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Bacterial communities in the rumen and feces of lactating Holstein dairy cows are not affected when fed reduced-fat dried distillers' grains with solubles

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ABSTRACT

Reduced-fat dried distillers' grains with solubles (**RF-DDGSs**) are co-products of ethanol production and contain less fat than traditional distillers' grains. The fat in corn is ~91% unsaturated, and it is toxic to rumen microorganisms so it could influence the composition of the rumen microbiome. It has been demonstrated that RF-DDGS is a suitable ration ingredient to support the high-producing dairy cow, and this feedstuff is a promising alternative protein source for lactating dairy cows. The current study aims to better understand the effect of RF-DDGS on the rumen and fecal bacterial composition in lactating dairy cows. Thirty-six multiparous (two or three), mid-lactation Holstein cows (BW = 680 ± 11 kg; 106 ± 27 DIM) were randomly assigned to two groups which were fed a control diet made up of corn, corn silage, and alfalfa hay supplemented with expeller soybean meal or with added RF-DDGS (20% of the DM) containing approximately 6.0% fat. Whole rumen contents (rumen fluid and digesta; esophageal tubing method) and feces (free-catch method) were collected on day 35 of the experimental period, after the 14-d acclimation period. Rumen contents and feces from each cow were used for DNA extraction. The bacterial community composition in rumen and fecal samples was assessed via the 16S rRNA gene by using the Illumina MiSeq sequencing platform. Bacteroidetes, Actinobacteria, and Firmicutes were the most abundant phyla in rumen contents. The fecal microbiota was dominated by the phyla Firmicutes and Bacteroidetes, as well as Actinobacteria and Chloroflexi. RF-DGGS increased bacterial richness, evenness, and Shannon diversity in both rumen and fecal samples and was associated with several taxa that had different abundance in treatment versus control comparisons. The RF-DGGS, however, did not significantly alter the bacterial community in the rumen or feces. In general, these findings demonstrated that dietary inclusion of RF-DDGS did not impose any serious short-term (within 30 days) health or production consequences, as would be expected. With this study, we present further evidence that inclusion of 20% (DM basis) RF-DDGS in the diet of lactating dairy cows can be done without consequence on the microbiome of the rumen.
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Implications

Reduced-fat dried distillers' grains with solubles are a quality, economical, and readily available protein source demonstrated to support the protein needs of high-producing dairy cows. In this study, the rumen and fecal bacterial communities of lactating dairy cows were not significantly influenced by 20% (DM basis) reduced-fat dried distillers' grains with solubles and did not impose serious short-term (within 30 days) health or production consequences. This diet could potentially be introduced into Total Mixed Ration feeding of dairy cattle given the fact that it is readily available and relatively economical.

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Introduction

Distillers' grains plus solubles (**DGSs**) are co-products of ethanol production from various grains and have been used in dairy cattle ration formulation for over 100 years (Schingoethe et al., 2009). This feed is known to typically contain high concentrations of protein, lipids, phosphorous, sulfur, and non-forage fiber (Schingoethe et al., 2009; Min et al., 2019). The intermediate fat concentration (~10% of DM) and physically effective fiber (~39% neutral detergent fiber of DM) contribute to the high energy content in DGS (Schingoethe et al., 2009). DGS may contain an apparently sufficient fiber content based upon proximate analyses, yet the small particle size of the fiber may allow it to pass through the GI tract too quickly to make an appreciable difference on fermentation activity (Zebeli et al., 2012). Thus, because DGS is not necessarily a good form of "physically effective NDF" (Zebeli et al., 2012), there is the likelihood of milk fat depression (fast passage rate results in poor fermentability) when used as a concentrate replacer and diets are balanced using traditional metrics of proximate analysis that do not account for particle size (Schingoethe et al., 2009). Additionally, we have refuted what was previously reported, that dried DGS (DDGS) added to lactating dairy cow feed ration formulation is a major source of late blowing defect in the production of Swiss-style cheese (Testroet et al., 2018).

DDGS availability, however, is now limited because of the change in the economic value of corn oil extraction to the biodiesel industry, making traditional DGS not widely available, especially as ethanol plants improve the technology of production streams (Irwin and Good, 2013). Currently, the co-product in abundance is the reduced-fat dried distillers' grains with solubles (**RF-DDGSs**), which contains a decreased (~5 percentage points on DM basis) oil content (Peterson et al., 2003; Testroet et al., 2018). RF-DDGS, used as an alternative protein feed (~30% protein DM basis typically) source, may be advantageous in alleviating milk fat depression by limiting the supply of bacterially produced MFD-inducing precursor fatty acids (Testroet et al., 2018).

The accepted use of any feedstuff in animal feed formulation depends on the effects of that feedstuff on the animals' production efficiency and ultimately the quality of the products produced by that animal. For example, if an animal is fed an ingredient and produces milk that is either unsuitable for feed production or has undesirable off-flavors, dairy farmers will avoid that ingredient, which has been reported previously (Lettat et al., 2013; Schären et al., 2018). RF-DDGS has shown promising results concerning the improvement of some metrics of lactation performance (e.g., decreased milk urea nitrogen). No adverse effect on milk composition or measured blood metrics of energy balance (e.g., non-esterified fatty acids, blood glucose) but an increase in feed intake without accompanying change in milk yield resulted in decreased feed efficiency in lactating dairy cattle fed RF-DDGS (0 or 20%) (Testroet et al., 2018). Though the influence of dietary inclusion of RF-DDGS on lactation performance has been investigated to a moderate degree (Callaway et al., 2010; Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016; Ranathunga et al., 2019), relatively little is known about its effect on rumen microbiota and the interrelation of microbiota on lactation performance.

