Effect of hay type on cecal and fecal microbiome and fermentation parameters in horses and efficacy of varying protein sources on feedlot goat performance

by

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Abstract

Six cecally cannulated horses were used in a split plot design in a 2-period crossover. Each period consisted of a 21-d acclimation to hay type followed by a 24-h sample collection. Whole plot consisted of hay type (cool-season grass hay and legume hay) with subplots of sampling location (cecum and rectum) and hour. Fecal and cecal samples were collected every 3 h and analyzed for pH, volatile fatty acids (VFA) and the V4 region of the 16S rRNA gene was sequenced using Illumina HiSeq. Horses fed alfalfa had greater ($P \leq 0.05$) fecal than cecal pH, whereas horses fed brome had greater ($P \leq 0.05$) cecal than fecal pH. Regardless of hay type, total VFA concentrations were greater ($P \leq 0.05$) in cecal fluid than in feces, and alfalfa resulted in greater ($P \leq 0.05$) VFA concentrations than brome in both sampling locations. Alpha diversity was greater ($P \leq 0.05$) in fecal compared to cecal samples. Microbial community structure within each sampling location and hay type differed from one another ($P \leq 0.05$). In all, fermentation parameters and bacterial populations were impacted by hay type and sampling location.

Two experiments were conducted to evaluate impact of varying protein source on feedlot goat performance. In experiment 1 the effects of feeding dried distillers grains with solubles (DDGS) in place of soybean meal (SBM) on growth, economic efficiency, carcass characteristics, backfat fatty acid profiles, and fecal microbiome of Boer-type goats were evaluated. Forty-eight goats were assigned to 1 of 4 dietary treatments consisting of 0% (0DDGS), 10.3% (10DDGS), 20.5% (20DDGS), or 31.1% (30DDGS) DDGS replacing SBM in the total diet. Inclusion of DDGS linearly improved ($P = 0.02$) ADG, while feed cost/kg gain decreased ($P < 0.0001$). There were no discernible differences in fecal percentages of Bacteroidetes ($P = 0.36$) and Firmicutes ($P = 0.12$) among treatments. Polyunsaturated fatty acids tended to quadratically increase ($P = 0.06$) with increased DDGS inclusion. In experiment

2, 75 Boer-type goats were assigned randomly to 1 of 5 dietary treatments consisting of SBM with Ammonia Chloride (AmCl; SBM+AmCl), DDGS with AmCl (DDGS+AmCl), SoyPlus (Dairy Nutrition Plus, Ames, IA) with AmCl (SoyPlus+AmCl), SBM with SoyChlor (Dairy Nutrition Plus Ames, IA; SBM+SoyChlor), and SoyPlus with SoyChlor (SoyPlus+SoyChlor). SoyChlor improved ADG ($P = 0.01$), feed efficiency ($P = 0.04$), and value of gain ($P = 0.01$) when compared to AmCl. SoyPlus had no effect on ADG ($P > 0.10$) when compared to SBM. Protein source did not alter Firmicutes:Bacteroidetes ($P > 0.10$). Goats fed SBM+AmCl had greater ($P = 0.04$) abundance of Bacteroidetes than goats fed DDGS+AmCl. No differences were detected in alpha and beta diversity measures. Loin eye area was greater in goats fed SBM compared to SoyPlus ($P = 0.05$) or DDGS ($P = 0.04$), regardless of chloride source. Alternative protein sources in goats may improve feed cost/kg gain without negatively impacting goat performance.

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Dedication

I would like to dedicate my thesis to my parents, Michael and Debra Sorensen. Thank you for always encouraging me to reach my goals.

Chapter 1 - Review of Literature: Next generation sequencing and microbiome analyses in horses and goats

Introduction

Next-generation, high-throughput deoxyribonucleic acid (DNA) sequencing has made genome sequencing faster and easier compared to previous methods. By amplifying single DNA segments rather than cloning DNA fragments, as with older methods, more sequences are generated in a shorter period of time (Ansorge, 2009). Furthermore, large numbers of sequences are obtained in parallel rather than 96 sequences typically processed in older capillary electrophoresis-based Sanger methods (Mardis, 2007; Hutchison III, 2007). As such, high-throughput sequencing is the preferred method for sequencing large numbers of samples in a short amount of time.

History of DNA sequencing

Even though the structure of DNA was discovered in 1953 (Watson and Crick, 1953), it took nearly 15 years for the first DNA sequence technique to be developed. This was largely due to problems related to the chemical properties of DNA molecules, chain length, only 4 residues in DNA compared to 20 amino acids found in other proteins, and lack of knowledge of base-specific DNAases (Hutchison III, 2007). Initial efforts were made in sequencing RNA, largely transfer RNA (tRNA) or microbial ribosomal RNA (rRNA), as it is more available in relatively pure populations compared to DNA. Since RNA lacks a complimentary strand, its simplicity yields strands that are often shorter than most eukaryotic DNA molecules (Heather and Chain, 2016). Furthermore, RNAase enzymes were available to cut RNA molecules at specific points. Holley et al. (1965) was the first to determine the sequence of an alanine tRNA from

Saccharomyces cerevisiae, leading the way for the sequencing of other nucleic acids. Deoxyribonucleic acid sequencing followed similar methods, using enzymes to cleave strands into fragments of 10 to 20 base pairs (bp) that could then undergo 2-dimensional (2D) chromatography or electrophoresis followed by chromatography (Hutchison III, 2007). Yet, early researchers were unable to determine entire gene sequences.

Sanger et al. (1965) developed a method for 2D fractionation of radioactive nucleotides. This technique used RNA digests, prepared by the action of ribonuclease T_1 or pancreatic ribonuclease, to distinguish differences between di-, tri-, and most tetra-nucleotides based on nucleotide separation via diethylaminoethyl (DEAE)-paper. This technique enabled sequencing of small RNAs and ultimately lead to the sequencing of 5S ribosomal RNA from *Escherichia coli* (Brownlee et al., 1967), coat protein cistron of R17 bacteriophage RNA (Adams et al., 1969), a yeast tyrosine tRNA (Madison et al., 1966), and numerous others (Cory et al., 1968; Dube et al., 1968; Min Jou et al., 1972)

A decade after the fractionation method was introduced, Sanger and Coulson (1975) released a new technique known as the "plus and minus" method for determining nucleic acid sequences. The "minus" system used DNA polymerase I, which is added to DNA template with only 3 deoxyribotriphosphates, to synthesize a chain until it reaches the triphosphate that was left out during electrophoresis, thus terminating the chain at the 3' end before the missing residue. The "plus" system begins with a similar mixture of DNA templates as its counterpart, but T4 polymerase and only 1 triphosphate is added. This mixture is then fractionated by electrophoresis with bands indicating the location of the added residue on a radioautograph (Sanger and Coulson, 1975). Sanger and Coulsen's (1975) method was simpler and reasonably rapid

compared to previous techniques. However, both the “plus” and “minus” had to be used together to generate a complete sequence.

Sanger and colleagues (1977) developed a technique called the “chain-termination” or “dideoxy” method to solve problems associated with the plus and minus method. In short, 2’, 3’-dideoxythymidine (ddT) triphosphates were used to terminate the sequence in thymidylic acid’s (dT) position because ddT does not contain a 3’-hydroxyl group. Thus, when the mixture was fractionated using gel electrophoresis, the pattern of the bands showed the distribution of dT. Analogous terminators could then be used for the remaining nucleotides in separate incubations before running them in parallel and the pattern of bands could then be read off as the sequence (Sanger et al., 1977). This method proved to be simpler compared to older techniques as it required no preliminary DNA extension and produced fewer artifact bands (Sanger et al., 1977). This method, with some modifications as technology developed, dominated the field of DNA sequencing for more than a decade (Heather and Chain, 2016).

