With exception of Firmicutes populations, no large shifts in ruminal taxa were associated with any feed efficiency phenotype as modeled in these studies (Table 1; greater ADG and greater ADFI [$\mathbf{ADG_{Greater}}$–$\mathbf{ADFI_{Greater}}$], greater ADG and less ADFI [$\mathbf{ADG_{Greater}}$–$\mathbf{ADFI_{Less}}$] less ADG and greater ADFI [$\mathbf{ADG_{Less}}$–$\mathbf{ADFI_{Greater}}$], less ADG and less ADFI [$\mathbf{ADG_{Less}}$–$\mathbf{ADFI_{Less}}$]).

However, finer shifts in taxa and OTU in the rumen were implicated as associating with feed efficiency phenotypes. The $ADG_{Greater}$–$ADFI_{Greater}$ group included increases in *Prevotella* ($P = 0.015$), *Lactobacillus* ($P = 0.042$), and *Dialister* ($P = 0.006$) populations, whereas *Anaerovibrio* ($P = 0.029$) was least prevalent. Increases in *Butyrivibrio* ($P = 0.039$) and *Leucobacter* ($P = 0.022$) were identified in the $ADG_{Greater}$–$ADFI_{Less}$ group. The ADG_{Less}–$ADFI_{Greater}$ group saw increases in *Lysobacter* ($P = 0.046$), *Janibacter* ($P = 0.016$), and *Succiniclasticum* ($P = 0.028$). Finally, the ADG_{Less}–$ADFI_{Less}$ group had significant increases in abundance of *Ruminococcus* species ($P = 0.026$) but decreases in *Acidaminococcus* ($P = 0.031$). Based on their putative functions, these taxa and OTU may play important roles in the fermentative and cellulolytic capacity of the rumen, contributing to the observed association with the respective feed efficiency phenotype. For example, *Leucobacter* spp. contain genes that encode for glycoside hydrolases and carbohydrate-binding modules that target starch and oligosaccharides (Weon et al., 2012; Rui et al., 2015, Chase et al., 2016). *Dialister* genera, from the phylum Firmicutes, family Selenomonadales, have been associated with hyposalivation (Hayashi et al., 2014), which may alter the buffering capacity of the rumen and fluid turnover. This is further supported by their association with lower or less stable pH in the rumen (Wallace et al., 2015). However, the microbial community of the rumen remains largely unculturable, and many of the algorithms to estimate its functional capacity are based on functions of culturable bacteria present in the database, which, in the case of ruminal microbiota, is very low. These matches against the database are questionable and must be considered only as a prediction, making studies in this area challenging.

Although several taxa and OTU were associated with feed efficiency groups within the jejunal content, such as the AA-fermenting genus *Acidaminococcus* ($P = 0.018$; $ADG_{Greater}$–$ADFI_{Less}$) and the obligately oxalotrophic, ammonium-dependent, aerobic genus *Ammoniphilus* ($P = 0.022$; ADG_{Less}–$ADFI_{Greater}$), only the phylum Proteobacteria ($P = 0.030$) was associated with the feed efficiency phenotypes modeled in these studies ($ADG_{Greater}$–$ADFI_{Less}$). Proteobacteria have been demonstrated to negatively correlate with Firmicutes populations as well as positively correlate with feed conversion ratio (Cook et al., 1994; Jami et al., 2014). However, the most interesting and substantial finding was that most taxa and OTU significantly associated with feed efficiency phenotypes in the jejunum of steers were associated with the genus *Butyrivibrio* and its family Lachnospiraceae within the $ADG_{Greater}$–$ADFI_{Less}$ group. *Butyrivibrio* species of greatest abundance that were significant for this association ranged from 1.9 to 6.0% ($P = 0.041$). The hemicellulolytic *Butyrivibrio* can ferment a wide range of sugars as well as influence the energy pool to enterocytes, of which butyrate is known to be a primary metabolic fuel (Wächtershäuser and Stein, 2000; de Graaf et al., 2010).

