Cecum microbial communities from steers differing in feed efficiency[1,2,3]

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ABSTRACT: Apart from the rumen, limited knowledge exists regarding the structure and function of bacterial communities within the gastrointestinal tract and their association with beef cattle feed efficiency. The objective of this study was to characterize the microbial communities of the cecum among steers differing in feed efficiency. Within 2 contemporary groups of steers, individual feed intake and BW gain were determined from animals fed the same diet. Within both of 2 contemporary groups, BW was regressed on feed intake and 4 steers within each Cartesian quadrant were sampled ($n = 16$/group). Bacterial 16S rRNA gene amplicons were sequenced from the cecal content using next-generation sequencing technology. No significant changes in diversity or richness were detected among quadrants, and UniFrac principal coordinate analysis did not show any differences among quadrants for microbial communities within the cecum. The relative abundances of microbial populations and operational taxonomic units revealed significant differences among feed efficiency groups ($P < 0.05$). Firmicutes was the dominant cecal phylum in all groups and accounted for up to 81% of the populations among samples. Populations were also dominated by families Ruminococcaceae, Lachnospiraceae, and Clostridiaceae, with significant shifts in the relative abundance of taxa among feed efficiency groups, including families Ruminococcaceae ($P = 0.040$), Lachnospiraceae ($P = 0.020$), Erysipelotrichaceae ($P = 0.046$), and Clostridiaceae ($P = 0.043$) and genera *Coprobacillus* ($P = 0.049$), *Parabacteroides* ($P = 0.044$), *Blautia* ($P = 0.042$), *Ruminococcus* ($P = 0.040$), *Oscillospira* ($P = 0.042$), and *Prevotella* ($P = 0.042$). The study identified cecal microbial associations with feed efficiency, ADG, and ADFI. This study suggests an association of the cecum microbial community with bovine feed efficiency at the 16S level.

Key words: 16S ribosomal ribonucleic acid, cecum, feed efficiency, microbiome, operational taxonomic units

INTRODUCTION

Feed remains the largest variable cost in beef production (Arthur et al., 2005), which is concerning due to decreasing acreage for crop production (Wirsenius et al., 2010), increasing world population, and increasing diversion of traditional livestock feedstuffs for production of biofuels (Galyean et al., 2011). Improving feed efficiency would help meet the challenges of decreased feed resources and increased demand.

Previous studies of feed efficiency in beef cattle have primarily focused on host-related genetic improvement technologies (Sherman et al., 2010; Abo-Ismail et al., 2014; Saatchi et al., 2014). Yet there is limited understanding of the interplay between feed efficiency and the microbiome within the lower gastrointestinal tract (**GIT**) of beef cattle. Microbial-associated

feed efficiency studies in ruminants have largely concentrated on the microbiota of the rumen, due to its role in energy production and nutrient supply to the host (Kim et al., 2011; Hernandez-Sanabria et al., 2012; McCann et al., 2014; Myer et al., 2015). However, cattle intestinal microbial communities are distinct from those of the rumen and feces (de Oliveira et al., 2013), necessitating their examination to fully understand the relationship between microbial populations along the beef cattle GIT and feed efficiency, ADG, and average daily DMI (ADFI). This is especially emphasized due to the role the cattle cecum plays in postruminal degradation of cellulose and starch as well as the mucosal immune system.

To assess the association of the microbial community with variation in beef cattle feed efficiency, we examined the microbial community of the cecum from steers differing in feed efficiency using deep 16S rRNA gene-based community profiling, with the purpose to characterize the bacterial community of the cattle cecum among steers differing in feed intake and BW gain. We hypothesize that variation in the cecum microbial populations could contribute to variation in host feed efficiency.

MATERIALS AND METHODS

Ethics Statement

This experiment was conducted to conform to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010) and was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee.

Experimental Design and Cecum Sampling

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. Each year, 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; Myer et al., 2015). 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, steers were 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. Four steers with the greatest deviation within each Cartesian quadrant were sampled ($n = 16$/group; 2 groups). 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 result was a 2×2 factorial design consisting of greater and less ADFI and greater and less ADG (Myer et al., 2015). Steers were allowed ad libitum access to feed within 1 h before harvest. At the end of the feeding period, steers were harvested and approximately 15 mL of cecum contents were sampled. The 2 feeding studies yielded 32 animals for analysis. 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.

Deoxyribonucleic Acid Extraction, Amplification, and Sequencing

Deoxyribonucleic acid was extracted from cecum samples using a repeated bead beating plus column method (Yu and Morrison, 2004). Briefly, 0.3 g of sample was centrifuged at 4°C for 5 min at 16,000 × g to pellet solids including bacterial cells and then resuspended in 0.2 mL Tris-EDTA (pH 8.0) buffer. Cell lysis was achieved by bead beating 0.15 g of the resuspended sample in ZR BashingBead Lysis Tubes (Zymo Research Corp, Santa Ana, CA) using the TissueLyser II system (Qiagen, Hilden, Germany) for 3 min at 21 Hz in the presence of 4% (wt/vol) SDS, 500 mM NaCl, and 50 mM EDTA. After mechanical and chemical cell lysis, 10 M ammonium acetate (260 μL) was used to precipitate and remove the impurities and SDS followed by equal volume isopropanol precipitation for the recovery of the nucleic acids. Supernatants were treated

with 2 μL ribonuclease (10 mg/mL) and proteinase K (QIAamp DNA Stool Mini Kit; Qiagen) followed by the use of QIAamp columns from the Qiagen DNA Stool Mini Kit (Qiagen). Genomic DNA concentration was determined using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Amplicon library preparation was performed by PCR amplification of the V1 to V3 region of the 16S rRNA gene, using modified universal primers 27F (5′-Adaptor/Index/AGAGTTTGATCCTGGCTCAG) and 519R (5′-Adaptor/Index/GTATTACCGCGGCTGCTG) including TruSeq adapters sequences and indices as well as AccuPrime Taq high fidelity DNA Polymerase (Life Technologies, Carlsbad, CA). Amplification consisted of 23 cycles, with an annealing temperature of 58°C. Products were purified using AmPure bead purification (Agencourt, Beverly, MA), and all libraries were quantified by the PicoGreen double-stranded DNA quantitation kit (Invitrogen, Carlsbad, CA) and by real-time PCR on the LightCycler 480 system (Roche, Mannheim, Germany). The PCR amplicon libraries were sequenced using the 2 × 300, version 3 600-cycle kit and the Illumina MiSeq sequencing platform (Illumina, Inc., San Diego, CA).

Sequence Read Processing and Analysis

All sequences were processed using the QIIME-1.8.0 software package (Caporaso et al., 2010). Paired reads were joined using fastq-join (Aronesty, 2011) and filtered for quality (sequences that had a mean quality score below Q25) using the Galaxy server (Blankenberg et al., 2010). Sequences that contained read lengths shorter than 400 bp were removed and adapters/index sequences were trimmed. Chimeric sequences were checked using ChimeraSlayer (Haas et al., 2011). All cleaned sequences were classified into taxa using the Greengenes 16S rRNA Gene Database (DeSantis et al., 2006). Operational taxonomic units (**OTU**) were calculated using the uclust program (0.03 dissimilarity; Edgar, 2010). After calculating richness for each quadrant, singletons were removed from further diversity analyses. Based on rarefaction curves, the number of OTU was normalized via subsampling 75,000 sequences from each cecum sample. A phylogenic tree was built with FastTree (Price et al., 2010) to determine α- and β-diversity metrics.

