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Investigation of bacterial diversity in the feces of cattle fed different diets[1]

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ABSTRACT: The objective of this study is to investigate individual animal variation of bovine fecal microbiota including as affected by diets. Fecal samples were collected from 426 cattle fed 1 of 3 diets typically fed to feedlot cattle: 1) 143 steers fed finishing diet (83% dry-rolled corn, 13% corn silage, and 4% supplement), 2) 147 steers fed late growing diet (66% dry-rolled corn, 26% corn silage, and 8% supplement), and 3) 136 heifers fed early growing diet (70% corn silage and 30% alfalfa haylage). Bacterial 16S rRNA gene amplicons were determined from individual fecal samples using next-generation pyrosequencing technology. A total of 2,149,008 16S rRNA gene sequences from 333 cattle with at least 2,000 sequences were analyzed. Firmicutes and Bacteroidetes were dominant phyla in all fecal samples. At the genus level, *Oscillibacter*, *Turicibacter*, *Roseburia*, *Fecalibacterium*, *Coprococcus*, *Clostridium*, *Prevotella*, and *Succinivibrio* were represented by more than 1% of total sequences. However, numerous sequences could not be assigned to a known genus. Dominant unclassified groups were unclassified Ruminococcaceae and unclassified Lachnospiraceae that could be classified to a family but not to a genus. These dominant genera and unclassified groups differed ($P < 0.001$) with diets. A total of 176,692 operational taxonomic units (OTU) were identified in combination across all the 333 cattle. Only 2,359 OTU were shared across 3 diet groups. UniFrac analysis showed that bacterial communities in cattle feces were greatly affected by dietary differences. This study indicates that the community structure of fecal microbiota in cattle is greatly affected by diet, particularly between forage- and concentrate-based diets.

Key words: 16S ribosomal ribonucleic acid gene, diet, fecal microbiota, feedlot cattle, operational taxonomic units, pyrosequencing

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INTRODUCTION

The gastrointestinal tract serves as a habitat for a diverse and dynamic population of bacterial species that can affect growth, health, and well-being of the host. The fecal microbiota of cattle affects not only animal health but also food safety (Shanks et al., 2011). Therefore, a better understanding of the fecal microbiota structure would be important to reduce foodborne pathogens through dietary changes.

Traditional culture-based studies have allowed for the isolation of easy-to-grow bacterial strains that have contributed to underpinning metabolic functions of the fecal microbiota. However, isolates recovered from culture-based studies typically represent only a small portion of the total microbial population in an environment (Janssen, 2006).

The use of 16S rRNA gene (***rrs***) sequence data (Woese et al., 1983) has identified numerous unculturable microorganisms. Recently, an *rrs* amplification method that uses pyrosequencing was applied to compare the community structure of fecal microbiota in cattle that were fed different diets, indicating that diet greatly influences the fecal microbiota of cattle (Callaway et al., 2010; Shanks et al., 2011; Rice et al., 2012). In addition, the community structure of fecal microbiota was different among individual cattle fed the same diet (Dowd et al., 2008; Durso et al., 2010).

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Although these studies provided insight into the community structure of fecal microbiota using pyrosequencing, the number of samples analyzed in these studies was limited. Lack of power in observation groups prohibits an appreciation of sources of variation, and differences in targeted regions of the *rrs* genes sequenced complicate comparisons between studies. In the present study, 426 fecal samples from cattle fed 3 different diets were used to analyze the community structure of the fecal microbiota using pyrosequencing. We hypothesized that the community structure of the fecal microbiota would be highly variable across animals but influenced by diet.

MATERIALS AND METHODS

Animal Diets and Fecal Sample Collection

A total of 426 cattle were used to investigate the fecal microbiota composition in cattle fed different diets (Table 1). Fecal samples were collected from 426 cattle assigned to 1 of 3 diet groups as described previously (Wells et al., 2009): 1) 143 steers were fed a late growing diet, on a DM basis, consisting of 66.0% dry-rolled corn, 26.0% corn silage, 5.85% soybean meal, and 2.15% vitamin and mineral supplement with monensin (designated as "Moderate Grain"), 2) 147 steers were fed a finishing diet, on a DM basis, consisting of 82.75% dry-rolled corn, 12.75% corn silage, and 4.5% vitamin and mineral supplement with monensin (designated as "High Grain"), and 3) 136 heifers were fed an early growing diet, on a DM basis, consisting of 70% corn silage and 30% alfalfa haylage (designated as "Silage/Forage"). All cattle were adapted to the specified diets for 1 mo before fecal sampling. Fecal samples of cattle fed Moderate Grain were collected twice (July and August 2009). The 2 samples from each animal were subjected to pyrosequencing analysis separately, and the resultant 2 sequence datasets obtained from each animal were combined into 1 sequence dataset. Fecal samples from cattle fed High Grain or Silage/Forage were collected every 2 wk for a period of 10 wk between June and September 2010, and the 6 samples from each animal were pooled into 1 composite sample for pyrosequencing analysis.

Pyrosequencing

Total DNA was extracted from fecal samples using a Mini-Beadbeater-8 (BioSpec Products, Bartlesville, OK) and the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) as described previously (Martinez et al., 2010). Each DNA sample was amplified with universal primers 27F (5′-adaptor primer-AGAGTTTGATCMTGGCTCAG-3′) and 518R (5′-adaptor primer-barcode-ATTACCGCGGCTGCTGG-3′) targeting the V1 through V3 *rrs* region.

Table 1. Ingredient composition as percentage of diet (DM basis)[1]

Item	Late growing diet (Moderate Grain)	Finishing diet (High Grain)	Early growing diet (Silage/Forage)
Dry-rolled corn	66	82.75	–
Corn silage	26	12.75	70
Soybean meal	5.85	–	–
Alfalfa haylage	–	–	30
Supplement 1[2]	2.15	–	–
Supplement 2[3]	–	4.5	–

[1]All diets were formulated to exceed National Research Council recommendations for cattle (NRC, 1996).

[2]Custom supplement included (% DM) 62.55% limestone, 2.38% NaCl, 32.63% urea, 0.93% trace mineral mix (13% Ca, 12% Zn, 8% Mn, 10% Zn, 1.5% Cu, 0.2% I, and 0.1% Co), 0.56% vitamin mix (A 8,818,490 IU/kg, D 881,849 IU/kg, and E 882 IU/kg), and 0.95% Rumensin-80 (Elanco Animal Health, Indianapolis, IN).

[3]Commercial supplement (Biegert Feeds, Bradshaw, NE) included (% DM) 32% CP (28% NPN), 7.5% Ca, 0.8% P, 4.8% NaCl, 1.8% K, 55,116 IU/kg vitamin A, 105 IU/kg vitamin E, and 0.065% sodium monensin (Elanco Animal Health, Indianapolis, IN).

Equal amounts of amplicons were pooled and gel-purified using the QIAquick Gel Extraction Kit (Qiagen) and then sequenced using the 454 GS FLX Titanium system by the Core for Applied Genomics and Ecology (University of Nebraska).

