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Running head: Distillers solubles alters rumen microbiome

Increasing corn distillers solubles alters the liquid fraction of the ruminal microbiome

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Abstract

Five ruminally-fistulated steers were used in a 5 × 5 Latin square design to determine the effects of increasing dietary fat and sulfur from condensed distillers solubles (**CDS**) on the ruminal microbiome. Treatments included a corn-based control (**CON**) and 4 levels of CDS (0, 10, 19, and 27%) in a coproduct-based (corn gluten feed and soybean hulls) diet. Fat concentrations were 1.79, 4.43, 6.80, and 8.91%, respectively, for diets containing 0, 10, 19, and 27% CDS. Steers were fed for ad libitum intake once daily. After feeding each diet for 18 d, ruminal samples were collected 3 h post-feeding on d 19. Samples were separated into solid and liquid fractions. Microbial DNA was extracted for bacterial analysis using paired-end sequencing of the V3-V4 region of the 16S rRNA gene on the MiSeq Illumina platform and quantitative PCR (**qPCR**) of selected species. Orthogonal contrasts were used to determine linear and quadratic effects of CDS inclusion. Increasing CDS inclusion decreased (linear; $P < 0.05$) alpha-diversity and species richness in the liquid fraction. Analysis of Bray-Curtis similarity indicated a treatment effect ($P = 0.01$) in the liquid fraction. At the phyla level, relative abundance of Bacteroidetes decreased in steers fed increasing dietary inclusion of CDS as Firmicutes increased to 82% of sequences for the 27% CDS treatment. Family Ruminococcaceae increased (linear; $P < 0.01$) 2-fold in the liquid fraction when feeding CDS increased from 0 to 27% CDS, yet genera *Ruminococcus* tended ($P = 0.09$) to decrease in steers fed greater CDS. The most abundant family of sulfate-reducing bacteria, Desulfovibrionaceae, increased ($P < 0.03$) in the solid and liquid fraction in steers fed additional dietary CDS and sulfur. Relative abundance of family Veillonellaceae and *Selenomonas ruminantium* was increased (linear; $P \leq 0.02$) in the solid fraction as steers were fed increasing CDS. There were no effects ($P > 0.10$) of feeding increasing dietary fat from CDS on fibroylytic genus *Fibrobacter* in either fraction. Results

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40 demonstrate increasing fat and sulfur from CDS in a coproduct-based diet markedly alters the
41 liquid fraction ruminal microbiome, but does not elicit negative effects on relative abundance of
42 identified fiber-fermenting bacteria.

43 **Keywords:** distillers solubles, rumen, microbiome, bacteria

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Introduction

Dietary fat is a concentrated energy source and may be fed to growing or lactating cattle. Several studies suggest feeding coproduct-based diets to cattle during the growing phase results in similar marbling scores compared with those fed starch-based diets (Retallick et al., 2010; Meteer et al., 2011; Segers et al., 2012). Data suggest increased fat concentrations may be responsible for maintaining intramuscular fat deposition when cattle are fed coproducts, such as distillers grains with solubles. Lipids can affect ruminal fermentation by decreasing the acetate:propionate ratio (Chalupa et al., 1984; Boggs et al., 1987), but are also capable of reducing VFA production, ruminal digestion of structural carbohydrates (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984), and methane production (Grainger and Beauchemin, 2011). Significant variation exists in the fatty acid profile of various feedstuffs and corresponds to known toxic effects of particular unsaturated fatty acids on specific rumen bacteria (Maczulak et al., 1981). Thus, the source of the dietary fat can greatly affect the aforementioned effects on ruminal digestion.

Condensed distillers solubles (**CDS**) is the most common nonfat liquid feed used to provide supplemental fat in feedlot cattle diets (Samuelson et al., 2016), and CDS typically ranges from 9 to 25% fat on a DM basis (Lardy, 2009). Including CDS up to 30% of the diet without other coproducts, improved cattle performance, but data indicated less CDS should be used in coproduct-based finishing diets (Pesta et al., 2015). High inclusion rates of distillers coproducts and specifically CDS may result in suitable growth performance, but the understanding of the effect of CDS on ruminal fermentation and the corresponding bacteria composition is limited. Given the relevance of CDS in the beef industry as an individual feedstuff and component of distillers grains with solubles, the objective was to determine

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changes in the ruminal bacterial community associated with increasing inclusion of CDS in coproduct-based diets.

Materials and Methods

Experiment Design

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana Champaign. Five ruminally-fistulated Angus × Simmental steers (BW = 335 ± 56 kg) were used in a 5 × 5 Latin square design to determine effects of increasing dietary CDS on digestion and ruminal fermentation (Segers et al., 2015). Dietary treatments included a corn-based control (CON) and 4 coproduct-based diets (corn-gluten and soy hulls) with increasing levels of CDS (0, 10, 19, and 27% diet DM; Table S1 in E-Supplement). Animals were fed once daily for ad libitum intakes and allowed ad libitum access to water. The 5 experimental periods consisted of 21 d. Ruminal samples were collected 3 h post-feeding on d 19 via ruminal cannula from 3 locations in the rumen and separated into the liquid and solid fractions. Samples were immediately put on ice and kept at -20°C prior to extraction. Feed samples were composited across period and 50 mg of each dried feedstuff (20 mg for CDS) was analyzed for fatty acid composition (Table 1) as previously described (Masood et al., 2005). An internal standard, C17:0 triacylglycerol, was added at the extraction step and later used to quantify peak areas.

Bacterial DNA Extraction and qPCR Analysis

The solid fraction samples (25 g) were used for DNA extraction by first homogenizing the digesta followed by phenol/chloroform extraction as described by Stevenson and Weimer (2007). Liquid fraction samples (50 mL) were centrifuged at 15,000 × g for 15 min. Collected sediment was used for extraction using the ZR-96 Fecal DNA Kit (ZYMO Research, Irvine,

CA). Extracted DNA was standardized to 8 ng/µL concentration for quantitative PCR and 20 ng/µL for 16S rRNA sequencing. Extracted DNA was stored at -80°C for later use.

Bacterial quantitative PCR (qPCR) primers utilized are listed in Table 2 and were validated using gel electrophoresis and Sanger sequencing. Each 10 µL reaction consisted of 4 µL sample DNA, 5 µL 1× SYBR Green with ROX (Quanta BioSciences, Gaithersburg, MD), 0.4 µL each of 10 µM forward and reverse primers, and 0.2 µL DNase/RNase free water in a MicroAmp^{TM} Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA). All reactions were performed using an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA) with the following conditions: 5 min at 95°C, 40 cycles of 1 s at 95°C (denaturation) and 30 sec at 60°C (annealing). The presence of a single PCR product was verified with an additional dissociation stage. All reactions were run in triplicate. Relative abundance of bacterial species was calculated using the geometrical mean of 2 universal primers with the efficiency-corrected Δ^{-CT} method (Ramirez-Farias et al., 2009). The portion of the 16S rRNA gene corresponding to the target of the eubacterial primer 3 (Muyzer et al., 1993) was commercially synthesized (IDT, Coralville, IA). A standard curve from 9.5×10^7 to 3.0×10^4 molecules per µL was used to obtain the 16S copy number from each sample. Samples were diluted to 1 ng/µL for suitable qPCR performance of eubacterial primer 3.