Statistical Analysis

All analyses were conducted using SAS 9.4 (SAS Inst. Inc., Cary, NC). The mean abundances ($n = 8$) of data metrics and each taxon were compared among the feed efficiency groups using a model of contemporary group and Cartesian quadrant (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 less ADFI [$\mathbf{ADG_{Less}}$–$\mathbf{ADFI_{Less}}$], and less ADG and greater ADFI [$\mathbf{ADG_{Less}}$–$\mathbf{ADFI_{Greater}}$]) as fixed effects. Significant differences were determined at $P < 0.05$ with the Benjamini–Hochberg method used for multiple-testing corrections (Benjamini and Hochberg, 1995). Multiple-testing corrections were made for the number of phyla, the number of OTU groups, and other classified taxa groups. Linear contrasts were then applied to significant quadrants to separate whether microbial populations varied by less vs. greater ADG, less vs. greater ADFI, or their interaction ($P < 0.05$). Principal coordinates analysis (**PCoA**) was performed using weighted and unweighted UniFrac analyses (Lozupone and Knight, 2005).

RESULTS

Diversity of Cecum Bacterial Communities

The sampled cecal contents from the 32 steers grouped into 4 feed efficiency phenotypes produced 17,757,018 sequence reads after filtering for quality and removing apparent chimeras, for an average of 554,907 reads per sample (range 130,883 to 1,205,513). The average read length was 500 bp. Operational taxonomic units were defined as a bin of sequence reads sharing ≥97% nucleotide sequence identity, and a total of 378,243 OTU were detected with an average of 11,820 ± 3,337 OTU per individual sample. The average number of OTU detected from each Cartesian quadrant ranged from 2,252 to 18,533 OTU. Singletons accounted for approximately 34% of the OTU detected within the cecum content samples. Using Good's coverage estimator as a metric for determining coverage, the data set reported coverage ranging from 94.97 to 96.97%. Bacterial diversity, as determined by Shannon diversity index, ranged from 7.37 to 8.40.

The individual samples were normalized to accurately compare the results among feed efficiency phenotype groups. The OTU table for each sample was rarefied to 75,000 sequence reads, based on the sample rarefaction curves. The normalized samples were then used for analysis using the sample means within each quadrant. The normalized sequence reads were analyzed using α-diversity metrics of bacterial diversity (Shannon index), richness (Chao-1), and coverage (Good's coverage estimator; Table 1). The number of OTU detected within each feed efficiency group did not differ ($P > 0.05$), averaging 5,572 ± 1,428 OTU per group. The Chao-1 richness metric also did not differ, estimating 10,180 ± 2,800 OTU per group. Bacterial diversity did not indicate any

Table 1. Diversity statistics among reads from feed efficiency grouped samples

Feed efficiency group[1]	Sampling type[2]	No. of sequences	No. of OTU[3,4]	Chao-1[4]	Shannon diversity index[4]	Good's coverage, %
$ADG_{Greater}$–$ADFI_{Greater}$	Subsampled reads	75,000	5,342 ± 1,446	9,886 ± 1,614	7.65 ± 1.20	96.18 ± 2.21
$ADG_{Greater}$–$ADFI_{Less}$	Subsampled reads	75,000	5,881 ± 1,086	10,592 ± 2,494	8.14 ± 0.94	95.98 ± 2.22
ADG_{Less}–$ADFI_{Less}$	Subsampled reads	75,000	6,820 ± 1,415	13,025 ± 2,248	8.40 ± 0.94	94.97 ± 2.44
ADG_{Less}–$ADFI_{Greater}$	Subsampled reads	75,000	4,746 ± 1,450	7,983 ± 2,292	7.37 ± 1.54	96.97 ± 2.07

[1]n = 8 among groups. $ADG_{Greater}$–$ADFI_{Greater}$ = 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.

[2]Means among the groups were compared using ANOVA and the Tukey's test.

[3]OTU = operational taxonomic units.

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

differences among feed efficiency groups ($P > 0.05$), with a range of 7.37 to 8.40. Coverage was adequate, ranging from 94.97% for ADG_{Less}–$ADFI_{Less}$ to 96.97% for ADG_{Less}–$ADFI_{Greater}$

The phylogeny-based UniFrac method (Lozupone and Knight, 2005) was applied using the detected OTU to determine if the data separated into any sample clusters, via PCoA (Fig. 1). This β-diversity metric takes into account the phylogenetic divergence among the OTU to determine differences among the cecum microbial communities from each feed efficiency group (Lozupone et al., 2007). The analysis did not indicate any separation into clusters in either the weighted (quantitative) or unweighted (qualitative) UniFrac distances of the cecum microbial communities (Lozupone et al., 2011).

Taxonomic and Operational Taxonomic Unit Composition

The 17,757,018 sequence reads were classified using the Greengenes 16S rRNA Gene Database (DeSantis et al., 2006), resulting in 18 phyla, 40 classes, 75 orders, 148 families, and 225 genera. The unassigned taxa accounted for approximately 1.08% of the reads. At the pylum level, Firmicutes was the most abundant within each feed efficiency group, ranging from 68 to 81% of the total reads (Fig. 2a). These abundances are consistent with previous studies regarding the microbial abundances within the cecum contents of cattle (de Oliveira et al., 2013; Malmuthuge and Griebel, 2014). Other dominant phyla included Bacteroidetes (18 to 26 ± 3.9%), Spirochaetes (1.4 to 3.1 ± 0.9%), Tenericutes (0.7 to 1.2 ± 0.2%), Actinobacteria (0.2 to 0.4 ± 0.1%),