Sequence Processing and Phylogenetic Analysis

All sequence processing and phylogenetic analysis were conducted using the programs in QIIME software package 1.4.0 (Caporaso et al., 2010b). After barcode and primer sequences were trimmed off, sequences that had read lengths shorter than 200 nucleotides, mean quality score below Q25, and homopolymer stretches longer than 8 nucleotides were removed. The remaining sequences were aligned using PyNAST against the Greengenes Core reference alignment (DeSantis et al., 2006; Caporaso et al., 2010a), and sequences that could not be aligned were removed. Chimeric sequences were checked using the ChimeraSlayer program (Haas et al., 2011). Fecal samples represented by less than 2,000 cleaned sequences were excluded from analysis. All cleaned sequences across the 3 diet groups were merged and then classified into taxa using the Ribosomal Database Project (**RDP**) naïve Bayesian rRNA Classifier 2.2 (Wang et al., 2007). Percentage of total sequences was used to compare taxa among the 3 diet groups. Operational taxonomic units (**OTU**) were calculated at 0.03 dissimilarity using the uclust method (Edgar, 2010). The number of OTU was normalized by randomly subsampling 2,000 sequences from each fecal sample. A phylogenetic tree was built using FastTree 2.1.3 (Price et al., 2010) to calculate α diversity and β diversity indices.

Table 2. Diversity statistics

Diet type	Sample type	No. of cleaned sequences	No. of observed OTU[1]	Maximum no. of OTU		Shannon diversity index
				Chao1	ACE	
Moderate Grain (n = 142)	Pooled sample	1,035,186	109,984	214,992	303,111	8.64
	Range of individual samples	3,132–11,702	1,432–5,048	4,048–20,171	7,165–46,185	6.33–7.67
	Range of individual subsamples[2]	2,000	1,184 ± 11[a]	5,734 ± 90[a]	13,929 ± 254[a]	6.53 ± 0.03[a]
High Grain (n = 130)	Pooled sample	877,232	67,469	167,616	278,239	7.01
	Range of individual samples	2,055–14,770	584–3,643	1,101–11,977	1,330–23,750	4.34–7.00
	Range of individual subsamples[2]	2,000	690 ± 12[c]	2,253 ± 94[c]	4,382 ± 265[c]	5.40 ± 0.03[c]
Silage/Forage (n = 61)	Pooled sample	236,590	35,341	101,211	183,344	7.49
	Range of individual samples	2,004–13,598	632–4,238	1,575–15,101	2,543–35,795	4.15–7.39
	Range of individual subsamples[2]	2,000	943 ± 17[b]	3,570 ± 138[b]	7,566 ± 387[b]	5.88 ± 0.05[b]

[a,b,c]Within a column, means for the range of individual subsamples having differing superscripts differ ($P < 0.05$).

[1]OTU = operational taxonomic units.

[2]Means among 3 diet groups were compared using ANOVA followed by the Tukey's test.

Statistical Analysis

This study was done to better understand the variation in bacterial community structure and composition among animals. As such, individual animal was used as the experimental unit. To characterize variation across diet cohort groups, observational differences in the experimental units were analyzed. The mean abundance of each taxon was compared among the 3 diet groups using 1-way ANOVA followed by the Tukey's test in SAS (SAS Inst. Inc., Cary, NC). Significant difference was determined at $P < 0.05$. Principal coordinates analysis (**PCoA**) was performed using both the weighted and the unweighted UniFrac methods (Lozupone and Knight, 2005).

RESULTS

Collective Data Summary

Of samples from all 426 animals, 333 samples represented by ≥2,000 cleaned *rrs* sequences were used for phylogenetic analysis. A total of 2,149,008 cleaned *rrs* sequences with 500-nucleotide average read length were obtained from the 333 samples composed of 142 Moderate Grain samples (1,035,186 sequences), 130 High Grain samples (877,232 sequences), and 61 Silage/Forage samples (236,590 sequences; Table 2). The number of cleaned *rrs* sequences in individual samples ranged from 2,004 to 14,770. The 2,149,008 *rrs* sequences were classified into 21 phyla, 40 classes, 71 orders, 152 families, and 434 genera. Firmicutes and Bacteroidetes represented 63.41 and 23.13% of all cleaned *rrs* sequences, respectively (Table 3). The rest of the phyla each represented less than 2% of all cleaned *rrs* sequences except for Proteobacteria (3.79%). Candidate division TM7 and Actinobacteria accounted for 1.56 and 1.17% of all cleaned *rrs* sequences, respectively. Acidobacteria, Chloroflexi, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Fibrobacteres, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospira, Planctomycetes, candidate division SR1, Spirochaetes, Synergistetes, Tenericutes, and Verrucomicrobia represented less than 1.00% of all cleaned *rrs* sequences. However, about 6% of all cleaned *rrs* sequences could not be classified into a known phylum and were found across all the 333 samples. Abundant genera that represented more than 0.50% of all the 2,149,008 *rrs* sequences were *Prevotella* (7.82%), *Oscillibacter* (5.21%), *Turicibacter* (4.47%), *Roseburia* (3.58%), *Fecalibacterium* (2.65%), *Coprococcus* (2.37%), *Succinivibrio* (2.36%), *Clostridium* (1.90%), *Lactobacillus* (0.89%), *Blautia* (0.81%), *Bacteroides* (0.72%), *Parabacteroides* (0.64%), and *Anaerovibrio* (0.56%).

A total of 176,692 OTU were calculated at a 0.03 dissimilarity cutoff in combination across all the 333 samples. The number of singletons was 98,526 and accounted for 56% of all the OTU. The number of OTU shared across the 3 diet groups was only 2,359 whereas the number of OTU specific to only 1 of the 3 diet groups was 145,299 that consisted of 77,444 (Moderate Grain), 36,788 (High Grain), and 28,717 (Silage/Forage), respectively (Fig. 1A). To compare OTU shared among the 3 diet groups, we normalized the 3 diet groups based on the smallest number of sequences in Silage/Forage (236,590 sequences × 3 dietary groups = 709,770 sequences; Fig. 1B). The number of shared OTU was greater between Moderate Grain and High Grain than between Silage/Forage and Moderate Grain (or High Grain; Fig. 1B). The community structure of the fecal microbiota in Silage/Forage appears to be distinct compared to Moderate Grain and High Grain.

Taxonomic Composition of the Fecal Microbiota

Taxonomic composition of the fecal microbiota among the 3 diet groups was compared based on the mean of the relative abundance (reads of a taxon/total reads in a sample) in the 3 diet groups as described previously (Benson et al.,