The Human Genome Project, initiated in 1990, encouraged advancements in sequencing to produce high-throughput data. Prior to this project, slab gel electrophoresis was the primary method used to generate DNA sequences. However, capillary electrophoresis soon became the obvious choice to create the high-throughput data necessary for the Human Genome Project and subsequent projects (Carrilho, 2000). Capillary electrophoresis separates DNA fragments based on size within fused-silica capillaries that are modified to be chemically inert to DNA in a matter of minutes rather than hours (Smith and Nelson, 2004). The system could separate DNA fragments containing hundreds of base pairs into single bases by using high-separating voltages (Smith and Nelson, 2004). Continued advancements in sequencing techniques led to faster

throughput and decreased cost, all while maintaining or improving quality standards through massively parallel sequencing platforms (Rogers and Venter, 2005).

Next generation sequencing

The first next-generation sequencer (NGS) became available in 2005, known as the 454 GenomeSequencer FLX (454 GS FLX) originally developed by 454 Life Sciences and later acquired by Roche Applied Science (Morozova and Marra, 2008; Ansorge, 2009; Heather and Chain, 2016). This system attaches DNA fragments with specific adaptor sequences to a bead. Beads then undergo water-in-oil emulsion polymerase chain reaction (PCR) for fragment amplification to incorporate an average of 1 DNA molecule on each bead (Heather and Chain, 2016). Amplification of DNA is necessary to provide enough light signal intensity to detect sequences in subsequent steps (Ansorge, 2009). Prior methods relied largely on cloning DNA fragments rather than amplification via PCR, which is less labor intensive. Each bead is then placed into a well at the top of a picoliter reaction plate and pyrosequencing is performed by washing a homopolymer, containing bead-linked enzymes and deoxynucleotidetriphosphates (dTNPs), over the plate to catalyze a reaction (Huse et al., 2007). Pyrophosphate is released based on which dTNP is added, detected as emitted light, and measured using a charged couple device sensor placed beneath the wells (Ansorge, 2009; Heather and Chain, 2016). The sequence corresponding to the complementary nucleotide of the pyrophosphate released is read using a pyrogram (Morozova and Marra, 2008). Using this method, researches were capable of producing reads 400 to 500 bp long for each coated bead within each well, therefore providing massively parallel sequencing (Heather and Chain, 2016).

More NGS were developed following 454 GS FLX. One of the more popular was the Solexa platform, now known as Illumina, which became commercialized in 2006. Similar to 454

GS FLX, the Illumina platform utilizes PCR for amplification rather than cloning. The Illumina platform differs from its predecessor in that it incorporates a sequencing-by-synthesis chemistry via a fluorescent labeled reversible terminator for each nucleotide base (Ansorge, 2009). Single-stranded DNA fragments are attached to a flow cell and then adapter bracketed DNA molecules are passed over to create a subsequent DNA template. The bending of replicating DNA strands over original molecules is referred to as "bridge amplification" or "bridging" (Morozova and Marra, 2008; Heather and Chain, 2016). Once a nucleotide is incorporated into the DNA template, the sequence is read based on fluorescent dye associated with each nucleotide by a charged couple device camera. After the terminator nucleotide is read, located on the 3' end, it is removed and the synthesis cycle is repeated (Ansorge, 2009).

Limitations associated with next generation sequencing

While the introduction of NGS has been invaluable to genome sequencing, it also comes with the risk of high error rates. Since pyrosequencing relies on homopolymer runs, a major source of errors, such as insertions and deletions (indels), result from misjudging the length of the run (Hutchison III, 2007). Huse et al. (2007) obtained 340,150 reads using the Roche GS20 (454 Life Sciences) and found 89% of total errors are from homopolymer effects such as indels and insertions with substitutions. The authors also determined insertions were the most common error, with an error rate of 0.18% on 32,801,429 bases (Huse et al., 2007) using the same method. Gilles et al. (2011) reported a slightly greater insertion rate at 0.273% on 8,596,016 bases using the 454 GS FLX Titanium platform when compared to Huse and colleagues, who used the Roche GS20. Both Huse et al. (2007) and Gilles et al. (2011) reported error rates for deletions (0.13% and 0.23, respectively), mismatches (0.08% and 0.02%, respectively), and ambiguous base calls (0.10% and 0.01%, respectively). Despite these errors, the GS20 and GS-

FLX Titanium sequencers matched their reference sequences 82% (Huse et al., 2007) and 67.57% (Gilles et al., 2011) of the time. In fact, < 2% of all reads contained a disproportionate number of errors, which accounted for approximately 50% of miscalls (Huse et al., 2007).

Dohm et al. (2008) characterized 2 data sets generated by the Illumina 1G platform to analyze biases in output and found a reduced error rate (< 0.01%) of insertions and deletions when compared to the error rate reported for the 454 Roche GS FLX (Gilles et al., 2011) and Roche GS40 (Huse et al., 2007) platforms. Since the Illumina method utilizes a separate step for each homopolymer run, fewer indels are typically found (Hutchison III, 2007). Similar to Huse and colleagues, a majority (> 25%) of the total insertions were in homopolymer tracts, yet no clear trend was seen for deletions (Dohm et al., 2008).

Mismatches in base calls may also be a problem across NGS platforms. Dohm et al. (2008) reported adenine (A) or tyrosine (T) were most frequently substituted for cysteine (C) in Illumina runs and C to guanine (G) transversions were noted the least. Using 454 sequencing, Huse et al. (2007) reported A to G and T to C transition mismatches occurred more frequently than others. There also may be an interaction between mismatches and the position within the sequence or with the length of the final sequences. In fact, Gilles et al. (2011) reported longer sequences tended to produce greater error rates, as only 10.09% of full length sequences produced error-free reads.

In addition to errors associated with indels and mismatches, sequencing errors may lead to inflated estimates of diversity. Ideally, operational taxonomic units (OTU) should represent the number of phylotypes in a sample. However, Kunin et al. (2010) reported an overestimation in diversity when comparing 454 GS FLX reads from 2 regions – about 4,250 reads each – of the 16S rRNA genes of *Escherichia coli*. Chimeras may be a major factor, often causing an

overestimation of diversity and incorrect identification of taxonomic groups (Ashelford et al., 2006). While a majority of chimeras form between similar sequences, which affects diversity to a smaller degree, chimeras that form between different phyla may be considered novel organisms if not identified as an anomaly (Haas et al., 2011).

While NGS is prone to high error rates, assemblies of data can still be highly accurate due to the large quantity of obtainable sequences (Hutchison III, 2007). High error rates may be resolved by deep sequencing of the gene in question or by checking data with computer software (Ashelford et al., 2006). Furthermore, sequence quality does not degrade based on previous errors as the run continues (Huse et al., 2007).

Analysis of microbial communities

Once high-throughput sequencing is complete, generated sequences are subjected to downstream analysis. This is done through microbial analysis packages such as Qiime (Kuczynski et al., 2012) or Mothur (Schloss et al., 2009) which take raw reads from a high-throughput sequencer and implement analyses to classify data taxonomically and calculate alpha and beta diversity. Additionally, tables and graphs can be created to help visualize complex data. However, this review will focus on Qiime as it is the microbial analysis package for studies in the following chapters.

Qiime, pronounced “chime”, is an acronym for Quantitative Insights in to Microbial Ecology (Kuczynski et al., 2012). Raw sequence data are taken from Illumina, Roche 454, etc. and analyzed using a series of typed commands to produce graphical and textual output. The program clusters all samples in OTUs based on their similarity to other sequences. For instance, sequences that are clustered with 97% similarity are considered to represent a species, while others may only be classified to their phylum. A representative sequence from each OTU is

selected and then used for further analysis. The selected sequence is taxonomically classified against a known gene database, like Greengenes (McDonald et al, 2012). Phylogenic relationships are classified and used to develop a phylogenic tree which demonstrates relationships between OTUs. A heatmap showing the distribution of OTUs in a community can also be constructed to further visualize the make-up of communities. Qiime can compute alpha and beta diversity indices and generate rarefication curves or Principal Coordinate Analysis (PCoA) plots.

Alpha diversity refers to diversity within a microbial community, or within samples. This is achieved by measuring species richness alone, for example by using the total number of taxa found in a sample (Costa et al, 2018), and through phylogenic measures, which combines species richness and evenness in calculations (Knight et al., 2018). Species richness can be analyzed using measurments like observed OTUs or via the Chao-1 abundance estimator. Another common measurement is Faith's phylogenetic diversity measure, which accounts for evenness within samples. Evenness represents how evenly split individuals are among species, as low values indicate few species dominating all others (Morris et al., 2014). Faith's diversity measure is more sensitive to the number of sequences in samples compared to similar measures, such as the Shannon index and Simpson index (Knight et al., 2018).