No feed efficiency phenotypes were significantly associated with the cecum and colon microbial communities at the phylum level. Within the cecum, the genera *Prevotella* ($P = 0.042$), *Blautia* ($P = 0.042$), *Coprobacillus* ($P = 0.004$), *Dorea* ($P = 0.042$), *Clostridium* ($P = 0.044$), and *Parabacteroides* ($P = 0.027$) were present in greatest abundance within the $ADG_{Greater}$–$ADFI_{Greater}$ group, whereas *Ruminococcus* ($P = 0.040$) and *Oscillospira* ($P = 0.041$) were least abundant within the group. Reduced abundances of the species *Lactobacillus ruminis* ($P = 0.047$) were also detected in the ADG_{Less}–$ADFI_{Greater}$ group. Although present at low abundances in the GIT of mammals, the genus *Blautia* has garnered increased interest due to host preference and specificity. This genus in the bacterial family Lachnospiraceae may contribute to host production by providing energy to the host from polysaccharides that other gut microorganisms cannot degrade (Biddle et al., 2013) as well as other specialized functions such as H2 consumption by *Blautia hydrogenotrophica* during acetogenesis (Bernalier et al., 1996). The colon taxa significant for feed efficiency phenotype associations included *Anaeroplasma* ($P = 0.022$), *Paludibacter* ($P = 0.023$), *Faecalibacterium* ($P = 0.036$), *Succinivibrio* ($P = 0.041$), and *Pseudobutyrivibrio* ($P = 0.048$). *Anaeroplasma* and *Faecalibacterium* were associated with the $ADG_{Greater}$–$ADFI_{Greater}$ group, where they were present in greatest abundance; the greater abundance of *Paludibacter* was associated with the ADG_{Less}–$ADFI_{Less}$ group; and *Succinivibrio* and *Pseudobutyrivibrio* were least abundant within the $ADG_{Greater}$–$ADFI_{Less}$ and ADG_{Less}–$ADFI_{Greater}$ groups, respectively. The genus *Dorea* ($P = 0.023$) was least abundant within the $ADG_{Greater}$–$ADFI_{Greater}$ group, whereas *Coprococcus* ($P = 0.032$) and *Clostridium* ($P = 0.045$) were in greatest abundance. *Prevotella* ($P = 0.044$) and *Butyrivibrio* ($P = 0.024$) were greatest in the least efficient group, ADG_{Less}–$ADFI_{Greater}$, whereas *Oscillospira* ($P = 0.046$) was greatest within the ADG_{Less}–$ADFI_{Less}$ group. Importantly, *Butyrivibrio* and *Pseudobutyrivibrio* species significantly associating with these feed efficiency groups in the digesta of the large intestine may provide additional insight and future research direction toward understanding and developing strategies to optimize feed efficiency due to butyrate production and strong xylan-degrading activities of these species in the GIT of ruminants.

Although many differences in bacterial community composition among feed efficiency phenotypes have

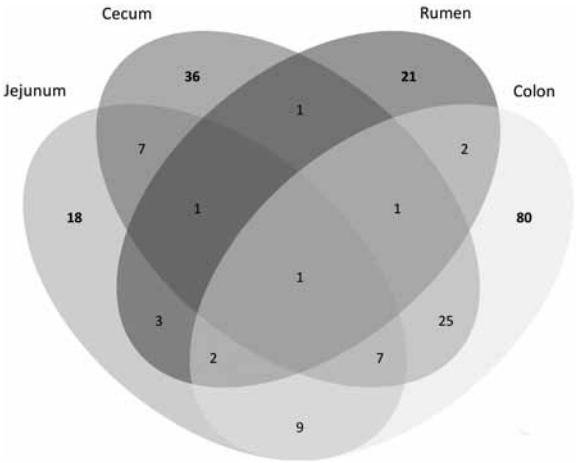

Figure 3. Venn diagram of the core operational taxonomic units (OTU) of each segment of the gastrointestinal tract (GIT). Each circle represents the absolute number of core OTU within each GIT segment and overlapping areas indicating shared OTU between core communities of the different GIT segments.