ADG_Greater -ADFI_Greater (○), ADG_Greater -ADFI_Less (▲), ADG_Less -ADFI_Less (■), ADG_Less -ADFI_Greater (▶)
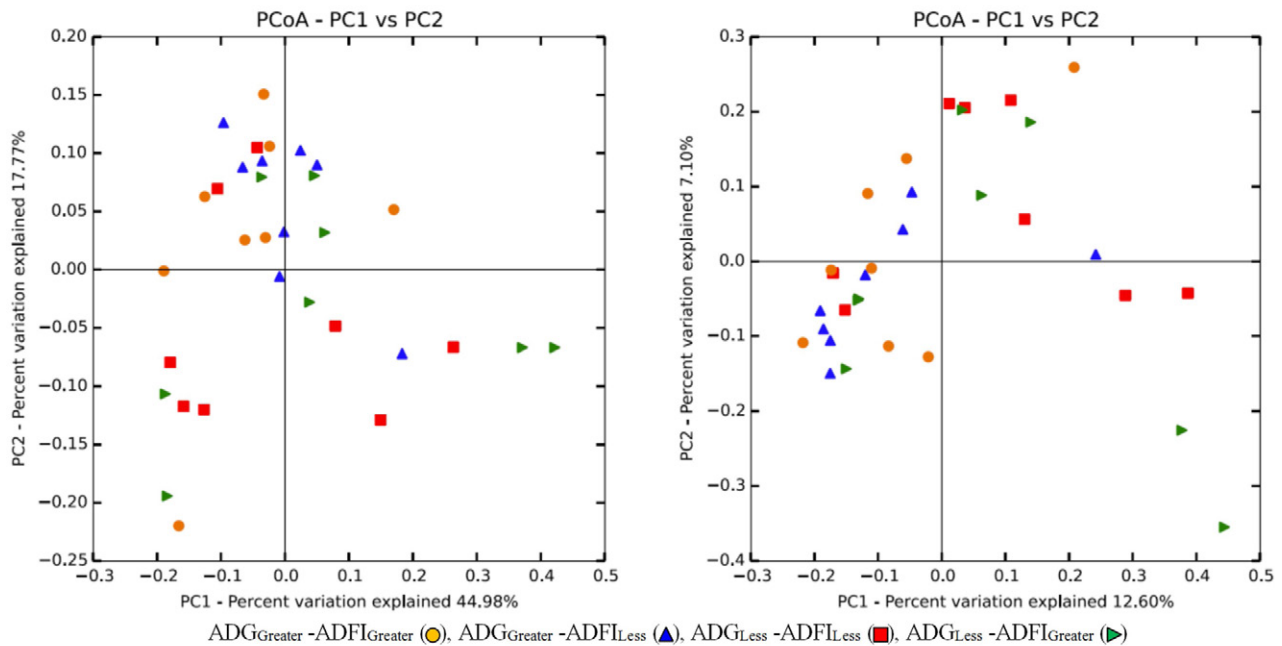
Figure 1. UniFrac (Lozupone and Knight, 2005) principal coordinates analysis (PCoA) displaying correlations among the bacterial communities of the 4 feed efficiency groups. A) Weighted PCoA analyzed from rarefied subsets of 75,000 sequences from each sample. B) Unweighted PCoA analyzed from rarefied subsets of 75,000 sequences from each sample. n = 8, represented by differing symbols. $ADG_{Greater}$–$ADFI_{Greater}$ = 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.

and Proteobacteria (0.3 to 0.8 ± 0.2%). No significant differences among the feed efficiency groups were observed within any of the phylum assignments. The remaining phyla accounted for less than 0.1% of the sequence reads, and no differences were observed among feed efficiency groups for the minor phyla abundances.

At the genus level, *Prevotella* (2.1 to 7.3 ± 2.8%), *Turicibacter* (4.6 to 6.7 ± 1.7%), *Coprococcus* (1.2 to 2.8 ± 0.5%), *Ruminococcus* (1.5 to 2.7 ± 0.4%), *Dorea* (2.2 to 3.3 ± 0.5%), *Blautia* (0.5 to 2.0 ± 0.3%), *Clostridium* (1.0 to 1.2 ± 0.1%), and *Oscillospira* (1.1 to 1.6 ± 0.2%) were present in the greatest abundance, each representing ≥1% of the total sequences (Fig. 2b). Of these genera, only *Blautia* differed among the feed efficiency groups (P = 0.036), with the $ADG_{Greater}$–$ADFI_{Greater}$ group having the greatest abundance (Table 2). There were several taxa that were not classified to the genus level but were present in abundances greater than 1% of the total sequences. These included families Ruminococcaceae (18 to 28 ± 3.2%), Lachnospiraceae (3.7 to 6.9 ± 0.9%), Clostridiaceae (5.2 to 14.0 ± 3.6%), Bacteroidaceae (2.0 to 4.5 ± 1.1%), and Paraprevotellaceae (0.9 to 2.5 ± 0.7%) as well as orders Clostridiales (5.1 to 9.1 ± 1.5%) and Bacteroidales (1.1 to 3.4 ± 0.5%). Bacteroidales was the only taxon at this level detected at differing abundances among the feed efficiency groups (P = 0.035), with the ADG_{Less}–$ADFI_{Greater}$ group having the greatest abundance (Table 2). Any remaining taxa were not listed and deemed nondetectable at abundances ≤0.001%.

Additional taxa were identified at low relative abundances, and differences were detected among feed efficiency phenotypes. These included the genera *Coprobacillus* (P = 0.004) and *Parabacteroides* (P = 0.027), with the greatest abundances within the $ADG_{Greater}$–$ADFI_{Greater}$ and ADG_{Less}–$ADFI_{Greater}$ groups, respectively. Differences among the groups were also detected within other low abundance taxa but not classified to the genus level. These included 2 identifications within the family Erysipelotrichaceae (P = 0.046 and 0.049) as well as the family Bifidobacteriaceae (P = 0.032), with the $ADG_{Greater}$–$ADFI_{Greater}$ group having the greatest abundance (Table 2). All taxa were defined as present in at least 50% of the samples.

The examination of OTU across all feed efficiency phenotype groups was also conducted to detect differences in abundance. Consideration was only given to OTU detectable at abundances >0.001% and present in at least 50% of the samples. Among the groups, 112 OTU were identified that differed in abundance (Table 3). The most commonly identified OTU were families Ruminococcaceae (OTU-19743; P = 0.040), Lachnospiraceae (OTU-67423; P = 0.020), and Clostridiaceae (OTU-82471; P = 0.043) as well as the order Clostridiales (OTU-61987; P = 0.040; Table 3).

At the genus level, *Prevotella* (OTU-63139; P = 0.042), *Blautia* (OTU-47330; P = 0.042), *Ruminococcus* (OTU-35773; P = 0.040), *Oscillospira* (OTU-37377; P = 0.041), *Dorea* (OTU-64423; P = 0.042), *Clostridium* (OTU-31826; P = 0.044), *Parabacteroides* (OTU-71177; P = 0.044), and *Coprobacillus* (OTU-74120; P = 0.049) as well as the species *Blautia producta* (OTU-65739; P = 0.042) and *Lactobacillus ruminis* (OTU-67464; P = 0.047) differed among groups (Table 3).

Effect of Gain and Intake

To examine the microbial population associations with the factors contributing to feed efficiency, the relationship of the microbial comminutes with ADG and ADFI were analyzed to determine whether the associated microbial populations differed by less vs. greater ADG, less vs. greater ADFI, or their interaction. The significant relative abundances of taxa and OTU between ADG and ADFI are listed in Tables 4 and 5, respectively. No taxa were associated with gain alone (Table 4), but 2 taxa were determined to have either a significant association with intake or the interaction. When examined using OTU, there was an even distribution of associations with both effects and their interaction. Pertaining to significant genera, *Prevotella* (OTU-63139; P = 0.049) was associated with gain whereas *Ruminococcus* (OTU-67544; P = 0.047), *Bacteroides* (OTU-39469; P = 0.048), and *Blautia* (OTU-47330; P = 0.047) were significant for the interaction (Table 5). Operational taxonomic units classified within families Ruminococcaceae, Lachnospiraceae, and Clostridiaceae were associated with both effects as well as their interaction (Table 5). The only classifications of OTU associated solely with intake were the order Bacteroidales (OTU-45474; P = 0.048) and the genus *Turicibacter* (OTU-56055; P = 0.048; Table 5).