Dietary composition and feed intake patterns serve as the major drivers that influence rumen microbiota composition (Perea et al., 2017; Schären et al., 2018; Ishaq et al., 2019). In cattle, digestion occurs mainly in the rumen by the metabolic activities of microorganisms which convert fermentable plant fiber into volatile fatty acids and microbial protein, thus providing nutrients and energy for the animal (Liu et al., 2016). However, depending on the composition of the ration, for example, one that reduces the pH of the rumen or includes plant secondary compounds, the normal activities of some rumen bacteria may be halted or inhibited. This was reported by feeding lactating cows with conventional distillers' grains produced from corn (Schären et al., 2018). Corn oil, which is derived from the whole corn kernel used in ethanol production, is comprised of ~91% unsaturated fatty acids, which have been shown to inhibit fiber digestion linearly as they are increased in the diet of ruminants (Drackley et al., 1992). Changes in rumen microbial populations have been associated with changes in production parameters such as feed efficiency and milk production and composition (Myer et al., 2015; Jewell et al., 2015; Shabat et al., 2016). To date, relatively little is known about the effect of dietary RF-DDGS inclusion in the ration of lactating dairy cattle on both ruminal and fecal microbiota. Our objective was, therefore, to investigate changes in the microbial communities in both the feces and rumen contents of lactating dairy cattle fed 20% (DM basis) RF-DDGS when compared with those fed a standard TMR consisting of corn, soy, and hay, and pending that, assess how those changes associated with overall lactation performance, previously reported in (Testroet et al., 2018).

Material and methods

Experimental design

The experimental design is described in a previous study (Testroet et al., 2018), with approval from the Iowa State University Animal Care and Use Committee (Protocol #7-15-8057-B). Briefly, 36 multiparous (parity 2 or 3), mid-lactation Holstein cows (BW = 680 ± 11 kg; 106 ± 27 DIM) were assigned randomly to one of two dietary treatment groups (n = 18 per group) in a 2 × 2 crossover design. The two crossover periods lasted 35 days each, in which the first 14 days was used for diet adaptation. Feed of both treatments was formulated to be isonitrogenous (with balanced limiting amino acids) and isoenergetic. The basal control diet was a total mixed ration (TMR) based on corn, corn silage, and alfalfa hay, supplemented with expeller soybean meal (SoyPlus, Landus Cooperative, Ames, IA) as a protein source. The RF-DDGS diet was formulated by using the same base ration as the control but with 20% of the DM expeller soybean meal being replaced with RF-DDGS (Poet Biorefining, Jewell, IA) containing approximately 6.0% fat DM basis. Details of the nutrient analysis of the diet have been previously published (Testroet et al., 2018). The RF-DDGS ration was supplemented with rumen-protected lysine to ensure that diets were similar in limiting amino acids (methionine in lysine content) (Kemin, Des Moines, IA).

Cows were housed together at the Iowa State University Dairy Farm (Ames, IA) in a 48-cow freestall pen and individually fed twice daily (0800 and 1600 h) through controlled-access gates (American Calan, Northwood, NH), allowing for approximately 10% refusals. Refusals were weighed and recorded daily. Feed ingredients in the TMR were mixed using a Patz V615 mixer (Patz Corp., Pound, WI). Cows were allowed *ad libitum* access to food and water except during their 3× daily milking (8 h apart; approximately 1 h of no access to feed or water per milking). Initially, cows were trained to use the Calan gates prior to the start of the acclimation period. Additionally, individual milk production was recorded daily using a Boumatic milking system (Boumatic LLC, Madison, WI). Animal intake and production results and analysis have been previously published (Testroet et al., 2018).

Sample collection and bacterial community processing

Whole rumen contents (rumen fluid and digesta) and feces were collected on day 35 of each of the experimental periods, yielding 36 rumen and 36 fecal samples total. Rumen contents

were collected via esophageal tube, and the first fraction (~100 mL) was presumed to contain a larger proportion of saliva and thus was discarded. Next, the subsequent portion (~100 mL) of rumen contents (pH 6–7) were collected, placed on ice, and frozen within 2 h of collection (−20 °C). Fecal samples were collected at the same time as collection of rumen samples by free-catch method from the rectum, placed on ice, and frozen at −20 °C until the time of microbial analysis.

Following thawing, each rumen or fecal sample (n = 72) was independently homogenized, and three grams (wet weight) of this homogenized material was used for the DNA extraction with a bead-beating method followed by phenol–chloroform extraction (Yeoman et al., 2013). The DNA was quantified by using a Nanodrop spectrophotometer following staining by using a Quant-it Pico Green dsDNA kit (Invitrogen, Paisley, UK). The DNA samples were stored at −80 °C until further processing. The V3–V4 region of the bacteria 16S ribosomal RNA (rRNA) gene was amplified by polymerase chain reaction (PCR) (95 °C for 2 m, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C of 30 s with a final extension at 72 °C for 5 m). The amplification used primers 338F (5′-ACTCCTRCGGGAGGCAGCAG-3′) and 806R (5′-GGACTACCVGGG TATCTAAT-3), with barcodes as an eight-base sequence unique to each sample. Amplicons were extracted from 2% agarose gels and purified by using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified by using QuantiFluorTM-ST (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar concentrations and paired end sequenced (2 × 300 nucleotides) on an Illumina MiSeq platform according to standard protocols (Caporaso et al., 2011). Raw sequence data (fastq files and metadata) are publicly available from the NCBI Sequence Read Archive (SRA) under Accession SRP271067 and BioProject PRJNA644954.