Library Construction and 16S rRNA Sequencing

Amplification of the V3-V4 region of the 16S rRNA gene used modified F338/R806 primers as described by Caporaso et al. (2012). The reverse PCR primer was indexed with 12-base Golay barcodes to facilitate multiplexing of samples. The PCR and sequencing protocol has been previously described in detail (Derakhshani et al., 2016). The 300 bp paired-end sequencing reaction was performed on a MiSeq platform (Illumina, CA, USA) at the Gut

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16S rRNA Read Analysis

The PANDAseq assembler was implemented to merge overlapping paired-end Illumina fastq files (Masella et al., 2012). All the sequences with low quality base calling scores and uncalled bases (N) in the overlapping region were discarded. A minimum 50 bp overlap was required for read merging. The subsequent fastq file was processed using the QIIME pipeline v1.8 (Caporaso et al., 2010b). Assembled reads were demultiplexed and quality filtered; reads were truncated after 3 consecutive bases with a quality score below 1e-5 and discarded if shorter than 75 bases. Chimeric reads were filtered using UCHIME (Edgar et al., 2011), and reads were clustered into OTU (Operational Taxonomic Units) based on 97% similarity with UCLUST (Edgar, 2010). Representative sequences from each OTU were assigned a taxonomy using RDP Classifier (Wang et al., 2007) and aligned to the Greengenes 13_5 reference database (McDonald et al., 2012) using PyNAST (Caporaso et al., 2010a).

After sample size standardization to the smallest sample library size (23,000 sequences), OTU richness, and alpha- and beta-diversity metrics were estimated. Alpha rarefaction curves were generated using the Chao1 metric (Chao, 1984). Between sample comparisons of diversity (beta-diversity) were calculated using the Bray-Curtis metric (Beals, 1984). Bray-Curtis distance matrices were utilized in principal coordinate analysis (PCoA) to generate two-dimensional plots in PRIMER v6 software (Clarke and Gorley, 2006). Permutational multivariate analysis of variance (PERMANOVA) was implemented to test differences in beta-diversity among treatments.

Statistical Analysis

Relative abundance of bacteria present at > 0.1% at the phyla, family, and genus level were evaluated and logit transformed ($z = \log[p/(1-p)]$) if necessary to ensure normal distribution of the residuals, where p represents the relative abundance of a bacterial taxa. Bacterial relative abundance was analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment and period as fixed effects, and steer as a random effect. Treatment means were calculated using the LSMEANS option. Linear and quadratic orthogonal polynomial contrasts evaluated level of CDS inclusion. The IML procedure was used to determine the coefficients for the nonlinear inclusion of CDS in the diets. Significance was declared at $P \leq 0.05$ while tendencies are discussed at $0.05 < P \leq 0.10$.

Results

A total of 1,617,146 quality-filtered reads were generated with an average of approximately 33,000 reads per sample. Sequencing depth ranged from 23,229 to 122,894. An average of 1,483 OTUs based on 97% similarity were obtained for each sample. Within the Greengenes database, 89.9 and 53.5% of sequences were identified at the family and genera taxonomic level, respectively. At the community level, the largest effects were observed in the liquid fraction. The Chao1 index indicated a linear decrease ($P = 0.01$) in species richness when cattle were fed increasing concentrations of CDS in the diet (Table 3). Similarly, alpha-diversity decreased (linear; $P \leq 0.02$) with increased CDS inclusion as observed in the Shannon and Simpson's indices. Species richness and alpha-diversity for cattle fed CON was lower than 0 and 10% CDS ($P < 0.05$) in the liquid fraction and most similar to 27% CDS. In the solid fraction, no effect of CDS inclusion was observed on species richness. Analysis of beta-diversity, a comparative measure of diversity between samples, in the liquid fraction revealed a separation by treatment primarily by the second principal coordinate (Figure 1; $P = 0.01$). The

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first two principal coordinates collectively accounted for 64% of the observed variation between samples. A Spearman correlation greater than 0.8 indicated *Prevotella* was associated with the separation of 0% CDS samples (data not shown). However, there was no treatment effect (P = 0.2; data not shown) using the Bray-Curtis similarity of beta-diversity observed in the solid fraction by PERMANOVA analysis.

Liquid Fraction Microbiome Effects

Firmicutes was the most abundant phylum in the liquid fraction representing more than 70% of all sequences (Table 4). A linear increase ($P < 0.01$) in relative abundance of Firmicutes was observed in steers fed increasing concentrations of CDS. This increase in Firmicutes corresponded with a decrease (linear; $P \leq 0.01$) in Bacteroidetes, Cyanobacteria, and Spirochaetes. Within the phylum Firmicutes, the linear increase when greater CDS was fed was primarily driven by family Ruminococcaceae as its increase (linear; $P < 0.01$; Table 5) represented 75% of the increase in relative abundance at the phylum level. Phyla level effects of Bacteroidetes, Cyanobacteria, and Spirochaetes were observed at the family level ($P \leq 0.01$) in Paraprevotellaceae, order Bacteroidales, Spirochaetaceae, and order YS2, respectively. Phylum Fibrobacteres was not affected (P = 0.64) by dietary treatment fed to cattle. A quadratic increase (P = 0.03) in relative abundance of Desulfovibrionaceae was observed with the greatest abundance detected at 19% CDS. At the genus level (Table 6), *Prevotella* was most abundant and tended (linear; P = 0.08) to decrease with greater CDS. Relative abundance of *Ruminococcus* and *Oscillospira* tended (linear; P = 0.09) to decrease with increasing CDS which was the opposite response observed for all reads assigned to the family Ruminococcaceae. Although the majority (~87%) of reads assigned to Ruminococcaceae were unassigned at the genus taxonomic level, the percentage of reads that did assign to *Ruminococcus* ranged from

36% for 0% CDS, to 4% for 27% CDS. *Bifidobacteria* and *Treponema* also decreased (linear; $P \leq 0.02$) with greater CDS inclusion. A quadratic response ($P = 0.03$) in *Coprococcus* relative abundance was observed and peaked at 19% CDS.

Solid Fraction Microbiome Effects

In the solid fraction, Firmicutes and Bacteroidetes comprised 82 to 90% of reads in a treatment (Table 7). Overall, few dietary effects were observed at the phyla level. Cyanobacteria was affected by dietary treatment ($P = 0.02$) with the lowest relative abundance observed for 27% CDS. The relative abundance of Firmicutes tended ($P = 0.10$) to increase with greater CDS inclusion. At the family level (Table 8), Veillonellaceae and Ruminococcaceae, members of the Firmicutes phyla, increased linearly ($P \leq 0.04$) with greater CDS inclusion. Veillonellaceae linear effects were primarily driven by the genus *Succiniclasticum* (Table 9) where more than 75% of the Veillonellaceae sequences classified at the genus level. Within Bacteroidetes, family Paraprevotellaceae and unidentified sequences in order Bacteroidales decreased (linear; $P \leq 0.01$) with additional CDS. Cattle fed CON had 4-fold increase in phyla Fibrobacteres, but no overall effect ($P = 0.11$) of dietary treatment or increasing CDS ($P > 0.58$) in the diet was detected. Desulfovibrionaceae was affected by treatment ($P = 0.01$) with the lowest relative abundance observed for CON and the greatest for 19% CDS. A quadratic response ($P \leq 0.01$) was observed for *Moryella* with the lowest relative abundances observed for 10 and 19% CDS, while *Mitsuokella* and *Coprococcus* increased with greater CDS (linear; $P \leq 0.04$). A main effect of treatment ($P = 0.03$) was observed for *Corynbacterium* with the greatest relative abundance observed for 19% CDS which was 2-fold greater than any other treatment.