DISCUSSION

Research examining the interplay between microbial populations and the host with regard to feed efficiency primarily has been aimed at the rumen and feces. However, the sections of the bovine GIT are distinct in microbial composition and function (de Oliveira et al., 2013; Malmuthuge and Griebel, 2014) and should not be neglected when determining factors affecting feed efficiency. Postruminal degradation of cellulose and starch occurs in the cecum and is important in animal digestion (Armstrong and Smithard, 1979). The cecum and colon are also well supplied with gut-associated lymphoid tissue, of which the mucosal immune system interacts with host and intestinal microbiota, playing a role in the host defense against pathogenic

A

B
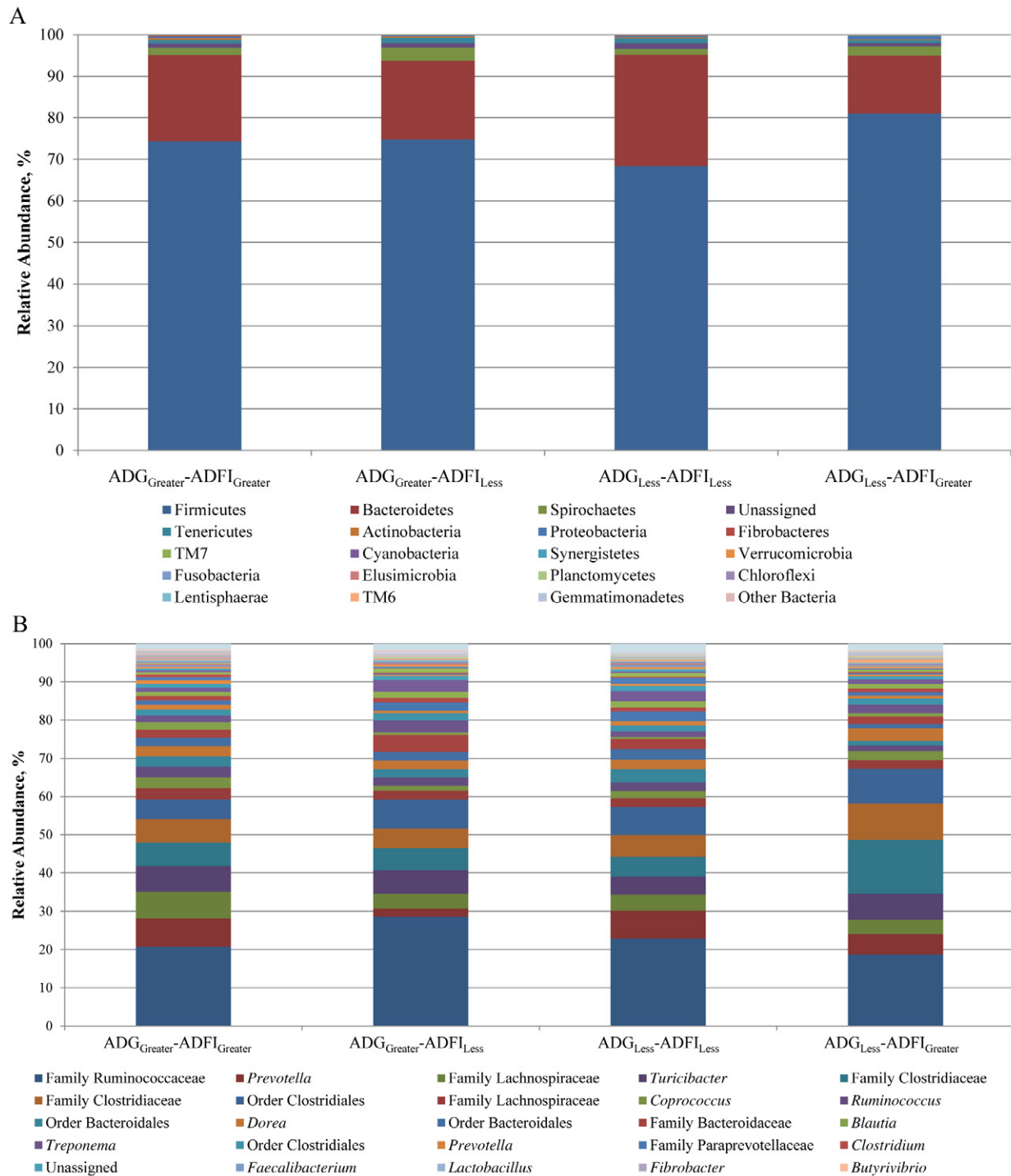
Figure 2. The taxonomic profiles for the relative phylum-level (A) and genus-level (B) abundance of each group classified by representation at ≥0.001% of total sequences. Taxonomic composition of the cecum microbiota among the 4 groups was compared based on the relative abundance (reads of a taxon/total reads in a sample). $ADG_{Greater}–ADFI_{Greater}$ = 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.

microorganisms (Moretó and Pérez-Bosque, 2009). Current studies also support that the initial acquisition of and exposure to microbes result in a host-specific gut microbiome, which plays important roles in the maturation of the mucosal immune system (Mulder et al., 2011; Chung et al., 2012). Highlighting the impor-

tance of the cecum in the comprehensive evaluation of feed efficiency in cattle, this study is among the first to examine the variation of microbial communities as a function of feed efficiency within the cecum of steers.

The microbial abundance and diversity is vastly greater within the cecum compared with that in the

Table 2. Relative abundance of significant taxa in the 4 feed efficiency groups

| Classification | Percentage of total sequences[1] | | | | SEM | P-value[2] | No. of steers with detectable taxon[3] |
	$ADG_{Greater}$–$ADFI_{Greater}$	$ADG_{Greater}$–$ADFI_{Less}$	ADG_{Less}–$ADFI_{Less}$	ADG_{Less}–$ADFI_{Greater}$			
Blautia	2.0002	0.6303	0.8668	0.4977	0.3508	0.036	32
Coprobacillus	0.1904	0.0264	0.0770	0.0238	0.0267	0.004	28
Family Bifidobacteriaceae	4.98×10^{-4}	0.0000	0.0000	0.0000	1.23×10^{-4}	0.032	16
Family Erysipelotrichaceae	0.7170	0.3058	0.3593	0.3487	0.1057	0.046	31
Family Erysipelotrichaceae	1.69×10^{-19}	2.16×10^{-3}	8.26×10^{-4}	8.26×10^{-4}	5.17×10^{-4}	0.049	16
Order Bacteroidales	2.7391	2.1340	1.1186	3.4556	0.5160	0.035	31
Parabacteroides	0.8073	0.3512	0.3449	1.5076	0.2577	0.027	31

[1]Data is shown as least squares means (n = 8/group). $ADG_{Greater}$–$ADFI_{Greater}$ = 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.

[2]Differences among the groups are significant at $P < 0.05$.

[3]The total number of steers was 32. All data are defined as taxa that are present in at least 50% of the samples.

rumen (Reti et al., 2013; de Oliveira et al., 2013; Myer et al., 2015). To accommodate for the increases in diversity and abundance, the study normalized the cecum samples to 75,000 sequences/sample, assuring the characterization of most of the bacterial OTU within the cecum contents of the steer. The liberal normalized depth was based on sample rarefaction curves and comparative studies using similar sequencing platforms (Kohl et al., 2014; Weese et al., 2014; Myer et al., 2015). The current study was able to recover approximately 96% of all OTU calculated at 0.03 dissimilarity, as determined by Good's coverage estimator, indicating adequate depth for cecum microbial community analysis.