Statistical analysis

The raw sequence dataset containing pair-ended reads of DNA sequences was analyzed by using the R statistical package version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Of the 72 samples collected, three failed in the sequencing run for unknown reasons, and did not generate usable data. In all, 69 samples (35 rumen and 34 feces) produced 4,279,120 raw reads for each the forward and reverse reads, via quality evaluation using the ShortRead package (Morgan et al., 2009). Based on visual assessment of the aggregated quality scores at each base from all samples (plotQualityProfile in DADA2), only the raw sequences from forward sequences were retained and were filtered by using the DADA2 package (Callahan et al., 2016). Reads were trimmed with the first 10 bases truncated, no ambiguous bases, a maximum of two errors were allowed in each read, and reads matching the phi X genome were removed as positive-control contamination. Eleven samples did not pass the initial trimming step based on low-quality scores (based on expected error). It is presumed that these samples did not sequence well from the low number of raw reads.

The DADA2 package then was used to estimate the error rates for the sequencing run, dereplicate the reads, pick sequence variants, and remove chimeric artifacts from the sequence table. Taxonomy was assigned by using the Silva taxonomic training data version 132 (Pruesse et al., 2007) formatted for DADA2. Unwanted taxa (chloroplasts and mitochondria) were removed from the analysis using dplyr package (Muller et al., 2018). The sequence table, taxonomy, and metadata were combined into a phyloseq object by using the phyloseq package (McMurdie and Holmes, 2013). Differential abundance was calculated using the DESeq2 package (Love et al., 2014) on two subsets of non-rarefied data, to compare the effect of diet within the sample type collected (i.e. fecal and rumen samples). For differential abundance, significance (P < 0.05) was determined by a Wald test, which is corrected for multiple comparisons by default using the Benjamini-Hochberg method, and the abundance of the significant taxa of each subset comparison was plotted by using the ggplot2 package.

Sequences were rarefied to 4 000/sample (rarefaction curve; Supplementary Fig. S1), and a total of 50 samples contained sufficient sequences to meet this threshold and were retained in the rarefied dataset which were used in the following analyses. Statistical analysis was similar to approaches used previously (Ishaq et al., 2019) Normality tests were run by using the Shapiro-Wilkes test on the generated diversity metrics for these data, and statistical models were chosen to accommodate non-normal data. Linear models were run for comparisons of diversity metrics by using linear mixed effect models (lme4 package (Bates et al., 2015), in which treatment and sample type were fixed effects, and sample date was a random effect, and visualized with the ggplot2 package (Wickham, 2009). Bacterial diversity at the phylum level was visualized by using a stacked bar plot made in phyloseq. Jaccard unweighted similarity was used to calculate sample similarity based on community membership (species presence/absence) and clustering tested with permutational analysis of variance (**permANOVA**) by using the vegan package (Oksanen et al., 2018). This was used to determine if diet treatment was associated with a fundamental change in the cohort of rumen bacteria. Canonical correspondence analysis (**CCA**) was used to compare the microbial diversity with environmental factors, using weighted Bray Curtis dissimilarity to calculate the distance ordination based on membership and structure (abundance), to give a broader understanding on how bacterial communities were associated with treatments. Diet treatment and sample type were included as interactive fixed effects, and parity as an independent fixed effect, in the final model. Crossover block, parity, and treatment period were included as random effects during data exploration, but did not improve the model or explain additional variance, thus were not included in the final model. Effect of variables on sample clustering was analyzed by using analysis of variance (one-way ANOVA) with Tukey's HSD mean separation test at α = 0.05. The complete code for the analysis in the R platform is provided as Supplementary Material S1.

Results

Diversity of the bacterial community

In this study, 16S rRNA gene sequence analysis of the rumen and feces samples generated a total of 4 279 120 raw sequences with an average of 133 167 trimmed sequences per sample. The overall number of bacterial sequence variants (SVs) identified in the curated dataset was 14 523. Observed SV richness, Shannon evenness, and Shannon diversity are visualized in Fig. 1, and linear modeling comparison (Table 1) shows that rumen samples had (P < 0.05) higher number of bacterial species detected, as well as inequity in their abundances, and total diversity, respectively, than the fecal samples.

The relative abundance of bacteria and their distribution in rumen and feces samples are shown at the phylum level (Fig. 2). The phyla Firmicutes, Bacteroidetes, and Actinobacteria were predominant in both rumen and fecal samples. In addition to these phyla, Tenericutes were highly abundant in some fecal samples, and Proteobacteria were abundant in the several and rumen samples. The dominant SVs by relative abundance in the rumen samples were identified as the genera *Flavobacterium*, *Lactococcus*, *Prevotella*, *Rubrobacter*, *Clostridium* sp. *CAG-352* and unclassified genera belonging to the Ruminococcaceae family (Fig. 3). In the