The relative abundance of bacterial species measured using qPCR in the solid fraction revealed a linear increase ($P = 0.02$) of *Selenomonas ruminantium* with increasing CDS inclusion

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(Table 10). In contrast, a decrease (linear; $P = 0.01$) in relative abundance of *Streptococcus bovis* occurred with greater CDS primarily driven the 4-fold higher values observed for 0% CDS. Moreover, *S. bovis* populations in the CON diet were nearly 26-fold greater than the 0% CDS. Although no effect of CDS inclusion was observed for *Megasphaera elsdenii*, there was a tendency ($P = 0.09$) for a 45-fold reduction for cattle fed CON compared with those fed any of the coproduct-based diets. Variation observed in relative abundance of *Anaerovibrio lipolytica* led to no differences ($P > 0.11$) despite a nearly 9-fold increase for 0% CDS. A trend ($P = 0.06$) for an increase in 16S log copy number was observed with increasing CDS. Additional results for bacterial families and genera with non-significant responses are listed in the E-supplement in Table S2, S3, S4, and S5.

Discussion

Many studies have evaluated the effects of supplemental fat on ruminal fermentation and biohydrogenation (Sackmann et al., 2003; Atkinson et al., 2006; Hess et al., 2008). However, the variation in basal diet composition, saturation of the supplemented fatty acids, and the amount of additional fat provided all contribute to differences observed for fermentation and bacterial effects. The fatty acid content of the CDS used in this experiment was similar to a previous report by Sasikala-Appukuttan et al. (2008). The data from Sasikala-Appukuttan et al. (2008) revealed that the addition of CDS from 10 to 20% of the diet increased ruminal ammonia and the molar proportion of butyrate, but propionate and acetate concentrations were not affected. Similarly, the corresponding ruminal fermentation results for the present study reported by Segers et al. (2015) indicated neither acetate, propionate, and ruminal pH nor total tract NDF digestion were affected by dietary treatment. However, greater NDF digestion has been observed when cattle fed diets containing wet distillers grains with solubles were compared with

cattle fed a corn bran and gluten meal diet with corn oil at similar levels of ether extract (Vander Pol et al., 2009). Although dietary fatty acid composition was not reported, the fatty acid profile of corn oil (Gillis et al., 2004) is similar to CDS with C18:2 representing more than 50% of fatty acids. Cattle fed the diet containing wet distillers grains with solubles had a greater proportion of unsaturated fatty acids (18:1 trans, 18:1, 18:2, and 18:3) flowing to the duodenum compared with cattle fed the corn oil diet suggesting differential levels of biohydrogenation (Vander Pol et al., 2009). Variation in biohydrogenation of feedstuffs with similar fatty acid content is supported by the difference in biohydrogenation observed between corn and corn oil (Duckett et al., 2002). Collectively, the data suggest reduced biohydrogenation and increased lipid digestibility likely contribute to positive animal responses to wet distillers grains with solubles compared with corn oil (Klopfenstein et al., 2008). Considering the varied effects of different fat sources with a similar fatty acid profile, the effect of CDS on the ruminal microbiome is an important piece to understand the effects of high levels of coproduct inclusion in beef cattle diets.

The first evaluation of CDS effects on rumen bacteria in vivo revealed a tendency to increase counts of total culturable, amylolytic, and lactilytic bacteria (Fron et al., 1996). Despite the increased inclusion of CDS in ruminant diets with greater ethanol production, this is the first study since Fron et al. (1996) to evaluate the effect of CDS on ruminal bacteria. Our 16S rRNA $\log_{10}$ copy number results support their findings suggesting an increase in bacteria in the liquid fraction with greater CDS. Compared with other fat sources, the sulfur and phosphorus concentrations and low pH of CDS make it a unique supplemental fat source among those fed to ruminants. While most of the lipids in CDS are incorporated into triacylglycerol, it does contain much greater concentrations of free fatty acids compared with corn oil (Moreau et al., 2011).

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The effect of CDS inclusion was greater in the liquid fraction due to observed changes in community level measures of alpha-diversity, species richness, and beta-diversity. While unsaturated fatty acids have long been known to inhibit fiber-degrading bacteria (Henderson, 1973), recent studies have observed no effect on community alpha-diversity with additional dietary lipids in rumen fluid (Zened et al., 2013; Huws et al., 2015). However, Huws et al. (2010) observed decreased denaturing gradient gel electrophoresis (DGGE) band numbers in liquid-associated bacteria, but not solid-associated bacteria, when cows fed a red clover silage diet were supplemented with fish oil. Supplemental fish oil also reduced DGGE band number in the liquid fraction when cattle were fed a grass silage diet, but alpha-diversity was not affected (Kim et al., 2008).

Within the liquid fraction, greater CDS inclusion increased relative abundance of Firmicutes and decreased Bacteroidetes primarily driven by corresponding changes in Ruminococcaceae and *Prevotella*, respectively. Adding sunflower oil to a silage-based diet fed to cattle caused similar numerical effects as relative abundance of Firmicutes increased while Bacteroidetes decreased (Zened et al., 2013); however, authors suggested large variation within these low starch diets prevented detection of statistical differences. A comparison of the data suggests more than 12 d may be needed for some animals to fully adapt to dietary changes as our samples were collected on d 19 of each period. Furthermore, despite the fact that the diets had similar NDF concentrations, sources of NDF varied significantly from silage and alfalfa hay in diet of Zened et al. (2013) compared with a mixture of silage, soy hulls, and corn gluten feed in this experiment. Our results agree with previous findings for *Treponema*, as it was decreased by the addition of fat as sunflower oil (Zened et al., 2013) and CDS in our experiment. Although

cultured strains are not cellulolytic, *Treponema bryantii* increased fiber degradation in co-culture with *Fibrobacter succinogenes* (Stanton and Canale-Parola, 1980).

At the family taxonomic level, Ruminococcaceae increased with CDS inclusion, but the opposite tendency occurred within the genus *Ruminococcus* as it decreased with greater CDS. Ruminococcus assigned reads likely correspond to a greater proportion of cultured Ruminococci with cellulolytic capabilities and known sensitivities to unsaturated fatty acids (Maczulak et al., 1981; Maia et al., 2007). The fact that a large proportion of Ruminococcaceae reads unidentified at the genus level have been commonly observed in 16S rRNA sequencing studies (Zened et al., 2013; McCann et al., 2014) indicates many Ruminococcaceae members remain uncultured.

The phylum Cyanobacteria increased significantly in the 0% CDS diet with nearly all reads assigned to the order YS2 which consisted of 330 OTUs. Although prior work on the ruminal microbiome has identified 16S rRNA reads as Cyanobacteria (Mao et al., 2013; Zhao et al., 2015), typically the reported relative abundances are under 1% compared with the 7% we observed in the liquid of fraction ruminal fluid from cattle fed the 0% CDS diet. Classically considered to be photosynthetic organisms, a new lineage within Cyanobacteria, Melainabacteria, has recently been observed in human fecal samples and is nonphotosynthetic (Di Rienzi et al., 2013; Soo et al., 2014). Previous studies have been able to assemble draft genomes from metagenomic DNA extracted from koala feces with a high prevalence of Melainabacteria (Soo et al., 2014). FeFe hydrogenases observed in the gut associated genomes suggest Melainabacteria may produce hydrogen and interact with hydrogenotrophic methanogens or acetogens (Di Rienzi et al., 2013). In addition, the Melainabacteria genomes encoded for the complete biosynthesis pathways of 4 B vitamins and may indicate a mutualistic relationship with the host (Di Rienzi et al., 2013).