The use of next-generation sequencing technologies allowed for deeper sequencing and greater coverage of the microbial communities within the cecum contents than previously reported (de Oliveira et al., 2013). However, the α-diversity metrics among the 4 feed efficiency phenotypes did not differ with regard to the number of observed OTU, richness (Chao-1), or diversity (Shannon index). Using the weighted and unweighted UniFrac (Lozupone and Knight, 2005) PCoA, which relies on the phylogenetic divergence among the OTU, the microbial communities within the cecum did not cluster according to host feed efficiency phenotype. These results support the phylogenetic similarities among the cecal microbial populations among groups. These results may be anticipated, as bacterial communities have been shown to separate and cluster based on diet (Kim et al., 2014; Patel et al., 2014), which have profound effects on the structure and diversity of the respective microbial communities. In addition, the microbial communities within the GIT can be extraordinarily stable, due to both functional redundancy and resilience to perturbation (Weimer, 2015). Yet microbial community effects on host feed efficiency or the effect of host feed efficiency on the

microbial community may be a function of finer shifts in population dynamics, which may not be reflected in phylogenetic analyses. The data presented here support the latter. Microbial community variation within the relative OTU and taxonomic abundances may not exhibit any changes within the α-diversity metrics of the populations associated with the groups, but changes in specific OTU and taxa may have important functions. It was also anticipated that host specificity might play a role in the similarities observed within the cecal microbial communities, which has been demonstrated in the rumen (Weimer et al., 2010).

The 16S sequences observed from the cecum content were primarily associated with the phyla Firmicutes, Bacteroidetes, Spirochaetes, Tenericutes, Actinobacteria, and Proteobacteria, with Firmicutes being the most abundant within each feed efficiency group. The dominance of Firmicutes and the remaining major phyla represent the majority of gut-associated phylotypes in a variety of mammals (Ley et al., 2008; Shanks et al., 2011; de Oliveira et al., 2013). This suggests the role of host specificity on microbial communities within the gut as well as the role the abundant phyla have on the microbial ecology within the mammalian gut. These phyla are similarly present in great abundance within the rumen fed the same diet, with noted differences between Firmicutes and Bacteroidetes abundances (Myer et al., 2015). Changes within the Firmicutes:Bacteroidetes ratio have been the focus of microbiome-associated obesity research (Ismail et al., 2010), and increases of the ratio have also been shown to affect energy harvesting and to correlate with increased fat (Jami et al., 2014). However, there were no observable differences at the phylum level when examining the differing feed efficiency groups.

The subphylum abundances were primarily dominated by the unclassified families Ruminococcaceae, Lachnospiraceae, Turicibacter, and Clostridiaceae

Table 3. Relative abundance of significant operational taxonomic units (OTU) in the 4 feed efficiency groups

		Percentage of total sequences[2]						No. of steers with detectable taxon[4]
OTU ID[1]	Classification	$ADG_{Greater}-ADFI_{Greater}$	$ADG_{Greater}-ADFI_{Less}$	$ADG_{Less}-ADFI_{Less}$	$ADG_{Less}-ADFI_{Greater}$	SEM	P-value[3]	
denovo39469	*Bacteroides*	0.002	0.001	0.003	0.012	0.002	0.045	19
denovo47330	*Blautia*	0.347	0.071	0.048	0.089	0.072	0.042	30
denovo65739	*Blautia producta*	0.673	0.186	0.381	0.045	0.140	0.042	31
denovo80965	Class Mollicutes	0.008	0.024	0.008	0.006	0.004	0.049	22
denovo31826	*Clostridium*	0.038	0.006	0.001	0.003	0.008	0.044	22
denovo57851	*Clostridium*	0.004	0.010	0.003	0.003	0.002	0.045	23
denovo14203	*Clostridium*	0.185	0.123	0.044	0.032	0.039	0.047	29
denovo11101	*Clostridium*	0.001	0.003	0.001	0.002	0.001	0.048	24
denovo74120	*Coprobacillus*	0.101	0.012	0.038	0.006	0.020	0.049	22
denovo64423	*Dorea*	0.003	0.001	0.001	0.001	0.001	0.042	17
denovo82471	Family Clostridiaceae	0.025	0.006	0.001	0.002	0.006	0.043	18
denovo17753	Family Clostridiaceae	0.000	0.002	0.001	0.003	0.001	0.044	17
denovo49662	Family Clostridiaceae	0.004	0.012	0.003	0.008	0.002	0.044	28
denovo17106	Family Clostridiaceae	0.001	0.004	0.002	0.001	0.001	0.045	16
denovo67423	Family Lachnospiraceae	0.007	0.001	0.001	0.001	0.001	0.020	17
denovo52061	Family Lachnospiraceae	0.072	0.012	0.020	0.005	0.016	0.042	25
denovo23347	Family Lachnospiraceae	0.001	0.010	0.002	0.008	0.002	0.043	21
denovo28883	Family Lachnospiraceae	0.031	0.013	0.022	0.008	0.005	0.044	30
denovo37291	Family Lachnospiraceae	0.029	0.005	0.001	0.010	0.007	0.044	23
denovo3955	Family Lachnospiraceae	0.007	0.014	0.024	0.015	0.003	0.047	30
denovo847	Family Lachnospiraceae	0.001	0.003	0.001	0.002	0.001	0.048	19
denovo24421	Family Lachnospiraceae	0.002	0.006	0.028	0.010	0.007	0.048	21
denovo71912	Family Lachnospiraceae	0.001	0.005	0.002	0.005	0.001	0.049	17
denovo82789	Family Lachnospiraceae	0.024	0.004	0.002	0.004	0.006	0.049	18
denovo75529	Family Peptococcaceae	0.003	0.006	0.002	0.002	0.001	0.041	17
denovo84650	Family Peptococcaceae	0.002	0.008	0.003	0.002	0.001	0.043	21
denovo73800	Family Rikenellaceae	0.013	0.053	0.025	0.118	0.022	0.044	26
denovo19743	Family Ruminococcaceae	0.010	0.035	0.011	0.023	0.006	0.040	23
denovo71896	Family Ruminococcaceae	0.013	0.039	0.010	0.020	0.007	0.040	27
denovo54614	Family Ruminococcaceae	0.343	0.956	0.531	0.513	0.131	0.040	30
denovo34289	Family Ruminococcaceae	0.009	0.018	0.002	0.010	0.003	0.041	26
denovo69959	Family Ruminococcaceae	0.003	0.014	0.004	0.004	0.002	0.041	22
denovo23145	Family Ruminococcaceae	0.002	0.013	0.003	0.002	0.003	0.041	23
denovo47897	Family Ruminococcaceae	0.010	0.032	0.010	0.015	0.005	0.041	26
denovo7112	Family Ruminococcaceae	0.022	0.089	0.032	0.087	0.018	0.041	29
denovo15053	Family Ruminococcaceae	0.001	0.010	0.002	0.009	0.002	0.041	18
denovo14979	Family Ruminococcaceae	0.001	0.007	0.002	0.002	0.001	0.042	20
denovo65365	Family Ruminococcaceae	0.028	0.064	0.017	0.076	0.015	0.042	26
denovo37029	Family Ruminococcaceae	0.001	0.008	0.001	0.005	0.002	0.042	20
denovo11150	Family Ruminococcaceae	0.001	0.007	0.001	0.002	0.001	0.044	16
denovo52756	Family Ruminococcaceae	0.002	0.001	0.000	0.004	0.001	0.044	16
denovo55195	Family Ruminococcaceae	0.015	0.067	0.014	0.008	0.011	0.044	26
denovo21059	Family Ruminococcaceae	0.003	0.013	0.002	0.003	0.002	0.045	22
denovo84531	Family Ruminococcaceae	0.031	0.094	0.044	0.138	0.024	0.045	27
denovo29885	Family Ruminococcaceae	0.001	0.002	0.005	0.001	0.001	0.045	16
denovo46639	Family Ruminococcaceae	0.003	0.014	0.003	0.009	0.003	0.045	25
denovo581	Family Ruminococcaceae	0.002	0.010	0.007	0.012	0.002	0.045	26
denovo77292	Family Ruminococcaceae	0.013	0.031	0.011	0.018	0.004	0.046	27
denovo40701	Family Ruminococcaceae	0.008	0.006	0.002	0.007	0.001	0.046	25
denovo33227	Family Ruminococcaceae	0.006	0.013	0.004	0.003	0.002	0.046	25
denovo30081	Family Ruminococcaceae	0.003	0.005	0.001	0.001	0.001	0.047	17
denovo84036	Family Ruminococcaceae	0.001	0.006	0.001	0.006	0.001	0.048	20
denovo43825	Family Ruminococcaceae	0.000	0.004	0.002	0.002	0.001	0.048	19