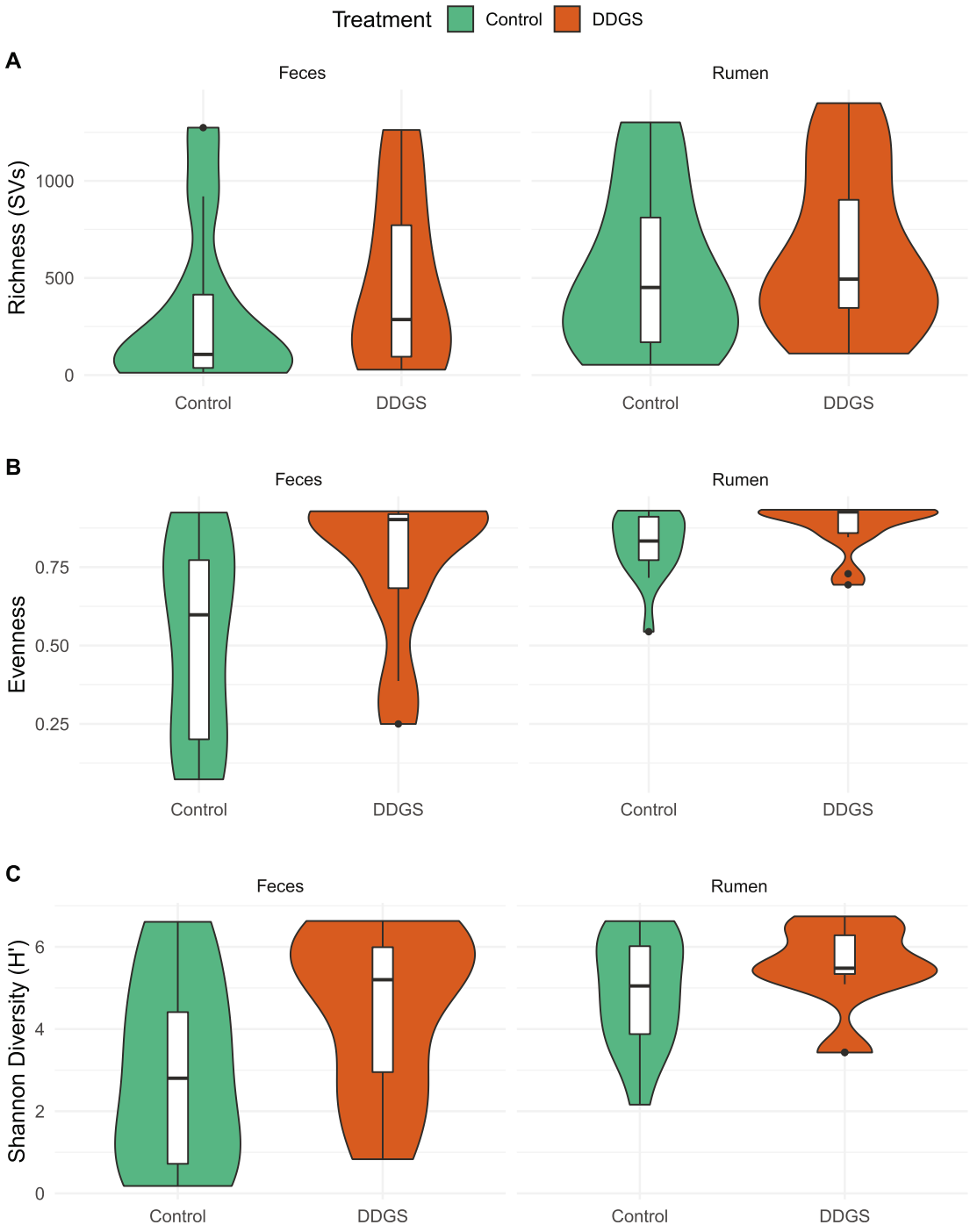

Fig. 1. Observed bacterial richness (A), evenness of abundance (B), and Shannon diversity (C) for bacteria in the rumen and feces of 36 lactating Holstein dairy cattle fed a diet containing reduced-fat dried distillers' grains with solubles (RF-DDGSs) compared to controls. Statistics are presented in Table 1.

fecal samples, the most abundant SVs were identified as the genera *Blastococcus*, *Acetitomaculum*, *Prevotella*, and unclassified genera belonging to the Ruminococcaceae family (Fig. 3).

Effect of reduced-fat dried distillers' grains with solubles on the bacterial community

The RF-DDGS diet treatment significantly increased bacterial richness, evenness, and diversity in both rumen and fecal samples (Fig. 1, Table 1). There was a significant interaction of diet and sample type on richness, but not on evenness or diversity. Dietary treatment increased bacterial richness when comparing between only fecal samples, or between only rumen samples.

Several bacterial SVs were differentially abundant (DESeq, $P < 0.05$) between the control and RF-DGGS diets in the rumen, and between the fecal samples (Fig. 4). In the rumen, members of the *Flavobacterium*, *Lactococcus*, *Prevotella*, and *Clostridium* (CAG-352) genera, as well as a member of the Ruminococcaceae family, were more abundant ($P < 0.05$) in the control diet. *Oribacterium*, *Ruminoclostridium*, and *Rubrobacter* genera, and a member of the Ruminococcaceae, were more abundant ($P < 0.05$) in the RF-DGGS diet in the rumen. In fecal samples, only 1 SV in the

Table 1
Effect of diet treatment or sample type on bacterial diversity metrics from rumen contents and feces of 36 lactating Holstein dairy cattle fed a diet containing reduced-fat dried distillers' grains with solubles (RF-DDGSs) compared to controls.