Beta diversity, on the other hand, compares diversity between microbial communities by measuring dissimilarity and creating a distance matrix. Analysis of beta diversity is either qualitative or quantitative. Qualitative analyses include binary-Jaccard or unweighted UniFrac while quantitative analyses include Bray-Curtis, Canberra, and weighted UniFrac (Knight et al., 2018). UniFrac has been found as a useful phylogenetic measure for environmental samples. Lozupone et al. (2007) determined unweighted UniFrac is helpful in detecting difference in the

presence or absence of bacterial lineages in different communities while weighted UniFrac is better suited for detecting differences in relative abundances between communities. Lozupone et al. (2007) also reported that weighted UniFrac may be better suited for analyzing transient changes, such as those related to nutrient availability, in microbial communities. However, UniFrac may be influenced by the number of sequences per sample, therefore rarefying or jackknifing sequences is recommended (Lozupone et al., 2011). Statistical analyses to determine beta diversity clustering between groups are typically performed using non-parametric permutation tests, such as PERMANOVA or ANOSIM. Furthermore, beta diversity is typically visualized using PCoA or principal coordinate analysis (PCA; Knight et al., 2018).

Using 16S rRNA to characterize bacterial communities

The 16S rRNA gene is widely sequenced to identify bacterial populations as it contains highly conserved and hypervariable regions that are useful in identifying bacteria (Brunstein, 2016). Both gram positive and gram negative bacteria can be detected and characterized rapidly with NGS (Jonasson et al., 2002). In a study by Tewari et al. (2011), 54 known bacterial isolates from animals were analyzed using both pyrosequencing and Sanger (500 bp) sequencing to evaluate each method's ability to identify the isolates and time to run sequences. The authors reported Sanger sequencing produced longer reads (484 ± 50 bp) than pyrosequencing (37 ± 7). Of the total isolates, 80% of reads from pyrosequencing could be identified to the genus order and 43% to the species level while 100% of reads from the Sanger method could be identified to the genus level and 87% to the species level. However, pyrosequencing was able to distinguish *Enterococcus cecorum* from *Streptococcus bovis* and other species that are of clinical importance. Tewari et al. (2011) also reported that pyrosequencing required 2.5 h to finish a run whereas Sanger sequencing required 7.5 h (Tewari et al., 2011).

Since NGS utilizes short reads, it is critical to understand which region(s) of the 16S rRNA gene should be amplified. Utilizing ruminal digesta, Myer et al. (2016) compared the V1 to V3 region using the Illumina MiSeq platform to the V1 to V8 regions sequenced by Pacific Biosciences RSII platform. Myer et al. (2016) determined that smaller regions (V1 to V3) of the 16S rRNA gene provided adequate depth, based on a rarefication curve, to characterize the bacterial community. While the smaller region produced 25,000 reads compared to 40,000 reads of the V1 to V8, diversity measures such as Shannon Diversity, Chao-1, and Good's coverage estimator were not different (Myer et al., 2016). However, sequencing the longer regions via Pacific Biosciences RSII platform produced greater phylogenic resolution and greater taxonomic accuracy (Myer et al., 2016).

To help characterize the function of select bacteria identified in microbial communities, culture methods have been used extensively to aid in understanding metabolic function. Bacteria are isolated and grown in pure or mixed cultures to study preferred substrates, end products, and synergistic relationships. Culture mediums may be nonselective such as cecal or ruminal fluid, which provide growth factors for multiple bacterial species. Selective mediums promote growth of a specific group of bacteria based on substrates added to a basal culture medium that is devoid of other sugars or carbohydrates (Dehority and Grubb, 1976). For instance, isolated cellulolytic bacteria may be added to a media of rumen fluid where its sugars are replaced with cellulose.

To maintain anaerobic conditions within a culture, systems such as anaerobic jars, roll tubes, or chambers may be used. Anaerobic jars utilize a GasPak and palladium catalyst to maintain anaerobic conditions; however, obligate anaerobes are unable to grow in anaerobic jars as strict anaerobic conditions are not continuous when the jar is opened and then resealed. Hungate's roll tube technique promotes greater growth of strictly anaerobic bacteria and archaea,

as the roll tubes contain strictly anaerobic medium. Unfortunately, Hungate's roll tube technique is a complex and time-consuming process. Lastly, anaerobic chambers allow for complete anaerobic conditions throughout incubation as every step of incubation may be performed within the chamber (Lagier et al., 2015). Not all bacteria are capable of being cultured, as some require strict anaerobic conditions, synergistic relationships with other microbes, or substrates that cannot be replicated in culture medium. While culture-based methods allow researchers to understand the functionality of certain bacteria that increase or decrease in an environment, only 10% of bacterial species in the rumen are believed have been identified thus far (Nagaraja, 2016). Through using NGS technologies to further identify which bacteria are present in a population, regardless of if they've been cultured or not, implications can be made to further understanding of complex interactions between microbes and substrates.

Detection of microbial communities in the horse

Fiber digestion and fermentation

As a grazer and hindgut fermenter, the horse has a highly functional cecum capable of fermenting structural carbohydrates into volatile fatty acids (VFA), which are then used for energy. The hindgut (cecum, large colon, small colon, and rectum) makes up approximately 62% of the volume of the gastrointestinal tract (GIT; Kararli, 1995). Liquid may pass from the stomach to the cecum in as little at 1.5 h (Argenzio et al., 1974) while most particulate matter takes 3 h to reach the cecum (Van Weyenberg et al., 2006). The cecum contains a large microbial population that maximizes bacterial fermentation by adapting to dietary substrates. Mackie and Wilkins (1988) reported total culturable cecal bacteria counts to be 21.20×10^8 per g of gut contents while digesta from the pelvic flexure of the colon contained 12.70×10^8 bacteria per g of gut contents. Mean retention time (MRT) in the cecum of horses consuming Timothy hay is

approximately 3 h (Miyaji et al., 2014). For horses consuming grass hay, the MRT of the large colon and small colon is 15 to 19 h and 5 to 8 h, respectively. Total MRT in the hindgut is approximately 21 to 29 h (Jensen et al., 2014; Miyaji et al., 2014).

Dry matter intake (DMI) significantly impacts passage rate and subsequent digestibility of forage. In horses consuming Timothy hay at 2.0 kg DM·100 kg body weight $(BW)^{-1}\cdot day^{-1}$, Miyaji et al. (2014) reported a shorter MRT through the hindgut than horses consuming 1.3 kg DM·100 kg $BW^{-1}\cdot day^{-1}$. Authors also found decreased total tract DM, neutral detergent fiber (NDF), and acid detergent fiber (ADF) digestibility at 2.0 kg DM forage·100 kg $BW^{-1}\cdot day^{-1}$, which is presumably due to decreased MRT.

The majority of energy absorbed from the equine GIT is supplied via glucose and VFA. Volatile fatty acids are the predominant energy source in forage-fed horses, while glucose increases and VFA decreased as concentrates are increased in a diet. Vermorel et al. (1997) fed horses various levels of grass or alfalfa hay and determined that VFA and glucose supplied 65 to 78% and 11 to 14% of absorbed energy, respectively. Of energy supplied by VFA, Gilinsky and colleagues (1976) determined approximately 30% of VFA were produced in the cecum. Similar to the rumen, acetate is the VFA found in greatest concentration in the equine hindgut, with propionate and butyrate following in smaller concentrations (Mackie and Williams, 1988).

Microbial community in the horse

Published literature which describes the microbial community structure of the equine cecum and colon is limited. The horse GIT contains communities of viruses, bacteria, archaea, protozoa, and fungi throughout the tract. This collection of microbiota, or microbiome, mirror the host's needs based on fermentable substrates (Costa et al., 2018). Therefore, dietary changes

can drastically alter the microbiome, thus improving or hampering overall health (Coverdale, 2016).