Continued

Table 3. (cont.)

OTU ID[1]	Classification	Percentage of total sequences[2]				SEM	*P*-value[3]	No. of steers with detectable taxon[4]
		$ADG_{Greater}-$ $ADFI_{Greater}$	$ADG_{Greater}-$ $ADFI_{Less}$	$ADG_{Less}-$ $ADFI_{Less}$	$ADG_{Less}-$ $ADFI_{Greater}$			
denovo68434	Family Ruminococcaceae	0.010	0.028	0.011	0.005	0.006	0.048	24
denovo10324	Family Ruminococcaceae	0.001	0.003	0.001	0.001	0.001	0.048	21
denovo56697	Family Ruminococcaceae	0.001	0.010	0.004	0.004	0.002	0.048	21
denovo27960	Family Ruminococcaceae	0.002	0.007	0.005	0.003	0.001	0.048	25
denovo29815	Family Ruminococcaceae	0.001	0.007	0.003	0.007	0.002	0.048	22
denovo57607	Family Ruminococcaceae	0.001	0.006	0.002	0.005	0.001	0.049	19
denovo1809	Family Ruminococcaceae	0.001	0.005	0.004	0.012	0.002	0.049	20
denovo83935	Family Ruminococcaceae	1.627	3.144	1.575	2.111	0.410	0.049	32
denovo63389	Family Ruminococcaceae	0.003	0.003	0.003	0.001	0.001	0.049	23
denovo57290	Family Ruminococcaceae	0.002	0.004	0.000	0.002	0.001	0.049	16
denovo36101	Family Ruminococcaceae	0.002	0.003	0.001	0.001	0.001	0.049	20
denovo79392	Family Ruminococcaceae	0.001	0.004	0.000	0.001	0.001	0.049	16
denovo36066	Family Ruminococcaceae	0.003	0.018	0.003	0.001	0.004	0.049	23
denovo62672	Family Ruminococcaceae	0.006	0.027	0.006	0.016	0.006	0.049	21
denovo67464	*Lactobacillus ruminis*	0.048	0.015	0.027	0.007	0.010	0.047	29
denovo45474	Order Bacteroidales	0.004	0.007	0.002	0.034	0.008	0.041	16
denovo79232	Order Bacteroidales	0.002	0.010	0.000	0.006	0.002	0.048	16
denovo61987	Order Clostridiales	0.004	0.009	0.003	0.002	0.002	0.040	22
denovo1815	Order Clostridiales	0.000	0.003	0.001	0.004	0.001	0.041	19
denovo13244	Order Clostridiales	0.321	0.569	0.244	0.259	0.078	0.041	31
denovo77042	Order Clostridiales	0.007	0.016	0.003	0.002	0.003	0.041	22
denovo3281	Order Clostridiales	0.002	0.007	0.004	0.004	0.001	0.042	25
denovo56131	Order Clostridiales	0.000	0.002	0.001	0.002	0.001	0.042	16
denovo55554	Order Clostridiales	0.001	0.003	0.001	0.002	0.001	0.042	18
denovo8481	Order Clostridiales	0.004	0.010	0.007	0.020	0.004	0.042	22
denovo76947	Order Clostridiales	0.002	0.007	0.002	0.004	0.001	0.042	20
denovo209	Order Clostridiales	0.000	0.004	0.001	0.003	0.001	0.042	16
denovo32474	Order Clostridiales	0.002	0.006	0.003	0.007	0.001	0.042	26
denovo41594	Order Clostridiales	0.024	0.006	0.010	0.007	0.004	0.042	29
denovo77809	Order Clostridiales	0.003	0.002	0.003	0.006	0.001	0.042	28
denovo25052	Order Clostridiales	0.004	0.006	0.001	0.002	0.001	0.044	19
denovo29731	Order Clostridiales	0.006	0.003	0.004	0.001	0.001	0.045	23
denovo18250	Order Clostridiales	0.030	0.010	0.016	0.008	0.005	0.047	30
denovo15108	Order Clostridiales	0.015	0.041	0.021	0.035	0.007	0.047	29
denovo34282	Order Clostridiales	0.002	0.006	0.000	0.003	0.001	0.047	17
denovo83207	Order Clostridiales	0.002	0.002	0.008	0.004	0.001	0.047	24
denovo41989	Order Clostridiales	0.002	0.007	0.003	0.002	0.001	0.047	20
denovo2832	Order Clostridiales	0.008	0.016	0.004	0.005	0.003	0.048	26
denovo76627	Order Clostridiales	0.001	0.003	0.000	0.002	0.001	0.049	16
denovo81859	Order Clostridiales	0.002	0.008	0.002	0.008	0.002	0.049	24
denovo18403	Order Clostridiales	0.001	0.006	0.002	0.005	0.001	0.049	18
denovo70538	Order Clostridiales	0.003	0.005	0.002	0.020	0.005	0.049	17
denovo20936	Order Clostridiales	0.001	0.003	0.001	0.003	0.001	0.049	23
denovo25838	Order Clostridiales	0.001	0.005	0.002	0.002	0.001	0.049	17
denovo70326	Order Clostridiales	0.001	0.004	0.002	0.001	0.001	0.049	22
denovo37377	*Oscillospira*	0.001	0.013	0.002	0.012	0.003	0.041	23
denovo85505	*Oscillospira*	0.003	0.002	0.001	0.010	0.002	0.042	17
denovo70565	*Oscillospira*	0.001	0.001	0.003	0.010	0.002	0.044	21
denovo43768	*Oscillospira*	0.002	0.008	0.003	0.007	0.001	0.046	22
denovo30472	*Oscillospira*	0.002	0.007	0.004	0.003	0.001	0.047	22
denovo71177	*Parabacteroides*	0.003	0.001	0.001	0.001	0.001	0.044	16
denovo63139	*Prevotella*	0.107	0.023	0.021	0.021	0.021	0.042	20

Continued

Table 3. (cont.)