	Estimate (Regression Coefficient)	SE	*T* value	*P*-value	Significance
Observed richness					
Treatment (RF-DDGS vs. Control)	0.476	0.022	21.199	<0.001	***
Sample type (Rumen vs. Feces)	0.558	0.281	1.983	0.047	*
Treatment * Sample type	−0.254	0.028	−9.249	<0.001	***
Significant pairwise contrasts					
Control Feces * RF-DDGS Feces	−0.476	0.023	−21.199	<0.0001	***
Control Feces * RF-DGGS Rumen	−0.800	0.281	−2.774	0.028	*
Control Rumen * RF-DGGS Rumen	−0.222	0.016	−14.034	<0.0001	***
Evenness					
Treatment (RF-DDGS vs. Control)	0.241	0.085	2.833	0.007	**
Sample type (Rumen vs. Feces)	0.302	0.080	3.793	<0.001	***
Treatment * Sample type	−0.183	0.112	−1.639	0.108	ns
Shannon Diversity					
Treatment (RF-DDGS vs. Control)	1.553	0.734	2.116	0.040	*
Sample type (Rumen vs. Feces)	1.967	0.686	2.867	0.006	**
Treatment * Sample type	−0.974	0.964	−1.011	0.317	ns

Richness, evenness, and diversity values are visualized in Fig. 1, and assessed here with linear mixed effect models in which treatment and sample type were fixed effects and sample date was a random effect. Ration information is provided in Testroet et al. (2018). Significance is designated as $P > 0.05$ = not significant, ns; $0.05 > P > 0.01$ = *; $0.01 > P > 0.001$ = **, $P < 0.001$ = ***.

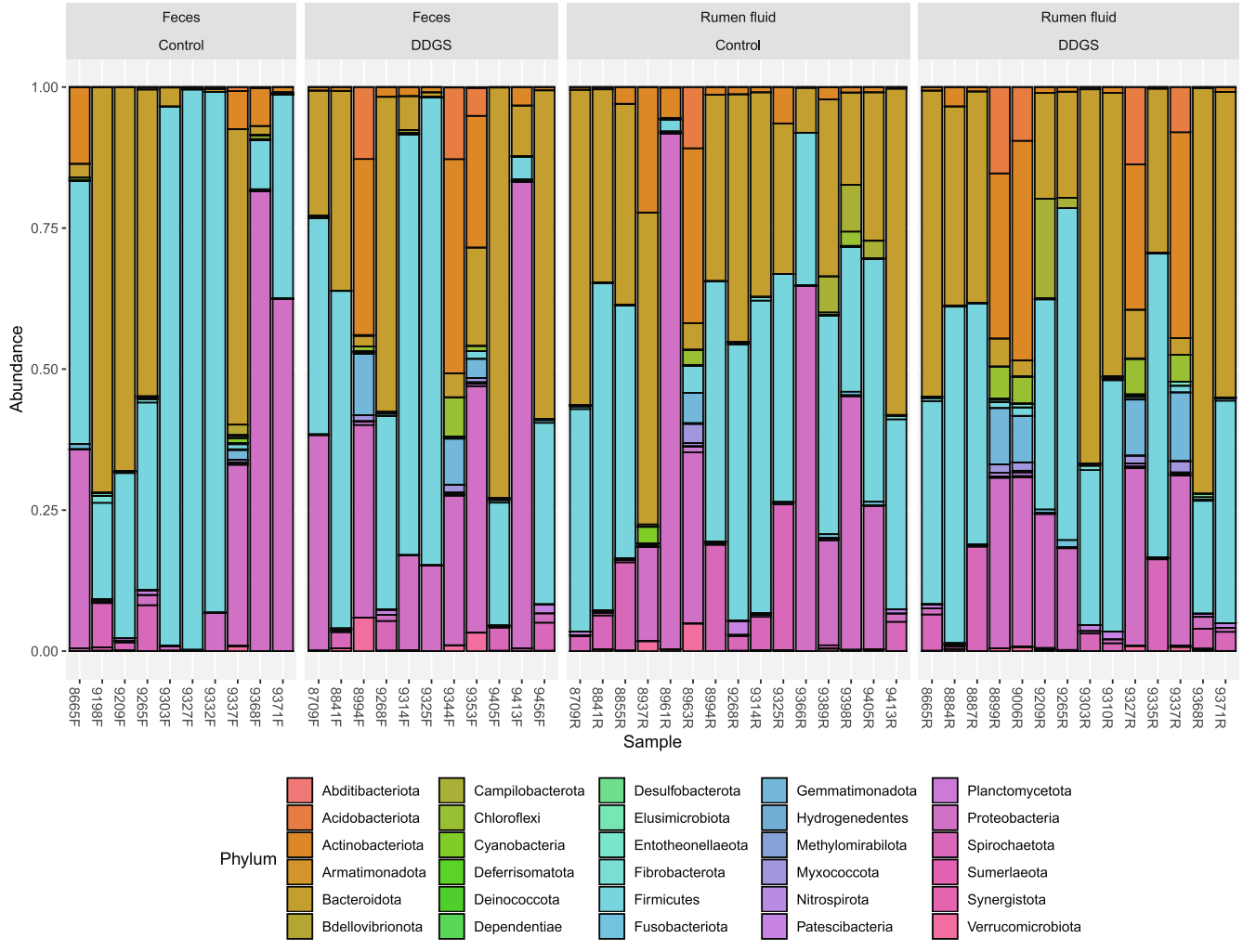

Fig. 2. Relative abundance of bacterial at the phylum level in the rumen and feces of 36 lactating Holstein dairy cattle fed a diet containing reduced-fat dried distillers' grains with solubles (RF-DDGSs) compared to controls.