Studies designed to characterize the gut microbiome of the horse are typically achieved through cannulation, euthanasia, or fecal sampling. Fecal samples have been the most widely used as the collection process is the least invasive method; however, feces only represent the microbial community in the distal hindgut and do not reflect the microbial structure of the cecum (Coverdale, 2016). Several researchers have worked to characterize microbial populations throughout the GIT. Dougal et al. (2012) compared samples collected from the lumen of the cecum, right dorsal colon (RDC), and rectum to determine differences in bacterial communities with quantitative PCR (qPCR) using specific 16S rRNA gene-targeted primers. Dougal et al. (2012) reported that the cecum contained more bacterial DNA/g of digesta than digesta obtained from the RDC and feces. Additionally, the RDC contained more archaeal mcrA genes than either the cecum or feces. Based on the Shannon-Wiener diversity index, Dougal et al. (2012) reported the microbial community in the RDC was more diverse than that of the cecum. The authors also evaluated the metabolome, or metabolites in a biological system, and found an increase in total VFA and acetate concentration in the RDC compared to the cecum and rectum. Propionate and N-butyrate concentrations did not differ between the RDC and cecum, but were less in the feces (Dougal et al., 2012).

Dougal et al. (2013) went on to characterize the core microbiome of horses and ponies using the Roche 454 GS FLX Titanium platform. Based on Simpson and Shannon diversity measures, diversity between the cecum, right ventral colon (RVC), left ventral colon (LVC), left dorsal colon (LDC), and RDC were not different. However, using qPCR to report bacterial load (ng DNA/mg DM), authors found larger DNA counts in each subsequent compartment, likely

due to live and dead bacteria migrating down the tract until expelled in the feces. Using Illumina MiSeq sequencing to analyze diversity (Simpson's Diversity Index), Costa et al. (2015) reported that the microbial communities in the stomach and small intestine were less diverse compared to the cecum, colon, and rectum. Aside from the stomach and small intestine, no other adjacent compartments within the GIT differed in their microbial diversity measures.

When consuming a forage-based diet, the most abundant phyla from various regions in the equine hindgut is Firmicutes (Costa et al., 2012; Dougal et al., 2013; Costa et al., 2015). According to Dougal et al. (2013) and Costa et al. (2012) the second most abundant phyla is Bacteroidetes followed by Fibrobacteres, Spirochaetes, and Proteobacteria. Yet, other studies have reported Verrucomicrobia to be the second most dominant phylum in fecal samples of forage-fed horses (Costa et al., 2015; Shepard et al., 2012). Shepard et al. (2012) analyzed fecal samples from 2 Arabian geldings consuming orchardgrass hay using Roches 454 GS-FLX. Firmicutes (43.7% of total bacterial sequences) was the dominant phyla with Verrucomicrobia, Proteobacteria, and Bacteroidetes also present at approximately 3-4% of relative abundance. Differences in results between studies may result from sequencing different regions of the 16S rRNA gene, type of sequencing platform used, sampling location, or number of horses sampled.

Indeed, dietary changes alter the gut microbiota. Willard et al. (1977) reported lower cecal pH 4 to 6 h post feeding in concentrate only-fed horses consuming largely oats and corn compared to horses fed only a legume-grass hay. Cecal acetate concentration was greater post-feeding in legume-grass hay-fed horses compared to their counterparts whereas cecal propionate concentrations were greater in concentrate-fed horses, presumably due to a changes in cecal microbial populations (Willard et al., 1977). Daly et al. (2012) reported Bacteroidetes were greater in concentrate-fed horses compared to their grass-fed counterparts as determined by

oligonucleotide-RNA hybridization with seven oligonucleotide probes to target specific bacteria populations. This is likely explained by the fact that genera within Bacteroidetes favor non-structural carbohydrates for fermentation and are more resilient to acidic conditions. In the same study, *Fibrobacter spp*. and bacteria within *Ruminococcaceae* were greater in grass-fed horses as they degrade fiber and may be suppressed by more acidic environments (Daly et al., 2012).

Warzecha et al. (2017) characterized the short-term effects of introducing a low-starch (0.9 g non-structural carbohydrate (NSC)/kg BW) and a high-starch (1.8 g NSC/kg BW) diet on the cecal microbiome. For both treatments, Bacteroidetes was the most abundant phylum followed by Firmicutes, Proteobacteria, Verrucomicrobia, Tenericutes, Spirochaetes, and Fibrobacteres. Both diets resulted in decreased *Ruminococcus* and increased *Prevotella* numbers over the first 12 h after feeding. Bacteria in the genus *Prevotella* are largely hemicellulolytic and pectinolytic and are common in the rumen of concentrate-fed cattle (Nagaraja, 2016).

Using roll tubes to determine viable counts and quantification of bacteria via oligonucleotide probes, Julliand et al. (1999) reported *Ruminococcus flavefaciens* is the major cellulolytic bacteria in the cecum of the horse followed by *F. succinogenes*. *Ruminococcus albus* was detected at low (< 0.01% of RNA quantified) levels. *Ruminococcus flavefaciens*, *R. albus*, and *F. succinogenes* are common cellulolytic bacteria in the rumen (Julliand et al., 1999).

Many of the previous studies have a confounding effect of diet as the horses utilized were donated and euthanized for various other nondigestive tract disorders and diseases (Dougal et al., 2012; Dougal et al., 2013; Costa et al., 2015). Furthermore, sequence depth varies across studies, making inferences difficult. Lastly, while fecal samples give an insight as to what is happening distal colon, they are not sufficient to characterize microbial shifts that occur in cecum and proximal sections of the GIT.

Characterization of microbial communities in goats

Microbial fermentation

Goat populations across the Midwest have increased 110% from 2002 to 2019 (NASS, 2002; NASS, 2019). As a result, the demand for low-cost feedstuffs has increased. The goal for producers and nutritionists is to improve animal performance and subsequent profitability. In ruminants, microbial fermentation occurs in the rumen and hindgut to produce VFA which are absorbed and used as an energy source for the animal. While the rumen accounts for 52.9% of the relative capacity of the GIT in the goat, the hindgut (cecum and colon) accounts for 12.7% (Kararli, 1995) and contributes 13% of total apparent energy absorbed through the GIT (Arieli and Sklan, 1985).

Volatile fatty acids provide 50 to 70% of a ruminant animal's energy (Membrive, 2016). Volatile fatty acids are weak acids with a pKa $\leq$ 4.8 that exist in the rumen as anions (Bergman, 1990). The predominant VFA in the rumen are acetate, propionate, and butyrate. Ruminal pH is typically 5.8 to 6.8 and acetate is produced in the greatest concentration with high forage diets when pH is above 5.7 (Membrive, 2016). Increased inclusion of concentrates in a goat's diet will lead to increased propionate production and decreased pH, subsequently lowering acetate:propionate in the rumen. Acetate is used as the primary energy substrate in animal tissues and can be converted into triglycerides by adipocytes for storage. Propionate is gluconeogenic via the liver, thus providing another energy source for striated tissues or glucose can be converted to lactose in mammary glands. Butyrate, produced in the smallest concentration relative to acetate and propionate, is mostly (95%) utilized as an energy source by rumen epithelial cells. Remaining butyrate enters the bloodstream and is converted to ketone bodies or long chain fatty acids in the liver (Membrive, 2016).

As the percent of dietary concentrates increase, pH may drop below 5.7 which inhibits fibrolytic bacteria and subsequent acetate production. Propionate-producing bacteria are more resilient at this decreased pH which results in a decrease in acetate:propionate. As pH approaches 5, VFAs are present in their undissociated state and are more permeable to the epithelium (Bergman, 1990). In goats, increasing the concentrate:forage has been reported to upregulate SCFA-/HCO_3- transporters and genes that help maintain intracellular pH by removing VFA from the rumen (Yan et al., 2014).

Much of the dietary protein consumed by a ruminant is fermented in the reticulo-rumen as it contains proteolytic bacteria and protozoa which hydrolyze peptide bonds to produce peptides and amino acids. These peptides and amino acids are then transported into the bacterial cell where they undergo further hydrolysis and deamination. Few free amino acids are found in ruminal fluid, indicating rapid fermentation of amino acids in the rumen (Nagaraja, 2016). Within the bacterial cell, valine, leucine and isoleucine undergo deamination and decarboxylation to form ammonia, CO_2, and branched-chain fatty acids (BCFA) such as isobutyrate, isovalerate, and 2-methylbutyrate. Ammonia, CO_2, and BCFA are then utilized by fibrolytic bacteria for growth and subsequently increased fermentation processes (Tamminga, 1979; Membrive, 2016).