Table 3. Relative abundance of dominant taxa in 3 diet groups

Classification	Percentage of total sequences[1]				SEM	*P*-value[3]	No. of cattle with detectable taxon[4]
	Collective data[2]	Moderate Grain	High Grain	Silage/Forage			
Firmicutes	64.33	50.31^{c}	76.91^{a}	70.15^{b}	0.82	0.001	333
Oscillibacter	5.20	4.57^{b}	8.10^{a}	0.65^{c}	0.21	0.001	332
Turicibacter	4.62	1.26^{c}	8.40^{a}	4.37^{b}	0.29	0.001	333
Roseburia	3.39	3.94^{a}	4.21^{a}	0.38^{b}	0.11	0.001	327
Fecalibacterium	2.43	4.06^{a}	1.78^{b}	0.05^{c}	0.14	0.001	274
Coprococcus	2.33	2.37^{b}	2.94^{a}	0.95^{c}	0.07	0.001	330
Clostridium	1.86	1.13^{b}	2.92^{a}	1.29^{b}	0.07	0.001	333
Sporacetigenium	0.40	0.19^{c}	0.36^{b}	1.01^{a}	0.02	0.001	332
Blautia	0.78	0.22^{b}	1.73^{a}	0.06^{b}	0.06	0.001	294
Lactobacillus	0.72	0.27^{b}	1.50^{a}	0.13^{b}	0.09	0.001	315
Subdoligranulum	0.35	0.29^{b}	0.56^{a}	0.05^{c}	0.02	0.001	273
Microbacterium	0.35	0.05^{b}	0.54^{a}	0.63^{a}	0.04	0.001	188
Anaerovibrio	0.52	0.96^{a}	0.25^{b}	0.05^{c}	0.03	0.001	275
Anaerovorax	0.31	0.12^{c}	0.31^{b}	0.75^{a}	0.02	0.001	318
Ruminococcus	0.24	0.11^{b}	0.32^{a}	0.39^{a}	0.02	0.001	325
Unclassified Ruminococcaceae	15.19	8.12^{c}	14.63^{b}	32.86^{a}	0.59	0.001	333
Unclassified Lachnospiraceae	12.15	12.98^{a}	12.71^{a}	9.06^{b}	0.19	0.001	333
Unclassified Clostridiales	6.78	5.78^{c}	6.75^{b}	9.20^{a}	0.13	0.001	333
Unclassified Peptostreptococcaceae	2.57	0.68^{c}	4.40^{a}	3.07^{b}	0.14	0.001	333
Unclassified Erysipelotrichaceae	0.45	0.23^{c}	0.41^{b}	1.03^{a}	0.02	0.001	333
Unclassified Veillonellaceae	0.31	0.60^{a}	0.12^{b}	0.05^{b}	0.02	0.001	258
Unclassified Clostridia	0.39	0.20^{b}	0.57^{a}	0.45^{a}	0.02	0.001	332
Unclassified Clostridiaceae	0.40	0.48^{a}	0.31^{b}	0.39^{b}	0.01	0.001	330
Unclassified Firmicutes	1.16	0.90^{c}	1.06^{b}	1.96^{a}	0.03	0.001	333
Bacteroidetes	21.28	37.39^{a}	12.82^{b}	1.83^{c}	0.89	0.001	333
Prevotella	6.99	14.39^{a}	2.15^{b}	0.09^{c}	0.42	0.001	318
Bacteroides	0.72	0.97^{a}	0.77^{a}	0.06^{b}	0.05	0.001	277
Parabacteroides	0.61	0.88^{a}	0.59^{b}	0.05^{c}	0.03	0.001	274
Unclassified Prevotellaceae	5.89	10.69^{a}	3.37^{b}	0.07^{c}	0.29	0.001	301
Unclassified Porphyromonadaceae	2.23	2.84^{a}	2.55^{a}	0.12^{b}	0.11	0.001	301
Unclassified Bacteroidales	1.95	3.44^{a}	1.09^{b}	0.32^{c}	0.09	0.001	323
Unclassified Bacteroidetes	2.67	3.81^{a}	2.15^{b}	1.16^{c}	0.13	0.001	331
Proteobacteria	3.53	6.04^{a}	1.30^{b}	2.43^{b}	0.21	0.001	333
Succinivibrio	2.08	4.46^{a}	0.43^{b}	0.05^{b}	0.14	0.001	267
Pantoea	0.28	0.58^{a}	0.05^{b}	0.05^{b}	0.03	0.001	111
TM7	2.32	0.24^{b}	0.56^{b}	10.92^{a}	0.24	0.001	329
TM7 genera *incertae sedis*	2.32	0.24^{b}	0.56^{b}	10.92^{a}	0.24	0.001	329
Actinobacteria	1.28	0.14^{c}	1.77^{b}	2.93^{a}	0.11	0.001	330
Propionibacterium	0.18	0.05^{b}	0.08^{b}	0.68^{a}	0.04	0.001	123
Unclassified Coriobacteriaceae	0.20	0.06^{c}	0.22^{b}	0.51^{a}	0.01	0.001	269
Cyanobacteria	0.54	1.14^{a}	0.09^{b}	0.09^{b}	0.06	0.001	189
Streptophyta	0.54	1.14^{a}	0.09^{b}	0.09^{b}	0.06	0.001	189
Verrucomicrobia	0.27	0.06^{b}	0.10^{b}	1.15^{a}	0.04	0.001	123
Akkermansia	0.27	0.06^{b}	0.10^{b}	1.14^{a}	0.04	0.001	111
Unclassified Bacteria	6.22	4.51^{c}	6.28^{b}	10.07^{a}	0.17	0.001	333

^{a,b,c}Within a row, means with a different subscript were different ($P < 0.05$).

[1]Values indicate means.

[2]Sequences obtained from all 333 fecal samples.

[3]A *P*-value of 0.001 is less than or equal to 0.001.

[4]Percentage of total sequences for cattle with nondetectable taxon was treated as 0.05%.

2010). Firmicutes was the first dominant phylum in all the 3 diet groups as shown in collective data. The abundance of Firmicutes was different ($P < 0.001$) among the 3 diet groups with the greatest percentage (76.9%) in High Grain and the lowest percentage (50.3%) in Moderate Grain (Table 3). Only 13 genera within Firmicutes represented ≥0.5% of all the sequences in at least 1 diet group. The abundance of *Oscillibacter*, *Turicibacter*, *Coprococcus*, *Clostridium*, *Blautia*, *Lactobacillus*, and *Subdoligranulum* was different ($P < 0.001$) among the 3 diet groups with the High Grain diet having the greatest abundance. The abundance of *Roseburia* was greater ($P < 0.001$) in High