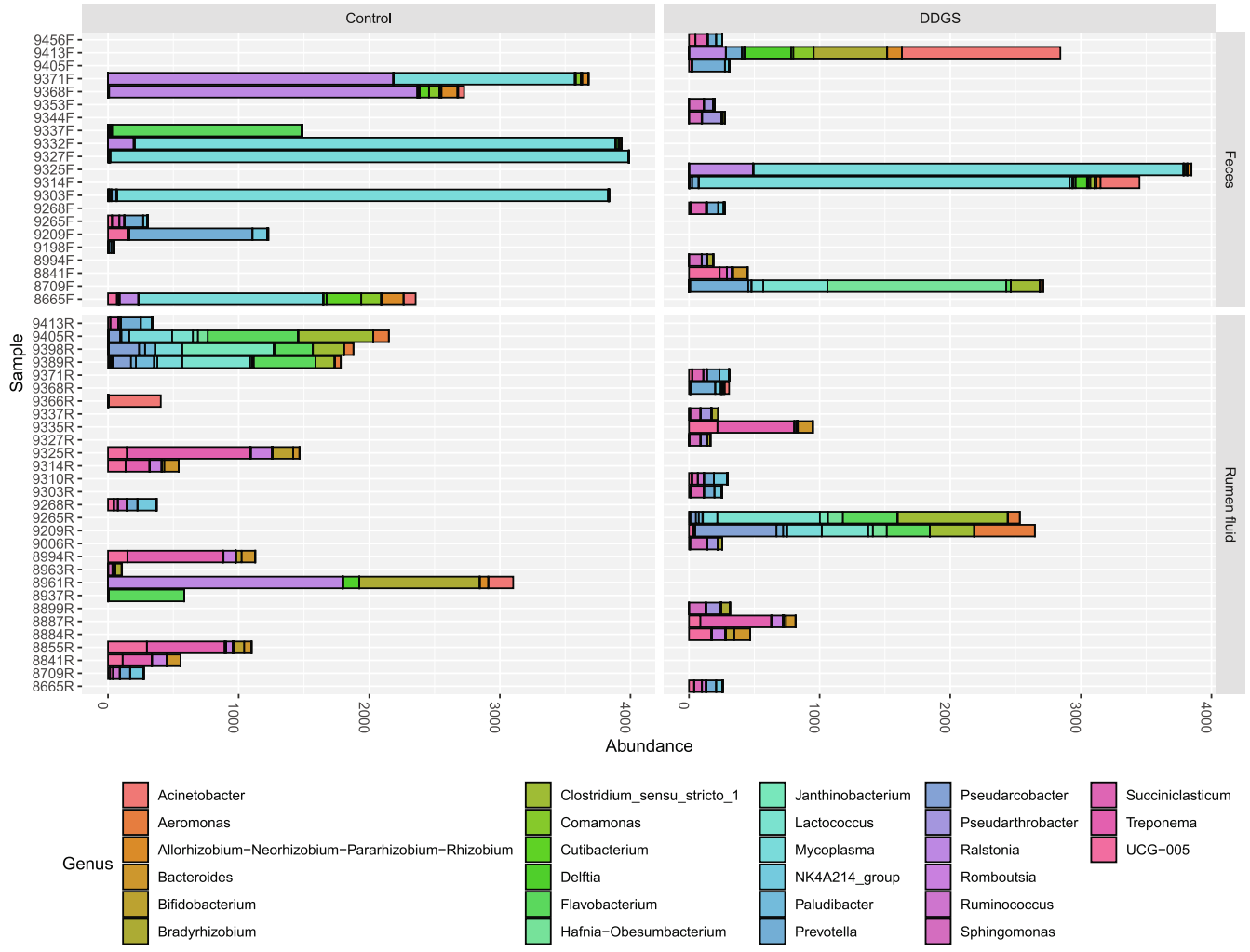

Fig. 3. Relative abundance of the top 50 bacterial sequence variants at the genus level in the rumen and feces of 36 lactating Holstein dairy cattle fed a diet containing reduced-fat dried distillers' grains with solubles (RF-DDGSs) compared to controls.

Prevotella genus was more abundant ($P < 0.05$) in control samples (Fig. 4). Several members of the genera *Prevotella*, *Blastococcus,* and *Acetitomaculum* and members of the families Lachnospiraceae and Ruminococcaceae were more abundant ($P < 0.05$) in the fecal samples of cows on the RF-DGGS diet.

The RF-DGGS, however, did not significantly affect the overall bacterial community, either collectively or when assessed within rumen or fecal samples (permANOVA, $P > 0.05$; Fig. 5). Sample type significantly ($P < 0.05$) affected the bacterial community similarity between samples, as did parity of the cow, which was treated as a fixed effect in the final model to evaluate effect of diet ($P < 0.05$; Fig. 5). No other experimental variables (period, treatment group) or animal digestion parameters (diet, rumen pH, rumen volatile fatty acid concentrations) affected the clustering of microbial communities of the rumen and feces.

Discussion

The objective of this study was to investigate changes in the microbial communities in both the feces and rumen contents of lactating dairy cattle fed RF-DDGS when compared with those fed a standard TMR consisting of corn, soy, and hay. There was very little effect of the diet on rumen bacteria, therefore, this rendered our second objective obsolete, thus we did not assess how changes associated with overall performance (objective two), reported previously in (Testroet et al., 2018). This finding agrees with previous studies that reported that very few phyla in the cow's rumen were affected by the addition of DGGS (Ishaq et al., 2017), or RF-DGGS, in the diet (Castillo-Lopez et al., 2017). This may reflect the similarity in nutrient composition of the DDGS to the portions of the diet that it typically replaces (i.e., soybean-derived products).