Protein that escapes microbial degradation within the rumen is termed ruminally undegraded protein (RUP). However, the majority of protein supplied to the small intestine is in the form of microbial cell protein (MCP). Ruminally undegradable protein and MCP undergo hydrolysis via proteolytic enzymes in the small intestine before being readily absorbed. Any substrate leaving the small intestine is subject to fermentation in the cecum and colon.

Depending on the amount of undegraded cellulose and hemicellulose reaching the cecum and colon, degradation in the hindgut may range from 18 to 27% and 30 to 40% of total cellulose and hemicellulose digested, respectively (Hoover, 1978). When sheep, also small ruminants, were fed a diet containing 8.3% cellulose and 18% crude protein, 26% of total cellulose was digested in the hindgut (Arieli and Sklan, 1985).

Microbial community in goats

While the gut microbiome of cattle has been extensively studied, there are relatively few studies regarding the microbial community of the GIT in goats. Much of the literature regarding the goat's microbiome as it relates to nutrition has centered around the impact of altering the concentrate:forage ratio in diets. Mao et al. (2014), using the 454 GS FLX Titanium platform, sequenced the ruminal microbiome of goats fed a total mixed ration (TMR) containing 0%, 25%, or 50% corn (DM basis). Goats fed 0% corn had greater alpha diversity, based on an analysis of richness and the Shannon index when compared to those consuming 50% corn. Furthermore, Firmicutes was the most abundant phylum (57%) and Bacteroidetes was the second most abundant (32.2%) in goats across treatments. Grilli et al. (2016) reported Firmicutes (54.3%) as the dominant phylum in the rumen of goats fed a diet of 60% alfalfa hay:40% corn or 100% alfalfa and Bacteroidetes was the second most dominant phylum at 41.4%.

Similar bacterial populations have been reported in the cecum and colon. Firmicutes was the most abundant phylum for the cecum and colon of goats consuming a TMR (corn silage, midicago sativa hay, corn, and wheat bran) at 80.38% and 81.87%, respectively. Similarly, Bacteroidetes (5.99% and 5.61%, respectively) was the second most dominant (Tao et al., 2017).

Goats consuming a diet of 60% alfalfa hay:40% corn had a greater ruminal abundance of an unclassified group from the *Bacteroidales* order and an unclassified group from the

Ruminoccaceae order when compared to those consuming only alfalfa. Additionally, the higher concentrate:roughage diet resulted in a reduced abundance of *Prevotella* and *Butyrivibrio* (Grilli et al., 2014). Mao et al. (2014) showed *Prevotella* and *Papillibacter* were less in goats fed 50% corn (DM basis) as compared to those consuming 0% and 25% corn diets. However, *Butyrivibrio* was reported to increase in goats as corn inclusion rates increased. *Butyrovibrio fibrisolvens* and *Prevotella sp*. are noncellulolytic degrading bacteria that can degrade hemicellulose. *Prevotella sp*. is also a major pectin-degrading bacterium (Nagaraja, 2016). Differences in the abundance of bacteria at the genus level between studies are likely influenced by dietary differences. Tao et al. (2017) found *Turcibacter*, *Clostridium*, *Oscillospira*, *Prevotella*, and *Bacteroides* to increase in the colon of goats fed a greater concentrate:roughage diet.

Archaea populations in the rumen are also changed in response to dietary substrates. Methanogens use end products of bacterial fermentation, specifically H_2 and CO_2, to help maintain the partial pressure of H_2 that may otherwise inhibit microbial enzymes (Morgavi et al., 2010). As more carbohydrates are degraded in the rumen methanogen populations increase, with the most noticeable increase in *Methanobrevibacter* (Mao et al., 2014).

Summary

While dietary effects on microbial populations in the gut and their subsequent end products in large ruminants are well understood, similar literature is lacking in horses. Similarly, relatively few researchers have evaluated the effect of diet on the goat hindgut microbiome. Compared to older methods, next generation sequencing provides larger data output to help identify alterations in the microbiota of the GIT. As quantitative methods continue to advance in high-throughput sequencing, the role of bacteria and archaea on animal health and performance become more apparent. Indeed, limitations exist with NGS but it continues to be on the forefront

of current research as there is much unknown about the microbial community within the GIT of horses and goats. Thus, the objectives of the following studies were to characterize the microbiome of the cecum and rectum in forage-fed horses and to evaluate the efficacy of various protein sources on feedlot goat performance by targeting the 16S rRNA gene for bacterial and archaeal identification.

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Chapter 2 - Effect of hay type on cecal and fecal microbiome and fermentation parameters in horses

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Abstract

Although forage is the primary component of equine diets, the effect of hay type on the microbiome of the gastrointestinal tract is relatively unexplored. The objective of this trial was to characterize the cecal and fecal microbiome of mature horses consuming alfalfa or smooth bromegrass (brome) hay. Six cecally cannulated horses (527 ± 16.3 kg; 12 ± 0.83 yr) were used in a split plot design run as a crossover in 2 periods. Whole plot treatment was *ad libitum* access to brome or alfalfa hay fed over two 21-d acclimation periods with subplots of sampling location (cecum and rectum) and sampling hour. Each acclimation period was followed by a 24-h collection period where cecal and fecal samples were collected every 3 h for analysis of pH and volatile fatty acids (VFA). Fecal and cecal samples were pooled and sent to a commercial lab (MR DNA, Shallowater, TX) for amplification of the V4 region of the 16S rRNA gene and sequenced using Illumina HiSeq. Main effects of hay on VFA, pH, and taxonomic abundances were analyzed using the MIXED procedure of SAS 9.4 with fixed effects of hay, hour, location, period, all possible interactions and random effect of horse. Alpha and β diversity were analyzed using the R Dame package. Horses fed alfalfa had greater ($P \leq 0.05$) fecal than cecal pH whereas horses fed brome had greater cecal than fecal pH ($P \leq 0.05$). Regardless of hay type, total VFA concentrations were greater ($P \leq 0.05$) in the cecum than in feces, and alfalfa resulted in greater ($P \leq 0.05$) VFA concentrations than brome in both sampling locations. Alpha diversity, measured using observed operation taxonomic units, Shannon, and Fisher alpha indices, was greater ($P \leq 0.05$) in fecal compared to cecal samples. Based on the Bray-Curtis distance matrix, microbial community structure within each sampling location and hay type differed from one another ($P \leq 0.05$). Bacteroidetes were greater ($P \leq 0.05$) in the cecum compared to the rectum, regardless of hay type. Firmicutes and Firmicutes:Bacteroidetes were greater ($P \leq 0.05$) in the

feces compared to cecal samples of alfalfa-fed horses. Cecal abundance of *YRC22*, *Prevotella*, and [*Prevotella*] were greater ($P \leq 0.05$) compared to feces regardless of hay type. *Ruminococcus* was unaffected ($P > 0.10$) by hay type, location, and any possible interactions. In all, fermentation parameters and bacterial populations were impacted by hay type and sampling location in the hindgut.

Introduction

Adapted for grazing and having a highly functional cecum capable of fermenting structural carbohydrates into volatile fatty acids (VFA), horses rely on forage consumption to maintain gut health. The gastrointestinal microbiome and fermentation parameters of horses on forage-only diets have been studied on a limited basis through the use of fecal samples or euthanasia. While fecal samples reflect changes in the distal part of the hindgut, they are not representative of the major fermentation chambers in the hindgut: the cecum and large colon (de Fombelle et al., 2003; Dougal et al., 2012). Studies performed with cannulated horses have focused predominantly on the impacts of dietary concentrates or abrupt shifts in diet. Limited literature is available on the microbiome of horses when compared to ruminants.