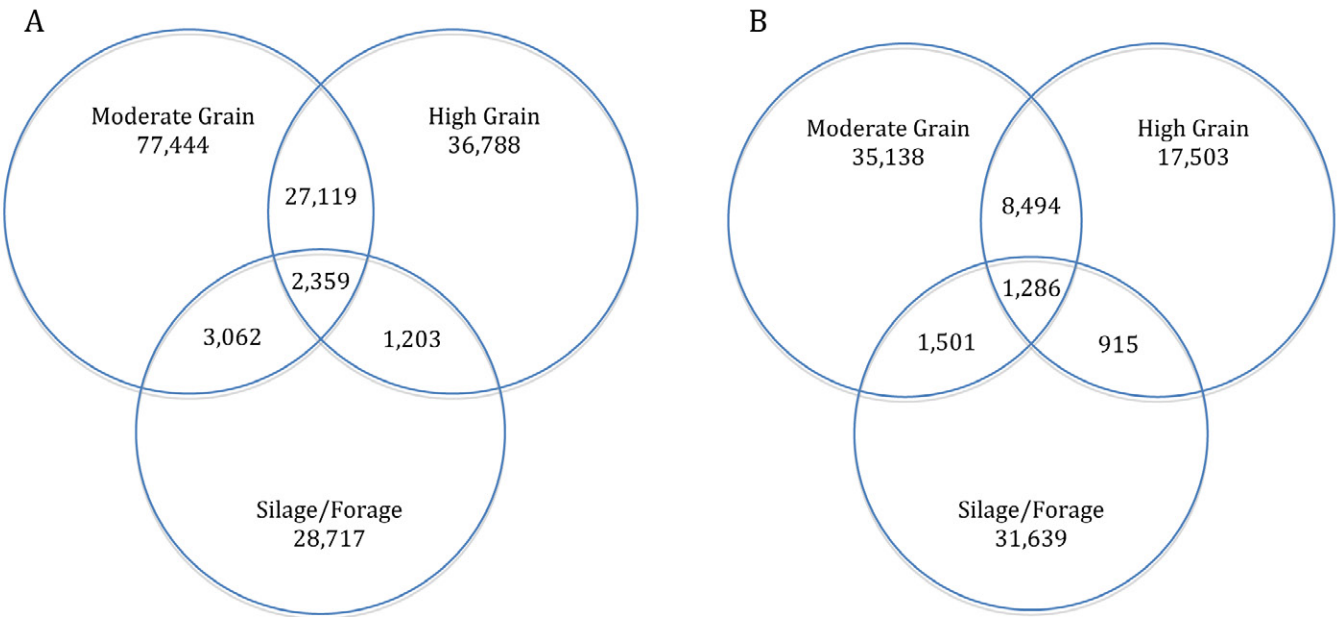

Figure 1. Venn diagrams showing the number of observed operational taxonomic units (OTU) shared across 3 diet groups. A) Only 2,359 OTU were shared in combination across 3 diet groups, and each group was represented by numerous unique OTU. B) The number of observed OTU was normalized based on the smallest number of sequences in Silage/Forage. The number of shared OTU was lower between Silage/Forage and Moderate Grain (or High Grain) than between Moderate Grain and High Grain. See online version for figure in color.

Grain than in Silage/Forage but not different from Moderate Grain. The abundance of *Microbacterium* was greater ($P < 0.001$) in High Grain than in Moderate Grain but not different from Silage/Forage. The abundance of *Fecalibacterium* and *Anaerovibrio* was different ($P < 0.001$) among the 3 diet groups with the Moderate Grain diet having the greatest abundance. The abundance of *Sporacetigenium* and *Anaerovorax* was different ($P < 0.001$) among the 3 diet groups with the Silage/Forage diet having the greatest abundance. However, most of the Firmicutes sequences could not be classified into a known genus in all 3 groups. Three dominant unclassified groups in collective data were unclassified Ruminococcaceae (15.19%), unclassified Lachnospiraceae (12.15%), and unclassified Clostridiales (6.78%) as shown in the composition of rumen microbiota (Kim et al., 2011). These 3 unclassified groups represented approximately 50% of all sequences in Silage/Forage. The abundance of unclassified Ruminococcaceae and unclassified Clostridiales was different ($P < 0.001$) among the 3 diet groups with the Silage/Forage diet having the greatest abundance whereas that of unclassified Lachnospiraceae was lower ($P < 0.001$) in Silage/Forage than in the other 2 groups. The rest of unclassified groups did not represent more than 1.20% of all sequences in collective data.

Bacteroidetes was the second dominant phylum in both Moderate Grain and High Grain whereas it was the fourth dominant in Silage/Forage and represented only 1.83% of all the sequences (Table 3). In Silage/Forage, Firmicutes was the first dominant phylum followed by TM7 and Proteobacteria. *Prevotella* was the most dominant among all known genera and represented 6.99% of all sequences in collective data. The abundance of *Prevotella* was different ($P < 0.001$) among the 3 diet groups and the greatest (14.46%) in Moderate Grain. The rest of the known genera within Bacteroidetes did not represent ≥0.5% of all sequences except for *Bacteroides* and *Parabacteroides*, representing 0.7 and 0.6% of all sequences, respectively. Both *Bacteroides* and *Parabacteroides* were lower in Silage/Forage than in the other 2 groups. Many Bacteroidetes sequences also could not be classified into a known genus, and unclassified Prevotellaceae was the first dominant group among unclassified groups. Unclassified Prevotellaceae, unclassified Bacteroidales, and unclassified Bacteroidetes were different ($P < 0.001$) among the 3 diet groups and the greatest in Moderate Grain with the Silage/Forage diet having the lowest abundance.

Of 19 minor phyla, only Proteobacteria, TM7, Actinobacteria, Cyanobacteria, and Verrucomicrobia represented ≥0.5% in at least 1 diet group (Table 3). The abundance of Proteobacteria and Cyanobacteria was greater ($P < 0.001$) in Moderate Grain than in the other 2 groups whereas the abundance of TM7, Actinobacteria, and Verrucomicrobia was greater ($P < 0.001$) in Silage/Forage than in the other 2 groups. At the genus level, the abundance of *Succinivibrio* and *Streptophyta* were greater ($P < 0.001$) in Moderate Grain than in the other 2 groups whereas the abundance of *Akkermansia* was greater ($P < 0.001$) in Silage/Forage than in the other 2 groups.

Analysis of Bacterial Diversity

The 333 individual subsamples with 2,000 sequences (333 × 2,000 = 666,000) were obtained to compare diversity of the fecal microbiota among the 3 diet groups. We calculated species-level OTU at a 0.03 dissimilarity cutoff from the 333 subsamples to estimate diversity of the fecal microbiota in the 3 diet groups. We identified a total of 90,386 OTU in combination across all the 333 subsamples. Fifty-six of the 90,386 OTU represented ≥0.5% of all sequences in at least 1 diet group, and 33 of the 56 OTU could not be classified to a known genus (Table 4).

One OTU (OTU-14605) classified to *Turicibacter* was the most dominant among the 56 OTU in collective data, and its abundance was the greatest ($P < 0.001$) in High Grain and the lowest ($P < 0.001$) in Moderate Grain (Table 4). Operational taxonomic unit-13387 (*Fecalibacterium*) and OTU-14977 (*Succinivibrio*) were the most abundant ($P < 0.001$) in Moderate Grain while OTU-7731 (*Clostridium*) was the most abundant ($P < 0.001$) in High Grain. These 3 OTU were also dominant and accounted for >1.0% of total sequences in collective data. Five OTU (from OTU-13994 to OTU-13998) assigned to *Oscillibacter* were more abundant ($P < 0.001$) in High Grain than in the other 2 groups and rarely detected in Silage/Forage. Three *Prevotella* OTU (from OTU-5060 to OTU-5062) were more abundant ($P < 0.001$) in Moderate Grain than in the other 2 groups and rarely detected in Silage/Forage. *Coprococcus* was represented by 3 OTU (from OTU-9760 to OTU-9762), and their abundances were different ($P < 0.001$) among the 3 diet groups. Operational taxonomic unit-9760, OTU-9761, and OTU-9762 were the most abundant in Silage/Forage, Moderate Grain, and High Grain, respectively. Operational taxonomic unit-5500 (*Lactobacillus*) and OTU-10257 (*Roseburia*) were the most abundant ($P < 0.001$) in High Grain and did not differ ($P > 0.05$) between Moderate Grain and Silage/Forage. Operational taxonomic unit-1194 (*Microbacterium*) was rarely detected in Moderate Grain while OTU-5135 (*Streptophyta*) and OTU-15034 (*Pantoea*) was rarely detected in High Grain and Silage/Forage.