Previous studies have demonstrated that DDGS itself contains live bacteria (Lehman and Rosentrater, 2007; Sankarlal et al., 2015), which have been demonstrated in human and mouse studies to temporarily increase gut diversity when the diet is regularly consumed. We presume that some of the increase in rumen bacterial species richness in the present study reflects the addition of these species in low abundance via the RF-DGGS diet. The taxonomic compositions of our rumen samples were consistent with several other studies, however, with varying rate of abundance (Liu et al., 2016; Castillo-Lopez et al., 2017; Xue et al., 2018; Hagey et al., 2019; Mu et al., 2019). The core genera abundant and prevalent in the Firmicutes phylum in these environments include *Ruminococcus*, *Butyrivibrio*, *Clostridium*, *Turicibacter, Saccharofermentans*, and *Syntrophococcus* (Liu et al., 2016; Castillo-Lopez et al., 2017; Xue et al., 2018; Hagey et al., 2019; Mu et al., 2019). In the Bacteroidetes phylum, this core is comprised mostly of the genus *Prevotella* (Xue et al., 2018).

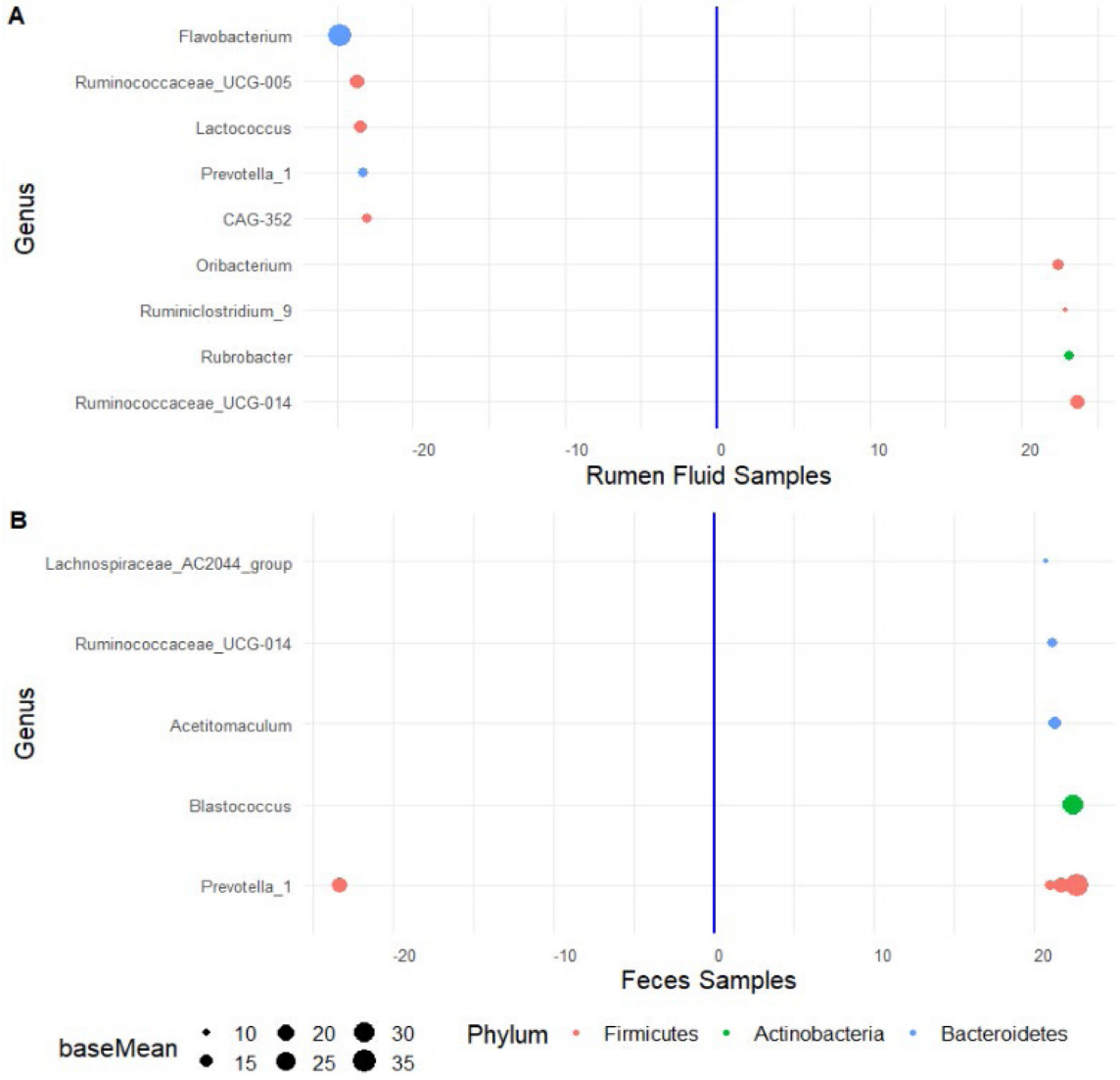

Fig. 4. Differentially abundant sequence variants in rumen (A) and feces (B) of 36 lactating Holstein dairy cattle fed a diet containing reduced-fat dried distillers' grains with solubles (RF-DDGSs) compared to controls. Controls are represented by the 0 line on the x-axis, and bacterial taxa which were significantly increased or decreased in the dietary treatment group are presented. Significance ($P < 0.05$) was determined by a Wald test.