Through the use of next generation sequencing, shifts in microbiota within the equine gastrointestinal tract can be detected efficiently and characterized through sequencing of the 16S rRNA gene. This gene contains highly conserved and hypervariable regions that are useful for bacterial identification (Brunstein, 2016). Bacterial populations in the hindgut largely dictate VFA concentrations. Firmicutes have been reported to be the most abundant phyla in fecal samples of live horses and all digestive compartments of euthanized horses fed various forage and forage plus grain diets (Fernandes et al., 2014; Costa et al., 2015). Our objective was to

evaluate fermentation parameters and characterize the fecal and cecal microbiome of mature horses consuming alfalfa or smooth bromegrass hay.

Materials and Methods

Animals

All animal protocols were approved by the Kansas State University Institutional Animal Care and Use Committee. Before initiation of the study, all horses were housed together in a single dry lot and fed *ad libitum* smooth bromegrass hay (brome). Experimental units consisted of 6 mature Quarter Horses (12 ± 0.83 yr; 537 ± 16.3 kg) previously fitted with cecal cannulae (Beard et al., 2011) that were housed within their respective treatment group in adjacent dry lots (21.6 × 22.6 m). Each lot was equipped with an automatic waterer, hay feeder, and salt block.

Experimental design and dietary treatments

Experimental design consisted of a split-plot, crossover where whole plot consisted of hay type (alfalfa or brome) with subplots of sampling time (hour) and sampling location within the gastrointestinal tract (cecum and rectum). On d 0, horses were randomly assigned to 1 of 2 dietary treatments: *ad libitum* brome (n = 3) or *ad libitum* alfalfa (n = 3) and remained on their respective diet for 22 d. Horses were group fed and hay was pitched into feeders at 0700 and 1900 as needed to allow horses *ad libitum* access to hay. Brome hay was pitched from a round bale while alfalfa was pitched from a large square bale. On d 22, cecal and fecal samples were collected. On d 23, horses were moved into the opposite pen to consume alternate hay type and the protocol repeated. Refusals of hay per pen were recorded at 0700 and 1900 on the final 4 d of each treatment period to determine dry matter intake (DMI).

Sample collection and laboratory analyses

Hay samples were collected prior to the beginning of period 1 with a hay core sampler (#07190, AgraTronix, Streetsboro, OH) and sent to a commercial laboratory (Dairy One Forage Lab, Ithaca, NY) for proximate analysis (Table 2.1).

Cecal and fecal samples were collected every 3 h for 24 h on d 22 of each period. Horses were placed into stocks, the cannula plug was removed, and cecal contents were collected via gravity flow. Fecal samples were collected via rectal grab. All samples for microbial analysis were collected in sterile 15-mL conical centrifuge tubes (Nunc Conical Sterile Polypropylene Centrifuge Tubes, Thermo-Fisher Scientific, Waltham, MA). Cecal samples were collected directly into conical tubes and approximately 10 mL of the inner portion of fecal samples were placed into conical tubes. Samples were immediately placed in a -20° C freezer for 24 h before being transported and stored in a -80° C freezer until DNA extraction and microbial sequencing were performed.

An additional sample of cecal fluid and fecal matter was collected at each time point and immediately strained through 4 layers of cheesecloth into 180-mL containers (Specimen Storage Containers, #4A0180, Thermo-Fisher Scientific, Waltham, MA). Strained cecal and fecal fluid were immediately measured for pH via a portable pH meter (Thermo Scientific Orion 3 Star Portable pH Meter, Waltham, MA). From each sample, three 1-mL aliquots of strained fluid were transferred by pipette into microcentrifuge tubes containing 0.25 mL of 25% metaphosphoric acid (wt/vol) for deproteination. Samples were then stored at -20° C until volatile fatty acid (VFA) analysis.

Deproteinated cecal and fecal fluid were thawed and centrifuged at 17,000 × g for 30 min. The aqueous supernatant were transferred to gas chromatography (GC) vials and analyzed

for VFA concentrations on an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA) fitted with a 15 m × 0.53 mm × 0.5 µm film thickness Nukol capillary column (Supelco columns; Sigma-Aldrich, St. Louis, MO) and flame ionization detector. Hydrogen was used as the carrier gas at a flow rate of 35 mL/min. Initial oven temperature was 80° C for 1 min and increased 20° C/min for 6 min to reach a final temperature of 200° C for 6 min. Inlet and detector temperatures were 250° C. Quantification of VFAs was completed by comparison against a known standard (Supelco Volatile Fatty Acid Standard Mix, Sigma-Aldrich, St. Louis, MO) containing acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate.

Cecal and fecal samples for deoxyribonucleic acid (DNA) extraction were pooled relative to feeding (0700 and 1900) within sampling location. In brief, cecal samples from time points 3 h, 6 h, 9 h, and 12 h were pooled in equal proportions as were samples 15 h, 18 h, 21 h, and 24 h per horse to represent the microbiome relative to the 0700 and 1900 feedings, respectively. To pool samples, individual samples were vortexed (Scientific Industries Vortex-Genie 2, Houston, TX) until thawed and kept on ice to minimize shifts in microbial populations. One g of each original sample was added into a sterile 15-mL conical centrifuge tube using sterilized lab scoops (Stainless Steel Lab Scoops, Thermo-Fisher Scientific, Waltham, MA). Pooled samples were vortexed to mix and frozen at -80° C. In total, 24 pooled cecal samples and 24 pooled fecal samples were shipped on dry ice to MR DNA (MR DNA, Shallowater, TX) for DNA extraction, amplification of the V4 region of the 16S gene, and sequencing using Illumina HiSeq protocols.

Deoxyribonucleic acid (DNA) extraction was performed using the Powersoil DNA Isolation Kit (Qiagen Inc., Valencia, CA) by following manufacturer's instructions. DNA was stored at -20° C until polymerase chain reaction (PCR) amplification.

The V4 region of the 16S gene underwent PCR amplification using the universal Eubacterial primers 515F (5'-GTGYCAGCMGCCGCGGTAA) and 806R (5'-GGACTACNGGGTWTCTAAT) with a barcode attached to the forward primer. All samples underwent a single step, 30-cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). Twenty-five µL of HotStarTaq Master Mix were added to samples followed by a diluted mix of primers (each primer had a final concentration of 0.1 to 0.5 µM) to reach a volume of 50 µL, minus the volume (< 1 µL/50 µL reaction) of template DNA added next. Samples then underwent PCR with the following conditions: 94° C for 3 min. Followed by 28 cycles of 94° C for 30 s, 53° C for 40 s and 72° C for 1 min; and lastly a final elongation step at 72° C for 5 min. After amplification, success of amplifications and relative intensity of bands was determined using electrophoresis in a 2% agarose gel. Amplicon products of different samples were pooled together based on molecular weight and DNA concentration and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, Beverly, MA). Sequencing was performed on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) following the manufacturers guidelines and based on a method originally described by Dowd et al. (2008).

Raw sequence data were processed through Qiime 1 (www.qiime.org). Raw sequences were joined and depleted of barcodes and primers. Data were then cleaned and checked for quality control using default settings in Qiime 1. Operational taxonomic units (OTU) were defined by clustering at 3% divergence (97% similarity) against an open reference and final OTUs were taxonomically classified against the 16S rRNA Greengenes database (http://greengenes.secondgenome.com/).

Statistical analyses

Data were analyzed utilizing the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Main and linear effects of hay type on VFA concentrations, pH, and microbial abundance using fixed effects of hay, period, location, hour (VFA and pH only) and all possible interactions, random effect of horse, and repeated measures of hour and location were analyzed. Degrees of freedom were determined using the Kenward-Rogers approximation. Differences were defined at $P \leq 0.05$; a tendency was determined at $0.05 < P \leq 0.10$. The PDiff option of SAS was used to determine differences between least-squares means.

Alpha and beta diversity were analyzed using the R Dame package (https://acnc-shinyapps.shinyapps.io/DAME/). Diversity indices used to evaluate alpha diversity included the observed OTU, Shannon index, and Fisher's alpha index with data at the OTU level. Comparisons were made via the Mann Whitney U test. Beta diversity was analyzed using PERMutational Multivariate Analysis of Variance to assess group differences. Data were plotted on a 2D principal coordinates analysis (PCoA) plot generated through DAME using the Bray-Curtis distance matrix.