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297 Overall effects of CDS inclusion in the solid fraction were more modest. Little change
298 was observed in community-level measures of diversity and phylum-level relative abundance.
299 Similar to the liquid fraction, Ruminococcaceae and Veillonellaceae increased slightly in the
300 solid fraction with greater CDS. Corresponding increases in *Succiniclasticum* and *Mitsuokella*
301 with increasing CDS fed in the diet agree with the description of cultured species in vitro. Out of
302 22 rumen bacteria cultures, *Mitsuokella multiacidus* was able to form oleic acid from linoleic
303 acid and ranked second in terms of membrane stability in presence of linoleic acid (Maia et al.,
304 2007). Another Veillonellaceae family member, *Selenomonas ruminantium* was detected using
305 qPCR and also increased linearly with CDS inclusion in the diet. While unaffected by
306 polyunsaturated fatty acids in vitro (Maia et al., 2007), the addition of oleic acid increases
307 growth of *S. ruminantium* in vitro (Maczulak et al., 1981).
308 Within the solid fraction, there was no effect of additional CDS on relative abundance of
309 *Fibrobacter succinogenes*, as indicated by qPCR and supported by 16S rRNA sequencing results
310 at the phyla level. This result agrees with the absence of any effect of greater CDS on total tract
311 NDF digestion in the corresponding study (Segers, et al., 2015). While Huws et al. (2010)
312 observed no effect on *F. succinogenes* in the rumen by the addition of fish oil to the diet, genus
313 Fibrobacter decreased in cattle fed a diet supplemented with flax oil (Huws et al., 2015). In
314 vitro, *F. succinogenes* is very sensitive to unsaturated fatty acids as C18:2 slowed growth and
315 C18:3 inhibited growth completely (Maia et al., 2007). Despite the increase in C18:2 and C18:3
316 with greater CDS inclusion in the diet, no effect of diet on Fibrobacter was observed in the
317 present study. In a 60% brome hay-based diet, total Fibrobacteres increased in cattle fed greater
318 dietary distillers grains with solubles, but a specific OTU classified as *F. succinogenes* remained
319 unaffected by diet (Castillo-Lopez et al., 2014). Overall, these findings suggest Fibrobacteres

may occupy a niche within the rumen that offers some protection from unsaturated fatty acids in CDS.

The most well-known rumen bacteria with lipolytic capabilities is *Anaerovibrio lipolytica*. Interestingly, *A. lipolytica* increased sharply on the 0% CDS diet which contained the least amount of fat. Early research on *A. lipolytica* indicated sensitivity to low pH for growth and lipase activity (Hobson, 1965; Henderson, 1971) and is supported by recent work in dual-flow fermenters (Fuentes et al., 2009). While Segers et al. (2015) reported no dietary effect on ruminal pH, results from 0% CDS revealed that it was the most stable throughout the day in addition to being the only diet without a distillers coproduct.

As the inclusion of CDS increased, dietary S increased and the relative abundance of sulfate-reducing family Desulfovibrionaceae increased in both the solid and liquid fractions. Recommendations for minimum S for growing beef cattle are 0.15% to meet the requirements of cellulolytic bacteria (NRC, 2016), while 0.3% has been suggested as a maximum to avoid reducing the risk of limiting DMI and occurrence of S-induced polioencephalomalacia (S-PEM; NRC, 2005). Loerch et al. (2012) observed a 15 d adaptation period for ruminal H_2S to increase after starting lambs on a diet with added sodium sulfate, thus suggesting our sampling on d 19 was sufficient time for Desulfovibrionaceae to respond. Although high dietary S has been shown to limit intake (Sarturi et al., 2013) which could affect the ruminal microbiome, a reduction in DMI was not observed (Segers et al., 2015) within the experimental period of 21 d. Overall, our data support preliminary results described by Drewnoski et al. (2014) that Desulfovibrionaceae is the most abundant sulfate-reducing bacterial family in the rumen and it responds to greater dietary S by increasing in relative abundance.

Conclusion

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343 Addition of CDS to a coproduct-based diet up to 27% caused the greatest change within
344 the liquid fraction of the ruminal microbiome. Specifically, greater CDS inclusion reduced
345 species richness, alpha-diversity, and relative abundance of Bacteroidetes while increasing
346 Ruminococcaceae. Overall alterations in the solid fraction microbiome were modest, but notable
347 increases in *Succiniclasticum*, *Mitsuokella*, and *S. ruminantium* were observed with greater CDS.
348 Desulfovibrionaceae increased with greater dietary S from CDS in both fractions with greatest
349 relative abundance observed at 19% CDS. An unusually large proportion of Cyanobacteria were
350 observed on the 0% CDS diet and suggest non-photosynthetic Cyanobacteria may have a niche
351 in the rumen. Overall, results indicate important alterations to the liquid fraction ruminal
352 microbiome when increasing dietary inclusions of CDS are fed in a coproduct-based diet without
353 significant alterations to fiber-fermenting bacteria.
354

355 **LITERATURE CITED**

356 Atkinson, R. L., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, B. W. Hess, and D. C. Rule.
357 2006. Site and extent of digestion, duodenal flow, and intestinal disappearance of total
358 and esterified fatty acids in sheep fed a high-concentrate diet supplemented with high-
359 linoleate safflower oil. J. Anim. Sci. 84: 387-396. doi:/2006.842387x
360 Beals, E. W. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate
361 ecological data. Adv. Ecol. Res. 14: 1-55. doi:10.1016/S0065-2504(08)60168-3
362 Boggs, D., W. Bergen, and D. Hawkins. 1987. Effects of tallow supplementation and protein
363 withdrawal on ruminal fermentation, microbial synthesis and site of digestion. J. Anim.
364 Sci. 64: 907-914. doi:10.2134/jas1987.643907x
365 Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight.
366 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment.
367 Bioinformatics 26: 266-267. doi:10.1093/bioinformatics/btp636
368 Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N.
369 Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights,
370 J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J.
371 Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J.
372 Zaneveld, and R. Knight. 2010b. QIIME allows analysis of high-throughput community
373 sequencing data. Nat. Meth. 7: 335-336. doi:10.1038/nmeth.f.303
374 Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M.
375 Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith, and R.
376 Knight. 2012. Ultra-high-throughput microbial community analysis on the Illumina
377 HiSeq and MiSeq platforms. ISME J 6: 1621-1624. doi:10.1038/ismej.2012.8

J. Anim. Sci Accepted Paper, posted 06/09/2017, doi:10.2527/jas2016.1361

Castillo-Lopez, E., H. A. Ramirez Ramirez, T. J. Klopfenstein, C. L. Anderson, N. D. Aluthge, S. C. Fernando, T. Jenkins, and P. J. Kononoff. 2014. Effect of feeding dried distillers grains with solubles on ruminal biohydrogenation, intestinal fatty acid profile, and gut microbial diversity evaluated through DNA pyro-sequencing. J. Anim. Sci. 92: 733-743. doi:10.2527/jas.2013-7223

Chalupa, W., B. Rickabaugh, D. Kronfeld, and S. D. Sklan. 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. J. Dairy Sci. 67: 1439-1444. doi:10.3168/jds.S0022-0302(84)81459-9

Chao, A. 1984. Nonparametric estimation of the number of classes in a population. Scand. J. Stat. 11: 265-270. doi:10.1214/aoms/1177729949

Clarke, K. R., and R. N. Gorley. 2006. PRIMER v6: User Manual PRIMER-E. Plymouth, UK.