been described, determining the stability of the microbial population is also critical in understanding the role and functions of these microorganisms within the GIT. Consequently, a core bacterial community was determined among all individual segments of the GIT (Fig. 3; see Supplemental Files S1-4). Those OTU occurring in 100% of samples within a segment were considered part of the core bacterial community. Across all segments, core bacterial communities consisted primarily of members of the phyla Bacteroidetes and Firmicutes. The majority of the ruminal genera belonged to the Bacteroidetes genus *Prevotella*, with the majority of the lower GIT taxa belonging to the Firmicutes genera *Butyrivibrio* and *Ruminococcus*. Studies identifying the core microbiomes of cattle have revealed differences in the number of genera identified (Jami and Mizrahi, 2012); however, these numbers are contingent on many factors such as experimental design and the number of animals interrogated, whereas increases in animal numbers would expectedly result in a more restrictive core microbiome. Interestingly, many of the organisms identified with the variation in feed efficiency are members of these core communities. Contributors to overall ruminal fermentation and gut function, variation in these core bacterial communities, and potential subsequent dysbioses likely contribute to these phenotypic differences. Identification of core bacterial communities aid to define a "normal" community and the potential to predict responses to perturbation or guided manipulation.

Aside from the static and dynamic characteristics of the ruminal and lower gut populations and their influences on feed efficiency, additional changes in these segments may be a result of other direct or indirect stimuli. Conditions such as acidosis have distinct influences on health and production due to shifts in ruminal and lower gastrointestinal (**GI**) microbial communities, ruminal fermentation, and ruminal and lower GI function, of which the microbiome and ruminal epithelia have been shown to acclimate in response to SARA (McCann et al., 2016). In turn, acclimation to SARA is facilitated by and linked to the immune system, through crosstalk of inflammatory mediators, endotoxins, epithelial remodeling, and microbial interactions (Dionissopoulos et al., 2012). Crosstalk mechanisms regarding the gut–brain axis are also associated with the potential to affect behavior, through nutritional effects on microbial ecology, ruminal fermentation and morphology, GI inflammation, barrier function, and epithelial gene expression (Devant et al., 2016). Ultimately, the examination of the relationship between the GIT microbiome and host feed efficiency has revealed the dynamic nature of the GIT microbiome and the matrix of interactions that may occur to impact production. The next imperative steps in this field will invariably move past the characterization of these factors and focus on analyzing the synergistic functions of these factors to develop a systems approach toward elucidating the molecular basis of feed efficiency in cattle.

SUMMARY AND CONCLUSIONS

The symbioses that occur in the GIT of cattle and other ruminants are essential for the structure and function of the tissues themselves as well as for overall health of the animal. Not only are the microbial communities responsible for providing energy to the host and synthesis of microbial protein in the rumen, which eventually flows into the intestine, but, similarly, are vital in countless functions and metabolic, physiological, and immunological processes within more distal segments of the GIT. These processes provide opportunities to reexamine and develop innovative methods to mitigate CH_4 emission, increase fiber digestibility, decrease N excretion, and optimize ADG, ADFI, and feed efficiency. It must be noted, however, that although the changes in taxa and OTU within the GIT are correlated with the observed differences in the phenotypes, it is not clear whether changes in the microbiome are contributing to differences in feed efficiency or host factors are driving changes in the microbiome.

Whereas the greatest of these efforts has been focused on the rumen due primarily to metabolic capacity of the rumen, the lower GIT of ruminants has received little attention. Fully interrogating these microbial symbioses has the potential to further define the underlying factors contributing to the efficiency of feed utilization and to explain additional sources of variation in feed efficiency and even to develop microbial markers to predict high-production phenotypes. Research using the convergence of traditional diet, management, and genetic approaches with bioinformatics, microbial ecology, and

metagenomics will provide great depth and new tools to optimize the efficiency of nutrient and feed utilization. The several studies described in this review are part of the beginning of a global effort to understand ruminant nutrition and feed efficiency through microbiome and metagenomic research. To address food security challenges, we must continue to aggressively pursue and develop novel tools and resources to produce beef.

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