Previous investigations also agree that gut microbiota composition is highly variable depending on the adaptation period allowed following diet introduction (Belenguer et al., 2010; Carda-Diéguez et al., 2014; Ishaq et al., 2019). This effect was originally reported in cows switched from a high-fiber diet to a high-grain diet on day 0-, day 3- and day 28 of a trial (Tajima et al., 2001). The gut microbiome may react to nuances in the diet over an unexpectedly long time, for example, even two diets with identical components but different particle sizes and processing caused shifts in rumen microbiota (Ishaq et al., 2019). The industry standard adaption period for cattle microbiota of 2–3 weeks (Weimer, 2015) may be too short to allow the gut microbiota to change and stabilize into a different community in some cases (Ishaq et al., 2019; Pinto et al., 2020), and may be an important factor in this study, as well.

Applicability of results to animal feed practices

The use of DGGS in animal feed has changed over time due to shifting production costs and demands, and what was considered "normal" used to be full-fat DDGS with 10–13 % Fat (DM basis), which our original study design included (Testroet et al., 2018). However, in the last few years in the United States, corn oil value dwarfs that of DDGS (Irwin and Good, 2013 and 2015) and the full-fat DGGS is no longer economically viable. Currently, to continue to produce full-fat DDGS would mean that the ethanol producer would either have old technology that does not include much oil removal or be very poorly managed. For this reason, in the United States' Midwest, "normal DDGS" has come to mean "RF-DDGS" with approximately 6% fat (DM basis), and one reason for a shift in research studies to using other alternatives in lieu of a full-fat DDGS feed comparison. Ultimately, our study design was revised to reflect actual feed practices based on current animal production economics. In addition, although the results may be perceived as a lack of finding (or negative results), null results are important in this context. No change to the rumen microbiota is also valuable information to producers, nutritionists, farmers, and dairy scientists supports the practice of substituting a more economical protein source in the place of extruded soybean meal.

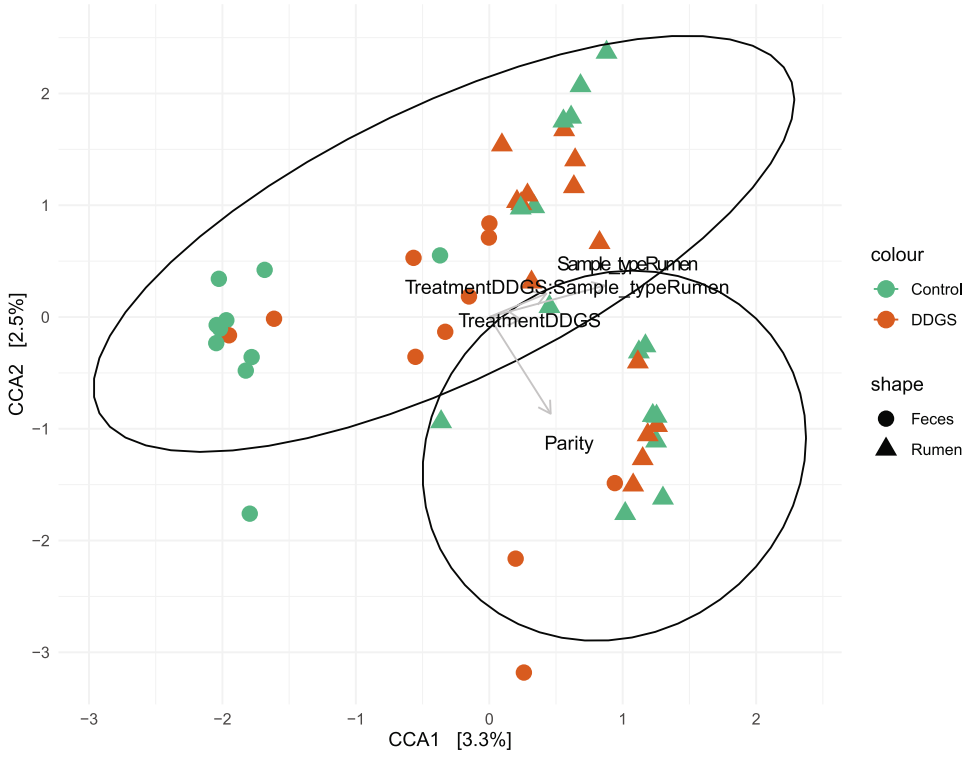

Fig. 5. Principal coordinate analysis (PCoA) plot of bacterial communities in the rumen and feces of 36 lactating Holstein dairy cattle fed a diet containing reduced-fat dried distillers' grains with solubles (RF-DDGSs) compared to controls. Bray Curtis was used to calculate distance ordination, and canonical correspondence analysis (CCA) was used to calculate significance for factors, including sample type (shape) seen on axis 1 and increasing parity (ellipses) seen on axis 2. Diet treatment and sample type were included as interactive fixed effects, and parity as an independent fixed effect, in the final model.

Conclusion

While not without any impact, presumably, this diet containing the RF-DDGS can be used without negatively impacting bacterial function. Given these and previous findings and based on the lack of alterations in rumen microbiota and in production parameters, RF-DDGS could potentially be utilized as an economical and readily available protein source for lactating dairy cattle. Moreover, this feed has the potential to increase the yield of animal and dairy products while cutting down on the cost of production.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100281.

Ethics approval

The experimental design is described in a previous study (Testroet et al., 2018), with approval from the Iowa State University Animal Care and Use Committee (Protocol #7-15-8057-B).

Data and model availability statement

Raw sequence data (fastq files and metadata) are publicly available from the NCBI Sequence Read Archive (SRA) under Accession SRP271067 and BioProject PRJNA644954.

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Declaration of interest

The authors have no conflicts of interest to declare.

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