Derakhshani, H., H. M. Tun, and E. Khafipour. 2016. An extended single-index multiplexed 16S rRNA sequencing for microbial community analysis on MiSeq illumina platforms. J. Basic Microbiol. 56: 321-326. doi:10.1002/jobm.201500420

Di Rienzi, S. C., I. Sharon, K. C. Wrighton, O. Koren, L. A. Hug, B. C. Thomas, J. K. Goodrich, J. T. Bell, T. D. Spector, J. F. Banfield, and R. E. Ley. 2013. The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. eLife 2: e01102. doi:10.7554/eLife.01102

Drewnoski, M. E., D. J. Pogge, and S. L. Hansen. 2014. High-sulfur in beef cattle diets: A review. J. Anim. Sci. 92: 3763-3780. doi:10.2527/jas.2013-7242

Duckett, S. K., J. G. Andrae, and F. N. Owens. 2002. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. 80. doi:/2002.80123353x

401 Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics
402 26: 2460-2461. doi:10.1093/bioinformatics/btq461
403 Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves
404 sensitivity and speed of chimera detection. Bioinformatics 27: 2194-2200.
405 doi:10.1093/bioinformatics/btr381
406 Fliegerova, K., I. Tapio, A. Bonin, J. Mrazek, M. L. Callegari, P. Bani, A. Bayat, J. Vilkki, J.
407 Kopečný, and K. J. Shingfield. 2014. Effect of DNA extraction and sample preservation
408 method on rumen bacterial population. Anaerobe 29: 80-84.
409 doi:10.1016/j.anaerobe.2013.09.015
410 Fron, M., H. Madeira, C. Richards, and M. Morrison. 1996. The impact of feeding condensed
411 distillers byproducts on rumen microbiology and metabolism. Anim. Feed Sci. Technol.
412 61: 235-245. doi:10.1016/0377-8401(95)00943-4
413 Fuentes, M. C., S. Calsamiglia, P. W. Cardozo, and B. Vlaeminck. 2009. Effect of pH and level
414 of concentrate in the diet on the production of biohydrogenation intermediates in a dual-
415 flow continuous culture. J. Dairy Sci. 92: 4456-4466. doi:10.3168/jds.2008-1722
416 Gillis, M. H., S. K. Duckett, and J. R. Sackmann. 2004. Effects of supplemental rumen-protected
417 conjugated linoleic acid or corn oil on fatty acid composition of adipose tissues in beef
418 cattle. J. Anim. Sci. 82: 1419-1427. doi:10.2527/2004.8251419x
419 Grainger, C., and K.A. Beauchemin. 2011. Can enteric methane emissions from ruminants be
420 lowered without lowering their production?. Anim. Feed Sci. Technol. 166: 308-320.
421 doi:10.1016/j.anifeedsci.2011.04.021
422 Henderson, C. 1971. A study of the lipase produced by Anaerovibrio lipolytica, a rumen
423 bacterium. Microbiology 65: 81-89. doi:10.1099/00221287-65-1-81

J. Anim. Sci Accepted Paper, posted 06/09/2017, doi:10.2527/jas2016.1361

424 Henderson, C. 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agr. Sci. 81:
425 107-112. doi:10.1017/S0021859600058378
426 Hess, B. W., G. E. Moss, and D. C. Rule. 2008. A decade of developments in the area of fat
427 supplementation research with beef cattle and sheep. J. Anim. Sci. 86: E188-E204.
428 doi:10.2527/jas.2007-0546
429 Hobson, P. N. 1965. Continuous culture of some anaerobic and facultatively anaerobic rumen
430 bacteria. Microbiology 38: 167-180. doi:10.1099/00221287-38-2-167
431 Huws, S. A., E. J. Kim, S. J. S. Cameron, S. E. Girdwood, L. Davies, J. Tweed, H. Vallin, and N.
432 D. Scollan. 2015. Characterization of the rumen lipidome and microbiome of steers fed a
433 diet supplemented with flax and echium oil. Microbial Biotechnology 8: 331-341.
434 doi:10.1111/1751-7915.12164
435 Huws, S. A., M. R. F. Lee, S. M. Muetzel, M. B. Scott, R. J. Wallace, and N. D. Scollan. 2010.
436 Forage type and fish oil cause shifts in rumen bacterial diversity. FEMS Microbiol. Ecol.
437 73: 396-407. doi:10.1111/j.1574-6941.2010.00892.x
438 Ikwuegbu, O., and J. Sutton. 1982. The effect of varying the amount of linseed oil
439 supplementation on rumen metabolism in sheep. Br. J. Nutr. 48: 365-375.
440 doi:10.1079/BJN19820120
441 Jenkins, T. C., and D. L. Palmquist. 1984. Effect of fatty acids or calcium soaps on rumen and
442 total nutrient digestibility of dairy rations. J. Dairy Sci. 67: 978-986.
443 doi:10.3168/jds.S0022-0302(84)81396-X
444 Kim, E. J., S. A. Huws, M. R. F. Lee, J. D. Wood, S. M. Muetzel, R. J. Wallace, and N. D.
445 Scollan. 2008. Fish oil Increases the duodenal flow of long chain polyunsaturated fatty

446 acids and trans-11 18:1 and decreases 18:0 in steers via changes in the rumen bacterial
447 community. J. Nutr. 138: 889-896.
448 Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board-Invited Review: Use of
449 distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86.
450 doi:10.2527/jas.2007-0550
451 Lardy, G. 2009. Feeding coproducts of the ethanol industry to beef cattle. Ext. Bull. No. AS-
452 1242 North Dakota State University, Fargo.
453 Loerch, S. C., F. L. Fluharty, L. A. Morrow, S. A. Metzger, and T. L. Felix. 2012. Effects of
454 dietary sulfur on ruminal hydrogen sulfide concentrations over time. J. Anim. Sci. 90(E.
455 Suppl):44–45.
456 Maczulak, A. E., B. A. Dehority, and D. L. Palmquist. 1981. Effects of long-chain fatty acids on
457 growth of rumen bacteria. Appl. Environ. Microbiol. 42: 856-862.
458 Maeda, H., C. Fujimoto, Y. Haruki, T. Maeda, S. Kokeguchi, M. Petelin, H. Arai, I. Tanimoto, F.
459 Nishimura, and S. Takashiba. 2003. Quantitative real-time PCR using TaqMan and
460 SYBR Green for *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*,
461 *Prevotella intermedia*, tetQ gene and total bacteria. FEMS Immunol. Med. Microbiol. 39:
462 81-86. doi:10.1016/s0928-8244(03)00224-4
463 Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of
464 polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van
465 Leeuwenhoek 91: 303-314. doi:10.1007/s10482-006-9118-2
466 Mao, S. Y., R. Y. Zhang, D. S. Wang, and W. Y. Zhu. 2013. Impact of subacute ruminal acidosis
467 (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. Anaerobe
468 24: 12-19. doi:10.1016/j.anaerobe.2013.08.003

J. Anim. Sci Accepted Paper, posted 06/09/2017, doi:10.2527/jas2016.1361

469 Masella, A. P., A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld. 2012.
470 PANDAseq: paired-end assembler for Illumina sequences. BMC Bioinformatics 13: 31.
471 doi:10.1186/1471-2105-13-31
472 Masood, A., K. D. Stark, and N. Salem. 2005. A simplified and efficient method for the analysis
473 of fatty acid methyl esters suitable for large clinical studies. J. Lipid Res. 46: 2299-2305.
474 doi:10.1194/jlr.D500022-JLR200
475 McCann, J. C., M. L. Drewery, J. E. Sawyer, W. E. Pinchak, and T. A. Wickersham. 2014.
476 Effect of postextraction algal residue supplementation on the ruminal microbiome of
477 steers consuming low-quality forage. J. Anim. Sci. 92: 5063-5075. doi:10.2527/jas.2014-
478 7811
479 McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L.
480 Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with
481 explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 6:
482 610-618. doi:10.1038/ismej.2011.139
483 Meteer, W. T., D. Faulkner, D. W. Shike, J. W. Adcock, and K. R. Retallick. 2011. Effects of
484 dry-rolled or steam-flaked corn finishing diets with or without twenty-five percent dried
485 distillers grains on ruminal fermentation and apparent total tract digestion. J. Anim. Sci.
486 89: E-Suppl. 2.
487 Minuti, A., A. Palladino, M. J. Khan, S. Alqarni, A. Agrawal, F. Piccioli-Capelli, F. Hidalgo, F.
488 C. Cardoso, E. Trevisi, and J. J. Loor. 2015. Abundance of ruminal bacteria, epithelial
489 gene expression, and systemic biomarkers of metabolism and inflammation are altered
490 during the peripartal period in dairy cows. J. Dairy Sci. 98: 8940-8951.
491 doi:10.3168/jds.2015-9722