OTU ID[1]	Classification	Percentage of total sequences[2]				SEM	P-value[3]	No. of steers with detectable taxon[4]
		$ADG_{Greater}$–$ADFI_{Greater}$	$ADG_{Greater}$–$ADFI_{Less}$	ADG_{Less}–$ADFI_{Less}$	ADG_{Less}–$ADFI_{Greater}$			
denovo35773	*Ruminococcus*	0.007	0.015	0.008	0.022	0.004	0.040	24
denovo36682	*Ruminococcus*	0.008	0.037	0.004	0.026	0.009	0.047	24
denovo67544	*Ruminococcus*	0.091	0.022	0.022	0.039	0.018	0.047	29
denovo56055	*Turicibacter*	0.001	0.004	0.001	0.001	0.001	0.041	18
denovo38404	*Turicibacter*	0.001	0.004	0.002	0.003	0.001	0.041	22
denovo40021	Unassigned	0.001	0.004	0.001	0.002	0.001	0.041	22
denovo73557	Unassigned	0.000	0.002	0.002	0.003	0.001	0.043	19

[1]ID = OTU identifier.

[2]Data is shown as least squares means (n = 8/group). $ADG_{Greater}$–$ADFI_{Greater}$ = 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.

[3]Differences among the groups are significant at $P < 0.05$.

[4]The total number of steers was 32. Percentage of total sequences for steers with nondetectable OTU were treated as 0.001%, and all data are defined as OTU that are present in at least 50% of the samples.

as well as the genera *Prevotella*, *Coprococcus*, and *Ruminococcus*. These results are in agreement with other studies regarding the microbial communities across the GIT, where families Ruminococcaceae, Lachnospiraceae, and Clostridiaceae were observed to be abundant in samples from the large intestine of the steer (de Oliveira et al., 2013).

Differences among the 4 feed efficiency groups could be detected in the relative abundance of specific taxa within the cecum. All differences were observed at the subphylum level. As anticipated, due to differences in the function and environment between the rumen and cecum, there is little overlap of the specific taxa and abundance between the cecum and rumen on the same diet (Myer et al., 2015), reaffirming that these populations are distinct from those of the rumen. For example, *Prevotella* abundances in the rumen vary between 45 and 57% (Myer et al., 2015), whereas in the cecum, they are generally <8%. This is likely primarily due to their ruminal role in the degradation and utilization of starch as well as the degradation of proteins and uptake and fermentation of peptides (Cotta, 1992). Digestive functions and processes within the cecum of cattle vary greatly from the rumen.

The functional and environmental differences between the rumen and cecum were also reflected within

several cecal populations that were in low relative abundance. *Coprobacillus*, which was observed to differ among the 4 groups, has been commonly found in the feces and cecum of chickens, although no association with differential feed conversion efficiencies was reported (Stanley et al., 2012). Interestingly, differences within the family Erysipelotrichaceae were also observed; the genus *Coprobacillus* belongs to this family. The family Erysipelotrichaceae has been associated with mice fed high-fat diets (Daniel et al., 2014). Although some associations are beginning to emerge as research allows for deeper sequencing, little is known about *Coprobacillus* beyond basic characterization studies of gut commensals. Found commonly in the rumen and large intestine, *Parabacteroides* has been found to be dominant when lower-forage diets are fed (Kim et al., 2014; Patel et al., 2014). The observed differences of the genus *Blautia* among the feed efficiency groups have been of particular interest. *Blautia* has been the focus of recent research regarding its ubiquitous presence among humans and other mammals, although at low abundance (Eren et al., 2015). *Blautia* is also a genus within in the family Lachnospiraceae, which can degrade complex polysaccharides to VFA, such as acetate, butyrate, and propionate, to be used for energy by the host (Biddle et al., 2013). The different *Blautia* strains have specialized

Table 4. Relative abundance of significant taxa within ADG and ADFI phenotypes

Classification	Phenotype[1]				SEM	Effect	P-value[2]
	$ADG_{Greater}$–$ADFI_{Greater}$	$ADG_{Greater}$–$ADFI_{Less}$	ADG_{Less}–$ADFI_{Less}$	ADG_{Less}–$ADFI_{Greater}$			
Coprobacillus	0.190	0.026	0.024	0.077	0.027	Intake	0.009
Coprobacillus	0.190	0.026	0.024	0.077	0.027	Gain × intake	0.048
Parabacteroides	0.807	0.351	1.508	0.345	0.258	Gain × intake	0.049

[1]Data is shown as least squares means (n = 16/phenotype). $ADG_{Greater}$–$ADFI_{Greater}$ = 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.

[2]Differences among the groups are significant at $P < 0.05$.

Table 5. Relative abundance of significant operational taxonomic units (OTU) within ADG and ADFI phenotypes

OTU ID[1]	Classification	$ADG_{Greater}-ADFI_{Greater}$	$ADG_{Greater}-ADFI_{Less}$	$ADG_{Less}-ADFI_{Less}$	$ADG_{Less}-ADFI_{Greater}$	SEM	Effect	P-value[3]
			Phenotype[2]					
denovo39469	*Bacteroides*	0.002	0.001	0.012	0.003	0.002	Gain × intake	0.048
denovo47330	*Blautia*	0.347	0.071	0.089	0.048	0.072	Gain × intake	0.047
denovo80965	Class Mollicutes	0.008	0.024	0.006	0.008	0.004	Gain × intake	0.047
denovo31826	*Clostridium*	0.038	0.006	0.003	0.001	0.008	Gain	0.048
denovo11101	*Clostridium*	0.001	0.003	0.002	0.001	0.001	Intake	0.047
denovo82471	Family Clostridiaceae	0.025	0.006	0.002	0.001	0.006	Gain	0.049
denovo17106	Family Clostridiaceae	0.001	0.004	0.001	0.002	0.001	Gain × intake	0.049
denovo24421	Family Lachnospiraceae	0.002	0.006	0.010	0.028	0.007	Gain	0.048
denovo37291	Family Lachnospiraceae	0.029	0.005	0.010	0.001	0.007	Gain × intake	0.049
denovo3955	Family Lachnospiraceae	0.007	0.014	0.024	0.015	0.003	Gain × intake	0.049
denovo52061	Family Lachnospiraceae	0.072	0.012	0.005	0.020	0.016	Intake	0.048
denovo847	Family Lachnospiraceae	0.001	0.003	0.002	0.001	0.001	Intake	0.049
denovo75529	Family Peptococcaceae	0.003	0.006	0.002	0.002	0.001	Gain	0.047
denovo84650	Family Peptococcaceae	0.002	0.008	0.002	0.003	0.001	Gain × intake	0.048
denovo34289	Family Ruminococcaceae	0.009	0.018	0.010	0.002	0.003	Gain	0.047
denovo1809	Family Ruminococcaceae	0.001	0.005	0.012	0.004	0.002	Gain	0.048
denovo36066	Family Ruminococcaceae	0.003	0.018	0.001	0.003	0.004	Gain	0.047
denovo69959	Family Ruminococcaceae	0.003	0.014	0.004	0.004	0.002	Gain × intake	0.049
denovo55195	Family Ruminococcaceae	0.015	0.067	0.008	0.014	0.011	Gain × intake	0.049
denovo40701	Family Ruminococcaceae	0.008	0.006	0.007	0.002	0.001	Gain × intake	0.047
denovo68434	Family Ruminococcaceae	0.010	0.028	0.005	0.011	0.006	Gain × intake	0.048
denovo54614	Family Ruminococcaceae	0.343	0.956	0.513	0.531	0.099	Intake	0.047
denovo47897	Family Ruminococcaceae	0.010	0.032	0.015	0.010	0.005	Intake	0.048
denovo14979	Family Ruminococcaceae	0.001	0.007	0.002	0.002	0.001	Intake	0.048
denovo21059	Family Ruminococcaceae	0.003	0.013	0.003	0.002	0.002	Intake	0.047
denovo10324	Family Ruminococcaceae	0.001	0.003	0.001	0.001	0.001	Intake	0.048
denovo56697	Family Ruminococcaceae	0.001	0.010	0.004	0.004	0.002	Intake	0.048
denovo83935	Family Ruminococcaceae	1.627	3.144	2.111	1.575	0.310	Intake	0.048
denovo79392	Family Ruminococcaceae	0.001	0.004	0.001	0.001	0.001	Intake	0.048
denovo45474	Order Bacteroidales	0.004	0.007	0.034	0.002	0.008	Intake	0.048
denovo61987	Order Clostridiales	0.004	0.009	0.002	0.003	0.001	Gain	0.048
denovo13244	Order Clostridiales	0.321	0.569	0.259	0.244	0.078	Gain	0.048
denovo2832	Order Clostridiales	0.008	0.016	0.005	0.004	0.002	Gain	0.048
denovo70326	Order Clostridiales	0.001	0.004	0.001	0.002	0.001	Gain	0.049
denovo77809	Order Clostridiales	0.003	0.002	0.006	0.003	0.001	Gain × intake	0.047
denovo41989	Order Clostridiales	0.002	0.007	0.002	0.003	0.001	Gain × intake	0.048
denovo25838	Order Clostridiales	0.001	0.005	0.002	0.002	0.001	Gain × intake	0.048
denovo3281	Order Clostridiales	0.002	0.007	0.004	0.004	0.001	Intake	0.047
denovo41594	Order Clostridiales	0.024	0.006	0.007	0.010	0.004	Intake	0.048
denovo70565	*Oscillospira*	0.001	0.001	0.010	0.003	0.002	Gain	0.048
denovo85505	*Oscillospira*	0.003	0.002	0.010	0.001	0.002	Gain × intake	0.048
denovo30472	*Oscillospira*	0.002	0.007	0.003	0.004	0.001	Gain × intake	0.047
denovo63139	*Prevotella*	0.107	0.023	0.021	0.021	0.015	Gain	0.049
denovo67544	*Ruminococcus*	0.091	0.022	0.039	0.022	0.018	Gain × intake	0.047
denovo56055	*Turicibacter*	0.001	0.004	0.001	0.001	0.001	Intake	0.048