Of the 33 OTU unclassified at the genus level, 10 OTU were assigned to unclassified Ruminococcaceae, and OTU-12973 of the 10 OTU accounted for 1.5% of total sequences in collective data (Table 4). Five of the 10 OTU were the most abundant ($P < 0.001$) in High Grain while another 5 of the 10 OTU were the most abundant ($P < 0.001$) in Silage/Forage. Unclassified Lachnospiraceae was represented by 7 of the 33 OTU, and OTU-9531 of the 7 OTU accounted for 1.65% of total sequences in collective data. Three of the 7 OTU were the most abundant ($P < 0.001$) in High Grain while another 3 of the 7 OTU were the most abundant ($P < 0.001$) in Silage/Forage. The remaining 1 OTU was the most abundant ($P < 0.001$) in Moderate Grain. Unclassified Prevotellaceae was represented by 3 of the 33 OTU, and 2 of the 3 OTU were the most abundant ($P < 0.001$) in Moderate Grain and rarely detected in Silage/Forage. The remaining 1 OTU was the most abundant ($P < 0.001$) in High Grain and rarely detected in Moderate Grain and Silage/Forage. Unclassified Bacteroidetes was represented by 3 of the 33 OTU, and 2 of the 33 OTU were rarely detected in Silage/Forage. The remaining 1 OTU was the most abundant ($P < 0.001$) in Silage/Forage and rarely detected in Moderate Grain and High Grain. The TM7 genera *incertae sedis* was represented by 2 OTU that were the most abundant ($P < 0.001$) in Silage/Forage and rarely detected in Moderate Grain and High Grain. The abundances of 2 OTU classified to unclassified Porphyromonadaceae were the lowest ($P < 0.001$) in Silage/Forage while those of 2 OTU classified to unclassified Peptostreptococcaceae were the lowest ($P < 0.001$) in Moderate Grain. Unclassified Clostridiales was represented by 1 OTU that was the most abundant ($P < 0.001$) in Silage/Forage. Unclassified Bacteria were represented by 3 of the 33 OTU, and 2 of the 3 OTU were more abundant ($P < 0.001$) in High Grain than in the other 2 groups. The remaining 1 OTU was the most abundant ($P < 0.001$) in Silage/Forage and rarely detected in Moderate Grain and High Grain.

Observed OTU, Chao1, ACE, and Shannon's diversity index were calculated from the 333 subsamples, and then their average values were compared among 3 diet groups. All these indices differed ($P < 0.001$) among the 3 diet groups and were the greatest in Moderate Grain and the lowest in High Grain (Table 2). These results indicate that bacterial diversity in the feces of cattle was the greatest in Moderate Grain but the lowest in High Grain. We also examined correlations among the 333 subsamples using UniFrac PCoA (Fig. 2). In both weighted and unweighted UniFrac, principal coordinate (**PC**) 1 separated bacterial communities based on diet. Unweighted UniFrac showed that spots of Silage/Forage were phylogenetically distinct from those of Moderate Grain and High Grain. Spots were more divergent in High Grain than in the other 2 groups, indicating that bacterial communities among individual animals are less similar in High Grain than in the other 2 groups.

DISCUSSION

Great diversity of the bovine fecal microbiota may be attributed to various factors such as diet, age, and geographic region. Large variation in fecal microbiota was observed among the 3 diet groups. While this project was not specifically designed to experimentally test the role of the diet on bacterial community composition, the observed differences among the groups were strik-

Table 4. Dominant operational taxonomic units (OTU) calculated from 333 subsamples in 3 diet groups