492 Moreau, R. A., K. Liu, J. K. Winkler-Moser, and V. Singh. 2011. Changes in lipid composition
493 during dry grind ethanol processing of corn. J. Am. Oil Chem. Soc. 88: 435-442.
494 doi:10.1007/s11746-010-1674-y
495 Muyzer, G., E. C. de Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial
496 populations by denaturing gradient gel electrophoresis analysis of polymerase chain
497 reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59: 695-700.
498 NRC. 2005. Mineral tolerance of animals. 2nd rev. ed. Natl. Acad. Press, Washington, DC.
499 NRC. 2016. Nutrient requirements of beef cattle. 8th ed. Natl Acad. Press, Washington, DC.
500 Pesta, A. C., B. L. Nuttelman, A. L. Shreck, W. A. Griffin, T. J. Klopfenstein, and G. E.
501 Erickson. 2015. Finishing performance of feedlot cattle fed condensed distillers solubles.
502 J. Anim. Sci. 93: 4350-4357. doi:10.2527/jas.2015-9247
503 Ramirez-Farias, C., K. Slezak, Z. Fuller, A. Duncan, G. Holtrop, and P. Louis. 2009. Effect of
504 inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and
505 *Faecalibacterium prausnitzii*. Br. J. Nutr. 101: 541-550.
506 doi:10.1017/S0007114508019880
507 Retallick, K., D. Faulkner, D. Shike, D. Parrett, L. Berger, J. Dahlquist, and T. Nash. 2010.
508 Effects of source of energy on performance, ultrasonic, carcass, and economic
509 characteristics of early-weaned steers. Prof. Anim. Sci. 26: 474-483.
510 doi:10.15232/S1080-7446(15)30634-3
511 Sackmann, J. R., S. K. Duckett, M. H. Gillis, C. E. Realini, A. H. Parks, and R. B. Eggelston.
512 2003. Effects of forage and sunflower oil levels on ruminal biohydrogenation of fatty
513 acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim.
514 Sci. 81: 3174-3181. doi:/2003.81123174x

Journal of Animal Science
J. Anim. Sci Accepted Paper, posted 06/09/2017, doi:10.2527/jas2016.1361

515 Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional
516 recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and
517 Texas Tech University survey. J. Anim. Sci. doi:10.2527/jas.2016-0282
518 Sarturi, J. O., G. E. Erickson, T. J. Klopfenstein, J. T. Vasconcelos, W. A. Griffin, K. M. Rolfe,
519 J. R. Benton, and V. R. Bremer. 2013. Effect of sulfur content in wet or dry distillers
520 grains fed at several inclusions on cattle growth performance, ruminal parameters, and
521 hydrogen sulfide. J. Anim. Sci. 91. doi:10.2527/jas.2012-5627
522 Sasikala-Appukuttan, A. K., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, K. Karges, and
523 M. L. Gibson. 2008. The feeding value of corn distillers solubles for lactating dairy cows.
524 J. Dairy Sci. 91: 279-287. doi:10.3168/jds.2007-0250
525 Segers, J. R., D. Faulkner, K. R. Retallick, and D. W. Shike. 2012. Effects of protein and fat
526 concentration in coproduct-based growing calf diets on performance and carcass
527 composition. J. Anim. Sci. 92: 5603-5611. doi:10.2527/jas.2014-7880
528 Segers, J. R., T. L. Felix, A. R. Green, G. N. Maia, B. C. Ramirez, and D. W. Shike. 2015. Effect
529 of dietary fat concentration from condensed corn distillers' solubles, during the growing
530 phase, on beef cattle performance, carcass traits, digestibility, and ruminal metabolism. J.
531 Anim. Sci. doi:10.3766/jas.2015-8917
532 Soo, R. M., C. T. Skennerton, Y. Sekiguchi, M. Imelfort, S. J. Paech, P. G. Dennis, J. A. Steen,
533 D. H. Parks, G. W. Tyson, and P. Hugenholtz. 2014. An expanded genomic
534 representation of the phylum Cyanobacteria. Genome Biol. Evol. 6: 1031-1045.
535 doi:10.1093/gbe/evu073

536 Stanton, T. B., and E. Canale-Parola. 1980. *Treponema bryantii* sp. nov., a rumen spirochete that
537 interacts with cellulolytic bacteria. Arch. Microbiol. 127: 145-156.
538 doi:10.1007/bf00428018
539 Stevenson, D. M., and P. J. Weimer. 2007. Dominance of *Prevotella* and low abundance of
540 classical ruminal bacterial species in the bovine rumen revealed by relative quantification
541 real-time PCR. Appl. Microbiol. Biotechnol. 75: 165-174. doi:10.1007/s00253-006-0802-
542 y
543 Vander Pol, K. J., M. K. Luebbe, G. I. Crawford, G. E. Erickson, and T. J. Klopfenstein. 2009.
544 Performance and digestibility characteristics of finishing diets containing distillers grains,
545 composites of corn processing coproducts, or supplemental corn oil. J. Anim. Sci. 87:
546 639-652. doi:10.2527/jas.2008-1036
547 Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian classifier for rapid
548 assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ.
549 Microbiol. 73: 5261-5267. doi:10.1128/aem.00062-07
550 Zened, A., S. Combes, L. Cauquil, J. Mariette, C. Klopp, O. Bouchez, A. Troegeler-Meynadier,
551 and F. Enjalbert. 2013. Microbial ecology of the rumen evaluated by 454 GS FLX
552 pyrosequencing is affected by starch and oil supplementation of diets. FEMS Microbiol.
553 Ecol. 83: 504-514. doi:10.1111/1574-6941.12011
554 Zhao, L., Q. Meng, L. Ren, W. Liu, X. Zhang, Y. Huo, and Z. Zhou. 2015. Effects of nitrate
555 addition on rumen fermentation, bacterial biodiversity and abundance. Asian. Austral. J.
556 Anim. Sci 28: 1433-1441. doi:10.5713/ajas.15.0091

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J. Anim. Sci Accepted Paper, posted 06/09/2017, doi:10.2527/jas2016.1361

Table 1. Dietary fatty acid composition

		CDS Inclusion[1]				CDS[2]
Item	CON	0%	10%	19%	27%	100%
Ether extract, % DM	5.53	1.79	4.43	6.80	8.91	27.94
Fatty acids, g/100 g of total fatty acids						
C16:0	15.73	21.79	16.48	15.29	14.70	13.39
C18:0	2.35	4.47	2.71	2.35	2.13	1.69
C18:1 n-9	24.59	20.67	23.48	24.26	24.58	25.27
C18:2 n-6	48.46	44.13	52.14	54.12	54.99	57.38
C18:3 n-3	1.78	5.49	2.84	2.22	1.94	1.18
C20:0	0.42	0.69	0.46	0.40	0.38	0.32
C20:1 n-9	0.62	0.57	0.39	0.35	0.33	0.27
C22:0	0.40	0.71	0.40	0.32	0.29	0.20

559 [1]CDS= condensed distillers solubles.

560 [2]Dietary fatty acid composition of the ingredient.

Table 2. Primers utilized for quantitative PCR of ruminal bacteria.