[1]ID = OTU identifier.

[2]Data is shown as least squares means (n = 16/phenotype). $ADG_{Greater}-ADFI_{Greater}$ = 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.

[3]Differences among the groups are significant at $P < 0.05$.

functions, which may be integral toward the metabolic capacity of the host, providing energy from polysaccharides that other gut microorganisms cannot degrade (Biddle et al., 2013; Eren et al., 2015). Its increasing focus pertaining to metabolism and health across different hosts and ecological niches suggests the potential effect *Blautia* may have on the feed efficiency of cattle.

The examination of OTU within the cecal contents revealed many differences among the feed efficiency groups. The majority that were differentially abundant belonged to the families Ruminococcaceae, Clostridiaceae, and Lachnospiraceae; the genera *Prevotella*, *Parabacteroides*, *Oscillospira*, *Turicibacter*, and *Blautia*; and the species *Blautia producta*. Ruminococci are known to include members that are cellulolytic and amylolytic as well as active in acetate, formate, and hydrogen production (Biddle et al., 2013). In this regard, its fiber-digesting contribution is anticipated from bypass substrate from the rumen. Family Lachnospiraceae is partially responsible for much of the potential energy available to the host intestinal tissues. This family also includes important colonic genera, such as *Butyrivibrio*, *Pseudobutyrivibrio*, and *Blautia*. Known for its degradation and utilization of polysaccharides, *Prevotella* was also associated with differing feed efficiency phenotypes. *Prevotella* spp. are common inhabitants of the GIT and feces of cattle and generally are also active fermenters of peptides and AA (Mao et al., 2012; de Oliveira et al., 2013). *Oscillospira* are members of the family Ruminococcaceae and are commonly found in the rumen and large intestinal microbial communities of cattle (Malmuthuge and Griebel, 2014). In the rumen, *Oscillospira* species are positively correlated with forage-rich diets (Mackie et al., 2003), but their abundance in feces has also been documented as being lowest in animals fed forage-rich diets (Kim et al., 2014). It is apparent that the association of *Oscillospira* with feed efficiency in the cecum of cattle needs much more examination. *Turicibacter* has been detected in the GIT of several mammals, including humans and cattle (Cuiv et al., 2011; Kim et al., 2014). Little is known about *Turicibacter*, but isolates have been presumed to be pathogens (Rettedal et al., 2009). Due to potential effects on cattle health and/or performance, further evaluation of *Turicibacter* is warranted, particularly its association with feed efficiency. Finally, the identification of *Parabacteroides* and *Blautia* within the significant OTU among differing feed efficiency groups is in agreement with the significant taxa data discussed previously.

Gain and intake are primary components influencing feed efficiency. Therefore, examining the individual components is imperative to help understand the influence the significant taxa and OTU identified have on the overall model of feed efficiency. Taxa and OTU that were significantly different in abundance among feed efficiency groups were examined to determine whether the microbial populations differed individually by gain (ADG) or intake (ADFI) or if an interaction was observed with the microbial group. Most of the taxa and OTU were evenly distributed among ADG, ADFI, and their interaction. *Prevotella* tended to be associated with ADG. This may be, in part, due to its great abundance and activity in the rumen as well as throughout the lower GIT. Additionally, it may be a result of spillover from the rumen, where the rumen microbial populations are primarily associated with ADG due to its metabolic activity (Myer et al., 2015). Conversely, members of the family Ruminococcaceae tended to associate with ADFI, which may be related to its activity on substrates escaping the rumen.

Many of the taxa and OTU identified as associating with changes in feed efficiency in this study are related to the cellulolytic, fermentative, and metabolic activities in the cattle cecum, and they previously have been demonstrated as common components in cecal microbial communities (de Oliveira et al., 2013). Although no changes were observed from the α- and β-diversity analyses, significant differences were identified when examining the relative abundance of specific taxa and OTU. It must be noted that although the study suggests the cecum microbial community differs at the 16S level in cattle that vary in feed efficiency, it is not clear whether changes in the microbial community are contributing to differences in feed efficiency or host factors are driving changes in the microbial community.

Associations between the bovine ruminal microbial community and feed efficiency have been reported, but few have examined the role of the microbial community on feed efficiency within distal portions of the GIT. Notably, the phylogenetic divergence among the microbial communities within the rumen and cecum highlight the specific roles of these communities within their GIT section (Myer et al., 2015). At high coverage and depth, this study was able to identify specific and significant cecal microbial associations with feed efficiency, ADG, and ADFI. Importantly, these data contribute to a comprehensive understanding of the impact that variation in microbial communities within the GIT have on feed efficiency, complementing previous studies that focused exclusively on the rumen (Myer et al., 2015).

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