No. OTU ID[2]	Classification	Percentage of total sequences[1]				SEM	*P*-value[4]	No. of cattle with detectable taxon[5]
		Collective data[3]	Moderate Grain	High Grain	Silage/Forage			
OTU-1153	Unclassified Bacteria	0.14	0.05^{b}	0.05^{b}	0.54^{a}	0.02	0.001	96
OTU-1157	Unclassified Bacteria	0.36	0.08^{b}	0.81^{a}	0.05^{b}	0.06	0.001	192
OTU-1158	Unclassified Bacteria	0.44	0.15^{b}	0.93^{a}	0.05^{b}	0.04	0.001	263
OTU-1209	*Propionibacterium*	0.15	0.05^{b}	0.07^{b}	0.55^{a}	0.04	0.001	98
OTU-1646	Unclassified Bacteroidetes	0.15	0.05^{b}	0.05^{b}	0.59^{a}	0.06	0.001	29
OTU-1648	Unclassified Bacteroidetes	0.51	0.65^{a}	0.59^{a}	0.05^{b}	0.03	0.001	268
OTU-1649	Unclassified Bacteroidetes	0.55	0.86^{a}	0.44^{b}	0.05^{c}	0.05	0.001	256
OTU-1194	*Microbacterium*	0.34	0.05^{b}	0.54^{a}	0.62^{a}	0.04	0.001	181
OTU-2601	Unclassified Porphyromonadaceae	0.29	0.20^{b}	0.52^{a}	0.05^{b}	0.03	0.001	234
OTU-2602	Unclassified Porphyromonadaceae	0.39	0.54^{a}	0.38^{b}	0.05^{c}	0.02	0.001	257
OTU-3869	Unclassified Prevotellaceae	0.37	0.72^{a}	0.15^{b}	0.05^{b}	0.03	0.001	238
OTU-3870	Unclassified Prevotellaceae	0.39	0.05^{b}	0.93^{a}	0.05^{b}	0.07	0.001	97
OTU-3872	Unclassified Prevotellaceae	0.65	1.39^{a}	0.12^{b}	0.05^{b}	0.05	0.001	227
OTU-5060	*Prevotella*	0.35	0.70^{a}	0.09^{b}	0.05^{b}	0.02	0.001	221
OTU-5061	*Prevotella*	0.39	0.77^{a}	0.13^{b}	0.05^{b}	0.03	0.001	247
OTU-5062	*Prevotella*	0.68	1.33^{a}	0.27^{b}	0.05^{b}	0.05	0.001	258
OTU-5135	*Streptophyta*	0.49	1.04^{a}	0.08^{b}	0.08^{b}	0.06	0.001	171
OTU-5500	*Lactobacillus*	0.57	0.19^{b}	1.22^{a}	0.08^{b}	0.08	0.001	296
OTU-7122	Unclassified Clostridiales	0.26	0.07^{c}	0.16^{b}	0.90^{a}	0.02	0.001	320
OTU-7331	*Clostridium*	1.12	0.45^{b}	2.19^{a}	0.39^{b}	0.06	0.001	331
OTU-9520	Unclassified Lachnospiraceae	0.15	0.05^{b}	0.06^{b}	0.57^{a}	0.02	0.001	71
OTU-9522	Unclassified Lachnospiraceae	0.16	0.05^{b}	0.05^{b}	0.64^{a}	0.02	0.001	117
OTU-9527	Unclassified Lachnospiraceae	0.30	0.19^{b}	0.52^{a}	0.11^{b}	0.03	0.001	306
OTU-9528	Unclassified Lachnospiraceae	0.36	0.29^{b}	0.57^{a}	0.05^{c}	0.03	0.001	259
OTU-9529	Unclassified Lachnospiraceae	0.40	0.05^{b}	0.06^{b}	1.94^{a}	0.05	0.001	134
OTU-9530	Unclassified Lachnospiraceae	0.43	0.75^{a}	0.26^{b}	0.05^{c}	0.02	0.001	263
OTU-9531	Unclassified Lachnospiraceae	1.65	0.86^{b}	3.25^{a}	0.05^{c}	0.12	0.001	274
OTU-9760	*Coprococcus*	0.15	0.05^{b}	0.06^{b}	0.58^{a}	0.02	0.001	106
OTU-9761	*Coprococcus*	0.36	0.58^{a}	0.27^{b}	0.05^{c}	0.02	0.001	265
OTU-9762	*Coprococcus*	1.10	0.89^{b}	1.82^{a}	0.05^{c}	0.06	0.001	272
OTU-10257	*Roseburia*	0.36	0.11^{b}	0.77^{a}	0.05^{b}	0.04	0.001	263
OTU-10377	Unclassified Peptostreptococcaceae	0.28	0.09^{c}	0.35^{b}	0.55^{a}	0.02	0.001	330
OTU-10378	Unclassified Peptostreptococcaceae	1.88	0.39^{c}	3.79^{a}	1.29^{b}	0.13	0.001	333
OTU-12960	Unclassified Ruminococcaceae	0.19	0.05^{b}	0.05^{b}	0.82^{a}	0.02	0.001	61
OTU-12962	Unclassified Ruminococcaceae	0.23	0.06^{b}	0.10^{b}	0.92^{a}	0.03	0.001	201
OTU-12965	Unclassified Ruminococcaceae	0.25	0.17^{b}	0.13^{b}	0.67^{a}	0.02	0.001	289
OTU-12966	Unclassified Ruminococcaceae	0.29	0.07^{b}	0.63^{a}	0.05^{b}	0.02	0.001	248
OTU-12968	Unclassified Ruminococcaceae	0.35	0.09^{b}	0.78^{a}	0.05^{b}	0.03	0.001	264
OTU-12969	Unclassified Ruminococcaceae	0.54	0.06^{b}	0.07^{b}	2.69^{a}	0.07	0.001	158
OTU-12970	Unclassified Ruminococcaceae	0.53	0.09^{b}	1.23^{a}	0.05^{b}	0.05	0.001	249
OTU-12971	Unclassified Ruminococcaceae	0.60	0.20^{b}	1.25^{a}	0.16^{b}	0.04	0.001	327
OTU-12972	Unclassified Ruminococcaceae	0.61	0.67^{b}	0.82^{a}	0.05^{c}	0.03	0.001	271
OTU-12973	Unclassified Ruminococcaceae	1.50	0.05^{b}	0.12^{b}	7.81^{a}	0.21	0.001	159
OTU-13387	*Fecalibacterium*	1.02	1.38^{a}	1.09^{b}	0.05^{c}	0.06	0.001	274
OTU-13994	*Oscillibacter*	0.27	0.15^{b}	0.52^{a}	0.05^{c}	0.02	0.001	270
OTU-13995	*Oscillibacter*	0.33	0.14^{b}	0.66^{a}	0.05^{b}	0.03	0.001	267
OTU-13996	*Oscillibacter*	0.36	0.33^{b}	0.54^{a}	0.05^{c}	0.02	0.001	266
OTU-13997	*Oscillibacter*	0.44	0.16^{b}	0.91^{a}	0.05^{b}	0.03	0.001	267
OTU-13998	*Oscillibacter*	1.00	0.29^{b}	2.23^{a}	0.05^{b}	0.08	0.001	271
OTU-14605	*Turicibacter*	3.56	0.71^{c}	6.72^{a}	3.44^{b}	0.24	0.001	333
OTU-14737	*Helicobacter*	0.26	0.05^{b}	0.05^{b}	1.18^{a}	0.14	0.001	19
OTU-14977	*Succinivibrio*	1.26	2.60^{a}	0.37^{b}	0.05^{b}	0.08	0.001	264
OTU-15034	*Pantoea*	0.24	0.50^{a}	0.05^{b}	0.05^{b}	0.03	0.001	108
OTU-15452	TM7 genera *incertae sedis*	0.20	0.05^{b}	0.08^{b}	0.81^{a}	0.02	0.001	171
OTU-15453	TM7 genera *incertae sedis*	0.22	0.05^{b}	0.05^{b}	0.94^{a}	0.02	0.001	131
OTU-15486	*Akkermansia*	0.17	0.05^{b}	0.10^{b}	0.60^{a}	0.03	0.001	84

[a,b,c]Within a row, means with a different subscript were different ($P < 0.05$).

[1]Values represent means.

[2] A total of 176,692 OTU were numbered in serial order.

[3]Sequences obtained from all 333 fecal samples.

[4]A *P*-value of 0.001 is less than or equal to 0.001.

[5]Percentage of total sequences for cattle with nondetectable taxon was treated as 0.05%.

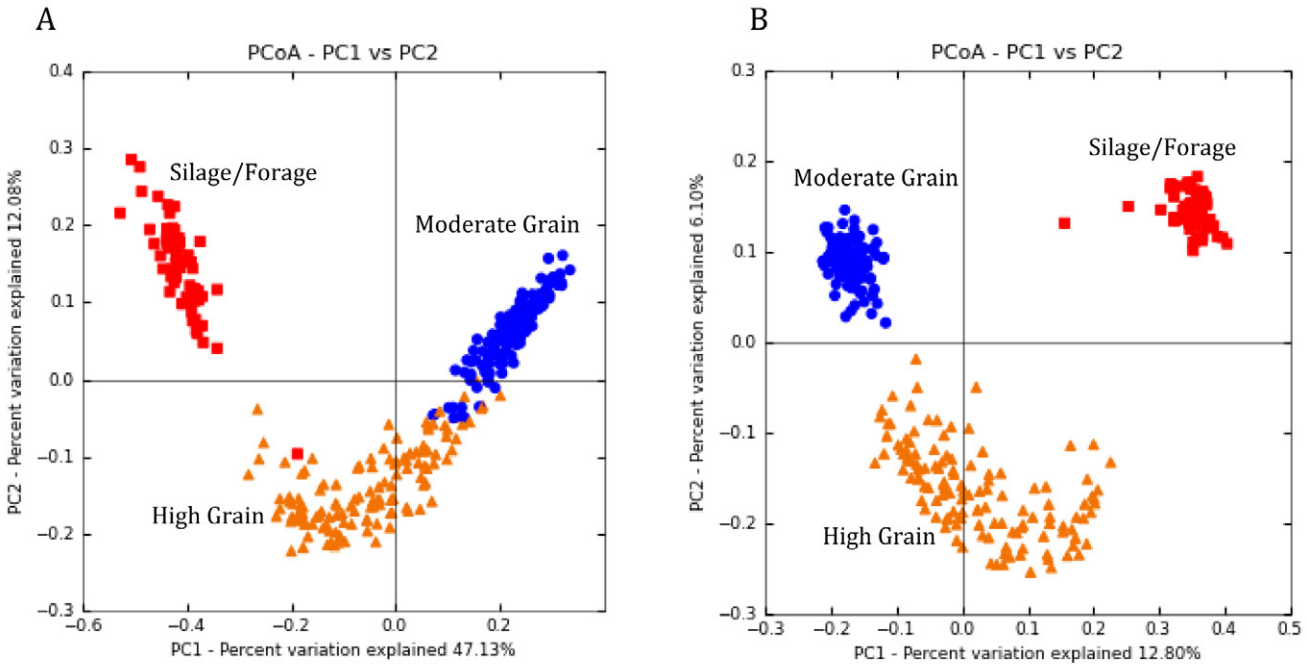

Figure 2. UniFrac principal coordinates analysis (PCoA) showing correlations among 3 diet groups. A) Weighted PCoA analyzed from 333 subsamples with 2,000 reads. B) Unweighted PCoA analyzed from 333 subsamples with 2,000 reads. PC = principal coordinate. See online version for figure in color.