Bacteria species		Primers (5` - 3`)	Source
Anaerovibrio lipolytica	F	GAAATGGATTCTAGTGGCAAACG	(Minuti et al., 2015)
	R	ACATCGGTCATGCGACCAA	
Butyrivibrio proteoclasticus	F	GGGCTTGCTTTGGAAACTGTT	(Minuti et al., 2015)
	R	CCCACCGATGTTCCTCCTAA	
Eubacterium ruminantium	F	CTCCCGAGACTGAGGAAGCTTG	(Stevenson and Weimer, 2007)
	R	GTCCATCTCACACCACCGGA	
Fibrobacter succinogenes	F	GCGGGTAGCAAACAGGATTAGA	(Stevenson and Weimer, 2007)
	R	CCCCCGGACACCCAGTAT	
Megaspheara elsdenii	F	AGATGGGGACAACAGCTGGA	(Stevenson and Weimer, 2007)
	R	CGAAAGCTCCGAAGAGCCT	
Prevotella bryantii	F	AGCGCAGGCCGTTTGG	(Stevenson and Weimer, 2007)
	R	GCTTCCTGTGCACTCAAGTCTGAC	
Selenomonas ruminantium	F	CAATAAGCATTCCGCCTGGG	(Stevenson and Weimer, 2007)
	R	TTCACTCAATGTCAAGCCCTGG	
Streptococcus bovis	F	TTCCTAGAGATAGGAAGTTTCTTCGG	(Stevenson and Weimer, 2007)
	R	ATGATGGCAACTAACAATAGGGGT	
Eubacterial primer 1	F	GGATTAGATACCCTGGTAGT	(Fliegerova et al., 2014)
	R	CACGACACGAGCTGACG	
Eubacterial primer 2	F	GTGSTGCAYGGYTGTCGTCA	(Maeda et al., 2003)
	R	ACGTCRTCCMCACCTTCCTC	
Eubacterial primer 3	F	CCTACGGGAGGCAGCAG	(Muyzer et al., 1993)
	R	ATTACCGCGGCTGCTGG	

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Table 3. Effect of increasing condensed distillers solubles (CDS) on alpha diversity in the liquid and solid fraction of the ruminal microbiome[1]

		CDS Inclusion				*P*-values[2]		
Item[3]	CON	0%	10%	19%	27%	Trt	L	Q
Liquid fraction								
Chao1	1407	2188	2013	1729	1463	0.03	0.01	0.67
Shannon	5.19	6.58	5.90	5.57	5.26	<0.01	<0.01	0.47
Simpson's	0.880	0.950	0.898	0.907	0.893	0.04	0.02	0.18
Solid fraction								
Chao1	2428	2476	2692	2454	2494	0.89	0.86	0.63
Shannon	7.24	7.19	7.46	7.29	7.48	0.64	0.37	0.79
Simpson's	0.98	0.96	0.97	0.97	0.98	0.37	0.07	0.80

[1]Number of observations: CON (n = 5), 0% (n = 4), 10% (n = 5), 19% (n = 5), 27% (n = 5).
[2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic contrast of CDS inclusion.
[3]Chao1 index describes species richness in a community. Shannon and Simpson's indices describe alpha diversity of a community that represent a combination of species richness and species evenness.

Table 4. Effect of increasing condensed distillers solubles (CDS) on relative abundance (% total reads) of bacterial phyla in the liquid fraction using 16S rRNA sequencing[1]

		CDS Inclusion				*P*-values[2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
Firmicutes	78.4	53.8	73.6	78.4	82.4	<0.01	<0.01	0.09
Bacteroidetes[3]	12.6	25.3	13.6	8.3	9.2	0.08	0.01	0.22
Actinobacteria	2.41	3.27	5.41	5.16	4.11	0.17	0.56	0.11
TM7	1.92	1.81	1.62	1.70	1.02	0.66	0.31	0.60
Cyanobacteria[3]	0.61	7.17	1.26	0.42	0.53	<0.01	<0.01	0.04
Proteobacteria	1.31	1.39	1.32	1.0	0.79	0.53	0.14	0.68
Spirochaetes[3]	0.17	1.23	0.37	0.09	0.24	0.01	<0.01	0.03
Fibrobacteres	0.04	0.08	0.03	0.04	0.06	0.65	0.68	0.16

570 [1]Phyla listed were detected at greater than 0.1% relative abundance averaged across all liquid
571 fraction samples.
572 [2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic
573 contrast of CDS inclusion.
574 [3]Data were logit transformed to ensure normality of residuals.
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Table 5. Effect of increasing condensed distillers solubles (CDS) on relative abundance (% total reads) of bacterial families in the liquid fraction using 16S rRNA sequencing[1]

		CDS Inclusion				*P*-value[2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
Firmicutes								
Ruminococcaceae	44.5	16.3	32.5	31.6	37.8	<0.01	<0.01	0.20
Mogibacteriaceae	1.35	0.79	1.04	1.03	1.51	0.35	0.09	0.63
Erysipelotrichaceae[3]	0.60	0.99	0.70	0.51	0.37	0.50	0.09	0.97
Clostridiaceae[3]	0.27	0.51	0.31	0.26	0.25	0.34	0.06	0.39
Bacteroidetes								
Prevotellaceae[3]	9.82	15.91	9.44	6.96	8.14	0.37	0.08	0.28
Bacteroidales[3,4]	1.14	4.59	1.61	0.40	0.39	<0.01	<0.01	0.34
Paraprevotellaceae[3]	0.66	1.42	0.82	0.15	0.38	0.01	<0.01	0.13
Proteobacteria								
Succinovibrionaceae	0.91	0.27	0.23	0.32	0.14	<0.01	0.57	0.53
RF-32[3,4]	0.10	0.16	0.11	0.05	0.04	0.11	0.01	0.91
Desulfovibrionaceae	0.014	0.0001	0.063	0.077	0.034	0.12	0.21	0.03
Other								
YS2[3,4]	0.53	7.19	1.24	0.40	0.51	<0.01	0.01	0.04
Bifidobacteriaceae	0.08	1.98	1.71	0.83	0.50	0.04	0.02	0.80
Spirochaetaceae	0.22	1.93	0.42	0.11	0.40	<0.01	<0.01	0.01
Corynebacteriaceae	0.11	0.18	0.12	0.26	0.32	0.09	0.07	0.25

[1]Families listed were detected at greater than 0.1% relative abundance averaged across all liquid fraction samples and were affected by dietary treatment ($P < 0.1$).
[2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic contrast of CDS inclusion.
[3]Data were logit transformed to ensure normality of residuals.
[4]Unidentifed sequences listed at the lowest level of taxonomic assignment (order).

Table 6. Effect of increasing condensed distillers solubles (CDS) on relative abundance (% total reads) of bacterial genera in the liquid fraction using 16S rRNA sequencing[1]

		CDS Inclusion				*P*-value[2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
Prevotella[3]	9.82	15.9	9.44	6.96	8.14	0.37	0.08	0.28
Ruminococcus	8.23	5.97	3.82	1.79	1.55	0.09	0.09	0.69
Bifidobacterium	0.08	1.94	1.67	0.82	0.50	0.04	0.02	0.81
Treponema[3]	0.17	1.27	0.36	0.09	0.24	0.01	<0.01	0.02
CF231[3]	0.23	0.94	0.47	0.04	0.22	0.03	0.02	0.12
Oscillospira	0.17	0.60	0.33	0.26	0.10	0.45	0.09	0.80
RFN-20	0.09	0.88	0.14	0.06	0.02	0.01	<0.01	0.62
Coprococcus[3]	0.10	0.16	0.26	0.44	0.10	0.07	0.73	0.03
Corynebacterium	0.11	0.18	0.12	0.26	0.32	0.09	0.07	0.25
Clostridium	0.03	0.36	0.11	0.08	0.08	0.03	0.02	0.21
Shuttleworthia[3]	0.18	0.04	0.05	0.27	0.03	0.03	0.62	0.05

583 [1]Genera listed were detected at greater than 0.1% relative abundance averaged across all liquid
584 fraction samples and were affected by dietary treatment ($P < 0.1$).
585 [2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic
586 contrast of CDS inclusion.
587 [3]Data were logit transformed to ensure normality of residuals.
588