Results

Dry matter intake

Dry matter intake per pen did not differ between hay types ($P = 0.64$; Figure 2.1). Horses consumed an average of 2.73% and 2.82% of their body weight in brome and alfalfa, respectively.

Cecal and fecal pH

When discussing the effect of time all timepoints (h) will be in relation to the morning feeding with h 0 at 0600. There were no observed main effects of location or hour on pH ($P >$

0.46), nor were there observed interactions between hay and hour ($P = 0.46$) or between hay, hour, and location on pH ($P = 0.62$; Table 2.2). An interaction between hay and location was detected ($P = 0.0003$) as horses consuming brome had a greater ($P = 0.01$) cecal fluid pH than fecal pH. Horses consuming alfalfa had greater fecal pH compared to cecal fluid pH ($P = 0.0004$). Within the cecum, horses fed brome had greater pH ($P = 0.05$) than those fed alfalfa. Fecal pH was greater ($P = 0.0004$) in alfalfa-fed horses compared to their brome-fed counterparts.

Cecal and fecal VFA

Interactions between hay, hour, and location were observed with acetate, propionate, butyrate, and total VFA concentrations ($P \leq 0.05$; Table 2.2). Cecal concentrations of acetate, butyrate, and total VFA were elevated ($P \leq 0.05$) in alfalfa-fed horses at all sample times compared to cecal samples from those consuming brome. In alfalfa-fed horses, acetate concentrations were greater ($P \leq 0.004$) in cecal samples at h 0, 9, 12, 15, 18, 21, and 24 compared to fecal samples. Propionate concentrations were greater ($P \leq 0.04$) in cecal samples of alfalfa-fed horses at h 0, 3, 15, 18, 21, and 24 compared to cecal samples of brome-fed horses and was greater ($P = 0.0007$) in fecal samples of alfalfa-fed horses than in fecal samples of brome-fed horses at h 18. Fecal concentrations of butyrate and total VFA in alfalfa-fed horses were greater ($P \leq 0.0001$) than brome-fed horses at h 18. Total VFA concentrations in alfalfa-fed horses were elevated ($P \leq 0.05$) in cecal samples compared to fecal samples at h 0, 9, 12, 15, and 21. No differences ($P > 0.05$) were detected in acetate, propionate, acetate:propionate (A:P), butyrate, and total VFA concentrations in brome-fed horses between cecal and fecal samples. A hay by hour interaction was detected ($P \leq 0.05$) for acetate, propionate, butyrate, and total VFA concentrations. Furthermore, a hay by location interaction ($P \leq 0.05$) was observed for acetate

and A:P, as both were elevated in cecal samples of alfalfa-fed horses compared to cecal samples of brome-fed horses ($P < 0.0001$) and fecal samples of alfalfa-fed horses ($P \leq 0.01$).

Microbial composition

A total of 3,201,298 reads were sequenced from 48 samples. The average number of reads per sample was 66,639 ± 17,767, with a minimum of 33,356 and a maximum of 107,390 reads per sample observed. Taxa that did not appear consistently through samples and unidentified genera and species were removed for statistical analysis.

Phylum

Approximately 11 phyla of bacteria were identified through taxonomic classification (Table 2.3). Bacteroidetes was the most abundant phyla detected in the cecum of brome-fed and alfalfa-fed horses (52 and 51.03%, respectively) while Firmicutes was the most abundant phyla detected in the rectum of brome-fed and alfalfa-fed horses (44.17 and 62.82%, respectively).

A hay by location interaction ($P \leq 0.05$) was noted for the phyla Bacteroidetes, Firmicutes, Spirochaetes, unassigned, Tenericutes, Actinobacteria, Cyanobacteria, and Firmicutes:Bacteroidetes (F:B), and there was a tendency ($P = 0.06$) for an interaction with Proteobacteria. Bacteroidetes was greater ($P \leq 0.0005$) in cecal samples of brome and alfalfa-fed horses compared to fecal samples and were more ($P = 0.002$) abundant in fecal samples of brome-fed horses compared to fecal samples of those consuming alfalfa. Fecal abundance of Firmicutes were greater ($P \leq 0.0001$) than the abundance detected in cecal samples within alfalfa-fed horses, which ultimately lead to an increased F:B ($P = 0.004$). Spirochaetes were observed in greater ($P \leq 0.003$) abundance in fecal samples of brome-fed horses compared to cecal samples of brome-fed horses and fecal samples of alfalfa-fed horses. Tenericutes were greater ($P \leq 0.0006$) in fecal samples of alfalfa-fed horses compared to cecal samples of alfalfa-

fed horses and fecal samples of brome-fed horses. Bacteria within Actinobacteria were more abundant ($P \leq 0.02$) in fecal samples of brome and alfalfa-fed horses compared to cecal samples, with a greater ($P = 0.01$) percentage in fecal samples of alfalfa-fed horses compared to brome. Cecal samples of brome-fed horses had greater ($P \leq 0.01$) abundance of Cyanobacteria than fecal samples of brome-fed horses and cecal samples of alfalfa-fed horses. Verrucomicrobia was greater ($P \leq 0.0001$) in fecal samples compared to cecal samples regardless of hay type. Fibrobacteres were greater ($P = 0.006$) in brome-fed horses than alfalfa-fed horses.

Class

Approximately 28 classes of bacteria were observed following taxonomic classification. Of those, only 10 classes had ≥ 1% relative abundance (Table A.1). Bacteroidia were observed in the greatest abundance in samples taken from the cecum of brome-fed and alfalfa-fed horses (52%% and 51.03%%, respectively), followed by Clostridia, Spirochaetes, Bacilli, Fibrobacteria, unassigned, Erysipelotrichi, and Mollicutes. Clostridia were the most abundant class in fecal samples of brome-fed and alfalfa-fed horses (41.58% and 52.93%%, respectively), followed by Bacteroidia, Sprirochaetes, Erysipelotrichi, Verruco-5, Bacilli, Unassigned, Mollicutes, and Methanobacteria.

Order

Approximately 35 orders of bacteria were identified through taxonomic classification. Of those, 10 demonstrated ≥ 1% relative abundance (Fig. A.2). *Bacteroidales* were observed in the greatest abundance in the cecum of brome- and alfalfa-fed horses (52% and 51.03%, respectively). *Clostridiales* were observed in greater abundance in samples collected from the rectum of brome-fed horses compared to alfalfa-fed horses (41.57% and 52.91%, respectively). The remaining orders that individually contributed to ≥ 1% abundance included *Spirochaetales*,

unassigned, *WCHB1-41*, *Lactobacillales*, *Erysipelotrichales*, *RF39*, *Fibrobacterales*, and *Methanobacteriales*.

Family

Approximately 52 families of bacteria were observed following taxonomic classification. Of those, 18 comprised ≥ 1% abundance (Table 2.4). A hay by location interaction ($P \leq 0.05$) was observed for *Mogibacteriaceae* and an unidentified family within the order *Bacteroidales*, which were more abundant in cecal samples compared to fecal samples of horses fed both hay types. *Lachnospiraceae* were greater ($P \leq 0.02$) in fecal samples of horses consuming alfalfa than cecal samples of alfalfa-fed horses and fecal samples of brome-fed horses. *Ruminococcaceae* tended to be affected ($P = 0.06$) by the interaction between hay type and location, as fecal abundance of this family was greater ($P = 0.0009$) than cecal abundance in horses consuming alfalfa. Cecal abundance of *Clostridiaceae* and an unidentified family within the *Clostridiales* order were less ($P \leq 0.02$) in horses fed alfalfa than the abundance in cecal samples of horses fed brome and fecal samples of alfalfa-fed horses. *Spirochaetceae* was greater ($P \leq 0.003$) in fecal samples of horses fed brome than fecal samples of alfalfa-fed horses and cecal samples of brome-fed horses. *Erysipelotrichaceae*, *Streptococcaceae*, and an unidentified family within *RF39* were greater ($P \leq 0.03$) in fecal samples compared to cecal in alfalfa-fed horses and greater than fecal samples of brome-fed horses. A tendency for a hay by location interaction ($P = 0.10$) was observed in *Lactobacillaceae*, as it was detected in greater ($P \leq 0.02$) abundance in cecal samples of horses consuming alfalfa compared to fecal samples of alfalfa-fed horses and cecal samples of brome-fed horses. Furthermore, *Lactobacillaceae* was greater ($P = 0.01$) in fecal samples of alfalfa-fed horses compared to fecal samples of brome-fed horses.