ing. As an observational study, the ANOVA methods and resulting statistical comparisons are not a test of an experimental null hypothesis regarding treatment differences; rather, they are presented as a tool to interpret the data. This is the first study of this magnitude of which we are aware that clearly demonstrates bacterial similarities within and compositional differences between groups of animals. Gender and year of study are additional compounding factors across these groups that could play a minor role in affecting bacterial community composition. Bacterial diversity was greater in Moderate Grain than in High Grain but not different among pens (data not shown). The diet composed of mostly dry-rolled corn appears to reduce bacterial diversity. Nonetheless, for all diets, Firmicutes was the first dominant phylum accounting for >50% of the total sequences. The dominance of Firmicutes corroborates previous studies (Durso et al., 2010; Shanks et al., 2011; Rice et al., 2012), indicating that this phylum is a resident member of the bovine feces irrespective of the factors affecting diversity of the bovine fecal microbiota. The present study indicates that diet is an important factor affecting diversity of the bovine fecal microbiota relative to other factors.

Durso et al. (2012) indicated that *Prevotella* and *Bacteroides* were commonly found in cattle feces and associated with dietary differences. *Prevotella* was more abundant in cattle fed a corn-based finishing diet than in cattle fed the diet including 40% wet distillers' grain with solubles (Durso et al., 2012). In the present study, *Prevotella* was the most abundant in cattle fed moderate grain with 66% dry-rolled corn. Although it seems that corn-based diets positively affect the abundance of *Prevotella* (Durso et al., 2012; Rice et al., 2012), *Prevotella* abundance was reduced in cattle fed High Grain with the greater level of dry-rolled corn (82.75%) compared to cattle fed the 66% dry-rolled corn. Both *Prevotella* and *Bacteroides* were rarely detected in cattle fed Silage/Forage (70% corn silage and 30% alfalfa haylage). Durso et al. (2012) observed an increase in abundance for *Bacteroides* when diets high in fat were fed, and in the present study, *Bacteroides* abundance seems to be negatively correlated with the diet rich in forage.

Oscillibacter was the most dominant genus in the phylum Firmicutes and the most abundant in cattle fed high grain (Table 3). *Oscillibacter* seems to be positively correlated with starch content. In humans, *Oscillibacter* increased in the diet of resistant starch (Walker et al., 2011). Cattle fed high starch diets can have high bypass starch from rumen (Wells et al., 2009) and *Oscillibacter* in the feces of cattle may be associated with the high levels of bypass starch. In the RDP database, *rrs* sequences recovered from ruminal *Oscillospira* were classified to *Oscillibacter*. Ruminal *Oscillospira* are positively correlated with diets rich in forage (Mackie et al., 2003), but the abundance of fecal *Oscillibacter* in the current study was the lowest in animals fed the diet rich in forage. Therefore, *Oscillibacter* present in the feces of cattle might not be represented by *rrs* sequences recovered from *Oscillospira* found in the rumen. *Turicibacter* also was a dominant genus as shown in mammals including

humans (Cuiv et al., 2011), and its isolate strain was presumed to be a pathogen (Rettedal et al., 2009). Because *Turicibacter* increased in the diet rich in dry-rolled corn, potential negative affects on cattle health or performance need to be evaluated. *Sporacetigenium* and *Anaerovorax* were abundant in the feces of cattle fed 40% wet distillers' grain with solubles compared to the corn-based finishing diet (Durso et al., 2012). *Sporacetigenium* ferments pentoses (Chen et al., 2006) and is likely abundant in Silage/Forage because forage diets are sources for hemicellulose that are rich in pentoses (Durso et al., 2012). The type strain of *Anaerovorax* ferments putrescine produced from amino acids and proteins (Matthies et al., 2000). *Anaerovorax* is likely abundant in Silage/Forage because the Silage/Forage diet includes the protein-rich alfalfa (Durso et al., 2012).

Roseburia, *Fecalibacterium*, and *Coprococcus* may contribute to producing butyrate that is used as the energy source for the mucosa (Pryde et al., 2002), and these 3 genera seem to be reduced by the diet rich in forage (Table 3). *Anaerovibrio* has been associated with lipid degradation (Henderson, 1971) and was abundant in cattle fed dry-rolled corn but rarely detected in cattle fed the diet rich in forage. *Blautia* can contribute to lactate and acetate production as described previously (Park et al., 2012) and was rarely found in cattle fed the diet rich in forage. Mucin-degrading *Akkermansia* (Derrien et al., 2004) was abundant in animals fed the diet rich in forage. The diet rich in forage may result in degradation of the protective mucus layer by *Akkermansia* in cattle.

Because *rrs* sequences that were recovered from the family Chloroplast originate from plant cells, Streptophyta placed within Chloroplast would be a taxon group present in consumed forage diets and was expected to not only be detected but also have a possible positive relationship with the amount of corn silage in the diet. Cattle fed the Moderate Grain diet, which had corn silage added to the diet, had abundant levels of this taxon group. However, the abundance of Streptophyta was lower in Silage/Forage (70% corn silage) than in Moderate Grain (26% corn silage). Factors affecting the abundance of Streptophyta in cattle feces will need to be elucidated in future studies.

Core taxa have been used to understand microbial ecology and hypothesize their function within a habitat (Shade and Handelsman, 2012). Although collective sequences were assigned to numerous taxa, only a small number of core taxa, which are 3 phyla, 3 classes, 2 orders, 6 families, and 2 genera, were detected across all the 333 cattle. Firmicutes and Bacteroidetes were major core phyla while Proteobacteria was a minor core phylum as described previously (Shanks et al., 2011; Rice et al., 2012). Only *Clostridium* and *Turicibacter* were identified as core taxa across all the 333 cattle. *Prevotella*, *Oscillibacter*, *Roseburia*, *Coprococcus*, *Lactobacillus*, *Anaerovorax*, *Sporacetigenium*, and *Ruminococcus* were detected across ≥95% of all the 333 cattle (Table 3). These 8 genera also are thought to be core taxa. A total of 10 core genera seem to be commonly found in cattle feces irrelevant to diets. These core genera might play a more central role in the fecal microbial ecosystem.