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Table 7. Effect of increasing condensed distillers solubles (CDS) on relative abundance (% total reads) of bacterial phyla in the solid fraction using 16S rRNA sequencing[1]

		CDS Inclusion				*P*-value[2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
Firmicutes	52.6	55.0	65.1	67.2	69.2	0.18	0.10	0.52
Bacteroidetes[3]	37.7	32.4	16.7	17.0	20.5	0.28	0.35	0.24
Actinobacteria	5.29	5.55	6.72	6.69	4.96	0.86	0.84	0.36
TM7[3]	0.48	1.32	0.58	0.75	0.41	0.42	0.12	0.81
Cyanobacteria[3]	0.08	0.11	0.07	0.11	0.02	0.02	0.01	0.09
Proteobacteria[3]	0.24	0.25	0.21	0.58	0.20	0.25	0.81	0.33
Spirochaetes[3]	0.10	0.19	0.06	0.08	0.04	0.36	0.10	0.57
Fibrobacteres[3]	0.23	0.04	0.06	0.04	0.04	0.11	0.97	0.58

589 [1]Phyla listed were detected at greater than 0.1% relative abundance averaged across all solid
590 fraction samples.
591 [2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic
592 contrast of CDS inclusion.
593 [3]Data were logit transformed to ensure normality of residuals.
594
595

Table 8. Effect of increasing condensed distillers solubles (CDS) on relative abundance (% total reads) of bacterial families in the solid fraction using 16S rRNA sequencing[1]

		CDS Inclusion				*P*-value[2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
Firmicutes								
Veillonellaceae	10.5	7.0	10.4	11.4	14.9	0.10	0.01	0.88
Ruminococcaceae	11.3	8.1	9.8	11.8	12.3	0.27	0.04	0.79
Bacteroidetes								
Paraprevotellaceae	1.69	2.90	0.71	0.94	0.39	0.05	0.01	0.17
Bacteroidales[4]	0.96	2.30	0.40	1.10	0.47	0.01	<0.01	0.05
S24-7[3]	0.89	0.21	0.56	0.48	0.57	0.09	0.07	0.24
Other								
Corynebacteriaceae	0.07	0.14	0.14	0.32	0.06	0.03	0.93	0.05
Succinivibrionaceae[3]	0.08	0.02	0.03	0.07	0.02	0.08	0.89	0.11
Desulfovibrionaceae[3]	0.008	0.013	0.052	0.214	0.093	0.01	0.01	0.09

[1]Families listed were detected at greater than 0.1% relative abundance averaged across all solid fraction samples and were affected by dietary treatment ($P < 0.1$).

[2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic contrast of CDS inclusion.

[3]Data were logit transformed to ensure normality of residuals.

[4]Unidentifed sequences listed at the lowest level of taxonomic assignment (order).

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Table 9. Effect of increasing condensed distillers solubles (CDS) on relative abundance (% total reads) of bacterial genera in the solid fraction using 16S rRNA sequencing[1]

		CDS Inclusion				*P*-value[2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
Succiniclasticum	9.4	5.2	8.4	9.3	11.5	0.16	0.02	0.86
Moryella	1.7	1.9	0.9	0.9	1.5	0.03	0.18	<0.01
Coprococcus[3]	0.23	0.40	0.71	0.97	0.81	<0.01	0.04	0.16
Shuttleworthia[3]	1.01	0.32	0.26	0.77	0.48	0.09	0.20	0.89
Mitsuokella	0.04	0.05	0.72	0.47	0.98	0.02	0.01	0.72
Corynebacterium	0.07	0.14	0.14	0.32	0.06	0.03	0.93	0.05

605 [1]Genera listed were detected at greater than 0.1% relative abundance averaged across all solid
606 fraction samples and were affected by dietary treatment ($P < 0.1$).
607 [2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic
608 contrast of CDS inclusion.
609 [3]Data were logit transformed to ensure normality of residuals.
610

Table 10. Effect of increasing condensed distillers solubles (CDS) on relative abundance of bacterial genera in the solid fraction using qPCR.

		CDS Inclusion				*P*-values[1,2]		
Item	CON	0%	10%	19%	27%	Trt	L	Q
A. lipolytica[3]	0.0001	0.0044	0.0002	0.0002	0.0005	0.27	0.21	0.11
B. proteoclasticus[3]	0.0158	0.0747	0.0381	0.0226	0.0537	0.36	0.54	0.23
E. ruminantium	0.2871	0.2089	0.2322	0.2455	0.1814	0.96	0.90	0.69
F. succinogenes[3]	0.0065	0.0058	0.0079	0.0026	0.0027	0.47	0.21	0.70
M. elsdenii[3]	2.8×10^{-5}	1.7×10^{-3}	1.3×10^{-3}	3.7×10^{-3}	1.3×10^{-3}	0.09	0.96	0.79
P. bryantii[3]	2.3×10^{-5}	2.5×10^{-5}	5.2×10^{-5}	1.1×10^{-5}	2.4×10^{-5}	0.69	0.64	0.95
S. ruminantium	0.54	0.83	1.56	1.20	2.18	0.02	0.02	0.69
S. bovis[3]	0.0907	0.0035	0.0006	0.0008	0.0005	<0.01	0.01	0.12
16S rRNA copy no.[4]	7.41	7.30	7.35	7.36	7.40	0.21	0.06	0.94

[1]No period effects were observed ($P < 0.05$).
[2]Trt = main effect of dietary treatment; L = linear contrast of CDS inclusion; Q = quadratic contrast of CDS inclusion.
[3]Data were logit transformed to ensure normality of residuals.
[4]16S rRNA $\log_{10}$ copy number/ng DNA.

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617 **Figure 1**. Principal coordinate analysis (PCoA) of beta-diversity in the liquid fraction using
618 Bray-Curtis similarity. Analysis by PERMANOVA revealed a treatment effect ($P = 0.01$).
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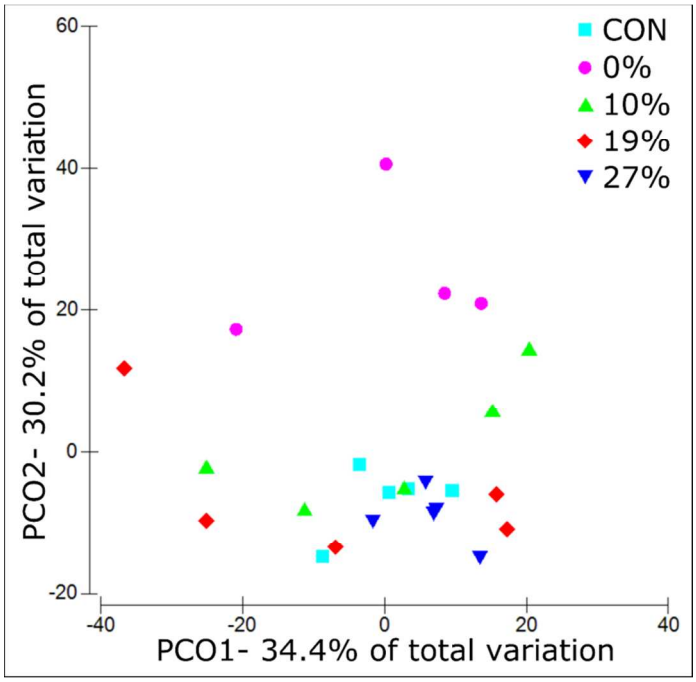
CON
0%
10%
19%
27%
PCO2- 30.2% of total variation
PCO1- 34.4% of total variation
60
40
20
0
-20
-40
-20
0
20
40

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