Core taxa for the 272 steers fed feedlot concentrate diets with Moderate Grain or High Grain diets were 3 phyla, 5 classes, 3 orders, 7 families, and 7 genera. The 7 genera were *Trucibacter*, *Subdoligranulum*, *Oscillibacter*, *Fecalibacterium*, *Roseburia*, *Coprococcus*, and *Clostridium* and were detected across all the 272 steers. In addition, *Sporacetigenium*, *Blautia*, *Prevotella*, *Coprobacillus*, *Lactobacillus*, *Bacteroides*, *Parabacteroides*, *Anaerovibrio*, *Ruminococcus*, and *Succinivibrio* were detected across ≥95% of all the 272 steers and appear to be core taxa. These 17 genera might play an important role in the fecal microbial ecosystem of cattle fed feedlot concentrate diets. This result is supported by the previous study (Durso et al., 2010) indicating that *Fecalibacterium*, *Ruminococcus*, *Roseburia*, *Clostridium*, *Prevotella*, and *Bacteroides* were core taxa for 6 cattle fed diet including 21% corn. Because Durso et al. (2010) used small numbers of sequences (11,171 sequences) for microbial analysis, the other genera seem not to be identified as core taxa.

Only 8 OTU were observed across ≥95% of all the 333 cattle and were composed of 2 *Clostridium*, 1 *Turicibacter*, 2 unclassified Clostridiales, 2 unclassified Peptostreptococcaceae, and 1 unclassified Ruminococcaceae. Species associated with these 8 core OTU might greatly contribute to the fecal microbial ecosystem irrespective of diet. Nine OTU including 6 of the 8 core OTU were observed across ≥95% of the 61 cattle fed Silage/Forage and were composed of 3 *Clostridium*, 1 *Turicibacter*, 1 Clostridiales, 3 Peptostreptococcaceae, and 1 Ruminococcaceae. Species associated with these 9 OTU might play an important role in the fecal microbial ecosystem of cattle fed Silage/Forage. Thirty-three OTU including the 8 core OTU were observed across ≥95% of the 272 cattle fed concentrate diets and were composed of 2 *Clostridium*, 2 *Coprococcus*, 5 *Roseburia*, 6 *Oscillibacter*, 1 *Turicibacter*, 1 *Fecalibacterium*, 1 *Succinivibrio*, 1 unclassified Bacteria, 1 unclassified Bacteroidetes, 2 unclassified Clostridiales, 5 unclassified Ruminococcaceae, 4 unclassified Lachnospiraceae, and 2 unclassified Peptostreptococcaceae. Species associated with these 33 OTU might play an important role in the fecal microbial ecosystem of cattle fed concentrated diets.

Numerous sequences could not be classified into a known genus. Dominant unclassified groups were unclassified Ruminococcaceae and unclassified Lachnospiraceae (Table 3). Most of the dominant OTU also were

assigned to these 2 unclassified groups. The dominance of OTU assigned to these unclassified groups will need to be identified using quantitative real-time PCR as described previously (Stiverson et al., 2011). These 2 unclassified groups also were dominant in the rumen (Kim et al., 2011), and some strains in cattle feces may have originated from the rumen. This assumption is supported by the study by Durso et al. (2013), where some OTU assigned to these 2 unclassified groups were shared between the rumen microbiota and the fecal microbiota. The OTU shared between the rumen microbiota and the fecal microbiota within the same cattle will need to be examined in future studies. Species associated with these dominant OTU might greatly affect animal health and meat safety, and isolation and characterization of these strains will need to be conducted to elucidate their function in the cattle gastrointestinal microbial ecosystem.

Hierarchical taxa classification based on naïve Bayesian rRNA Classifier (Wang et al., 2007) seems to be unreliable for some taxa. To minimize this issue, we searched for sequences recovered from isolates in the RDP database (release 10, update 29). *Roseburia* included 2 sequences recovered from *Butyrivibrio fibrisolvens* isolated from a Greenland ice sample (Sheridan et al., 2003) and the rumen (Wallace et al., 2006). The dominance of *Roseburia* in both Moderate Grain and High Grain might result from the increase of *B. fibrisolvens* in cattle feces. *Blautia* included many sequences recovered from *Ruminococcus* spp. isolated from human feces (Hayashi et al., 2002) and the rumen (Rieu-Lesme et al., 1996). Therefore, *Blautia*, which was dominant in High Grain, might be associated with the increase of *Ruminococcus*. *Anaerovorax* and *Coprococcus* included some sequences recovered from *Clostridium* spp. of nonfeces origin (Collins et al., 1994; Sheridan et al., 2003; Gourgue-Jeannot et al., 2006). The abundance of *Anaerovorax* and *Coprococcus* in cattle feces might be associated with the abundance of *Clostridium*. Unclassified Lachnospiraceae, which was highly abundant across all samples, included some sequences recovered from *Blautia* spp., *Clostridium* spp., *Eubacterium* spp., and *Ruminococcus* spp. isolated from feces (Lan et al., 2002; Brooks et al., 2003; Ballard et al., 2005; Roger et al., 2010; Park et al., 2012). The dominance of unclassified Lachnospiraceae in cattle feces might be associated with these 4 genera. This issue will need to be considered to investigate the taxonomic composition of the fecal microbiota in future studies.

Fecal samples of cattle fed Moderate Grain were collected in July and August under the same diet, and pyrosequencing analysis for the 2 replications was conducted separately. We compared bacterial communities between the 2 replications. The abundance of the phylum Firmicutes was greater ($P < 0.001$) in samples collected in August than in July. Some genera were different between the 2 replications, and most of the genera were assigned to Firmicutes. *Prevotella*, *Clostridium*, *Coprococcus*, *Roseburia*, *Fecalibacterium*, *Succinivibrio*, *Blautia*, *Subdoligranulum*, *Sporacetigenium*, *Ruminococcus*, *Coprobacillus*, *Lactobacillus*, and *Xylanibacter* were greater ($P < 0.05$) in samples collected in August than in July whereas *Parabacteroides*, *Oscillibacter*, *Bacteroides*, *Anaerovibrio*, *Anaerovorax*, *Alistipes*, and *Escherichia*/*Shigella* were lower ($P < 0.05$) in samples collected in August than in July. The taxonomic composition at the genus level appears to be affected by environmental factors. Although some genera were different between the 2 replications, unweighted PCoA based on species-level OTU separated the 2 replications by only 2% variation, indicating slight shifts at the species level. The taxonomic composition at the species level appears to be only slightly changed by normal environmental factors within the same group of animals over time.

Although we analyzed more than 2 million sequences across all the 333 samples, the percent coverage in collective data was still incomplete (77%) based on the rarefaction estimate of maximum number OTU (Larue et al., 2005). In the pooled sample of Moderate Grain, the number of sequences per a sample on the average was 7,300 and the percent coverage was 80%. On the other hand, 3,900 sequences per sample on the average resulted in 64% coverage in the pooled sample of Silage/Forage. To investigate minor taxa and OTU in the feces of cattle, it is apparent from current research that more than 7,300 sequences per sample will need to be obtained in future studies. Nonetheless, the present study had sufficient power in observation groups to enable a better understanding of the detailed list of fecal microbiota influenced by diet, core taxa irrespective of diets, and variation of fecal microbiota among individual cattle. This is the first study of this magnitude of which we are aware that clearly demonstrates bacterial similarities within and striking compositional differences between groups of animals. Diet appeared to have a large effect on fecal microbiota, particularly when comparing forage versus grain diets. In future studies, the role of diet will need to be considered when evaluating fecal microbiota and host relationships.

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