Paraprevotellaceae, *RFP12*, and *Prevotellaceae* were influenced ($P \leq 0.05$) by location as *Paraprevotellaceae* and *Prevotellaceae* were greater ($P \leq 0.05$) in cecal than fecal samples, regardless of hay type, and *RFP12* was greater ($P \leq 0.0001$) in fecal than cecal samples, regardless of hay type. *Veillonellaceae* tended to be greater ($P = 0.06$) in fecal compared to cecal samples, regardless of hay type. *Fibrobacteraceae* was more abundant ($P = 0.009$) in horses fed brome than those fed alfalfa, regardless of location. No differences ($P \geq 0.15$) were observed in *Methanobacteriaceae* between location, hay type, and any possible interactions.

Genus

Approximately 86 genera of bacteria were observed following taxonomic classification. Of those, 15 make up ≥ 1% relative abundance (Table 2.5). A hay by location interaction ($P \leq 0.05$) was observed for *YRC22*, *Lactobacillus*, *Fibrobacter*, and *Clostridium*. *Clostridium* and *Lactobacillus* were greater ($P \leq 0.0007$) in cecal samples of alfalfa-fed horses than fecal samples of alfalfa-fed horses and *Lactobacillus* was greater ($P = 0.005$) in cecal samples of alfalfa-fed horses compared to cecal samples of brome-fed horses while *Clostridium* was greater ($P < 0.0001$) in cecal samples of brome-fed horses compared to alfalfa-fed horses. Cecal abundance of *Fibrobacter* was greater ($P = 0.02$) in horses consuming brome compared to those fed alfalfa. Cecal abundance of *YRC22*, and [*Prevotella*] were greater ($P \leq 0.0006$) and *Prevotella* tended to be greater ($P = 0.06$) compared to fecal abundance, regardless of hay type. *Ruminococcus* was unaffected ($P > 0.17$) by hay type, location, and any possible interactions.

Species

Most bacteria were not identified at the species level. However, 2 species were indentifed and comprised ≥ 1% relative abundance (Table 2.6). *Fibrobacter succinogenes* was greater ($P = 0.006$) in horses consuming brome compared to alfalfa. Horses consuming brome had increased

Ruminococcus flavefaciens ($P \leq 0.004$) in cecal samples compared to fecal samples within brome-fed horses and compared to cecal samples of alfalfa-fed horses. The abundance of *R. flavefaciens* was greater ($P = 0.004$) in feces than in cecal samples of horses consuming alfalfa.

Alpha and beta diversity

Alpha diversity differed between locations within treatment at the OTU level (Figure 2.2). Fecal samples had greater diversity based on the Fisher alpha ($P < 0.0001$), observed OTU ($P \leq 0.002$), and Shannon ($P \leq 0.0001$) indices compared to cecal samples regardless of hay type. In cecal samples, OTU index did not differ ($P > 0.10$) between horses fed brome or alfalfa; however, the Shannon index was greater ($P = 0.0002$) and the Fisher alpha index tended to be greater ($P = 0.09$) in horses fed brome. No differences were detected ($P > 0.10$) in alpha diversity measures between hay types in fecal samples.

Based on dissimilarity and distant measures to assess beta diversity, cecal and fecal samples between hay types were different ($P \leq 0.05$). Furthermore, within cecal samples and fecal samples, hay types differed ($P \leq 0.05$) based on clustering in different quadrants of the plot in Figure 2.3.

Discussion

To date, evaluating the effects of hay type on the cecal and fecal microbiome and fermentation parameters in the horse has not been published. Cecal and fecal VFA concentrations did not differ in brome-fed horses and may be due to slower fermentation through compartments of the hindgut. However, horses consuming alfalfa had increased VFA concentrations in the cecum compared to fecal samples collected from the rectum. Dougal et al. (2012) and de Fombelle et al. (2003) also reported greater VFA concentrations in the cecum than the small colon of euthanized horses fed varied forage:concentrate diets. As expected, cecal pH

was lower than fecal pH in alfalfa-fed horses in the current study. Also, cecal pH of brome-fed horses was greater than alfalfa-fed horses. This was expected and most likely due to greater concentrations of VFA in the cecum of horses consuming alfalfa, which likely resulted from more rapid fermentation of readily fermentable structural carbohydrates, like pectin. Dry matter intake did not differ between hays, which was unexpected. Consequently, daily digestible energy intake was similar between brome and alfalfa (31.91 Mcal/d and 31.41 Mcal/d, respectively). Yet, increased fermentation of hay into VFA were reported in alfalfa-fed horses compared to brome-fed horses. Regardless of hay type, cecal and fecal pH values were similar to those reported in previous literature (Coverdale et al., 2004; Hussein et al., 2004; Jordan et al., 2019). It should be noted that cecal and fecal pH in brome-fed horses was expected to be similar, yet a lower pH was found in feces. This was largely attributed to 1 horse on trial who was an outlier with low fecal pH (pH < 6) while consuming brome.

Firmicutes were the dominant phyla in fecal samples obtained from horses consuming alfalfa and brome. Others have reported Firmicutes as the most abundant phyla in the feces of horses fed ryegrass-clover pasture (Fernandes et al., 2014) and orchardgrass hay (Shepard et al., 2012). Fernandes et al. (2014) reported similar observations in that Firmicutes increased in fecal samples due to greater abundances of *Lachnospiraceae*, *Ruminococcaceae*, and *Clostridiales*. Daly et al. (2001) observed a majority of sequences from luminal contents of the hindgut of euthanized grass-fed horses to be within the *Clostridiaceae* family, largely the cellulolytic *Clostridium* spp., along with *Butyrivibrio* spp., *Ruminococcus* spp. and *Eubacterium* spp. The current study also observed *Ruminococcus* and *Clostridium* to make up ≥ 1% of sequences.

In ruminants, *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes* are considered the most abundant cellulose degrading ruminal microbes (Nagaraja,

2016). Julliand et al. (1999) identified *R. flavefaciens* to be the most abundant cellulolytic bacteria followed by *F. succinogenes* in the cecum horses fed a 70% legume-orchardgrass hay:30% concentrate diet. *R. albus* was not detected in horses with oligonucleotide probes. In the current study, *F. succinogenes* was more abundant than *R. flavefaciens* in brome-fed horses and *F. succinogenes* was more abundant in brome-fed horses compared to alfalfa-fed horses. While *Ruminococcus* was unaffected by hay type or location, *Fibrobacter* was more abundant in cecal samples of brome-fed horses than alfalfa. *R. flavefaciens* was found to be more abundant in samples collected from cecum compared to the samples collected from the rectum of brome-fed horses; however, it was more abundant in fecal samples than cecal samples of alfalfa-fed horses. Even though the most bacteria were unable to be identified to the specie level in the current study, it appears *F. succinogenes* and *R. flavefaciens* play a role in cellulolytic degradation in the hindgut of hay-fed horses.

Stewart et al. (2018) reported Bacteroidetes as the dominant phylum followed by Firmicutes in fecal samples from horses consuming timothy hay. In the current study, Bacteroidetes was the dominant phylum in cecal samples, which is consistent with work by Warzecha et al. (2017) in cannulated horses fed up to 1.8 g nonstructural carbohydrates (NSC)/kg body weight (BW; as fed). Bacteroidetes was largely driven as the most abundant phylum in the cecum by increased *Paraprevotellaceae, Prevotellaceae*, and other unclassified *Bacteroidales*. *Prevotella* sp. are unable to digest cellulose but are capable of degrading hemicellulose and pectin (Nagaraja, 2016) and aid in peptide breakdown (Wallace et al., 1997). Therefore *Prevotella* sp. likely were elevated in the cecum of horses due to more structural carbohydrates and available protein.

Treponema was more abundant in fecal samples regardless of hay type. This may be due to the fact that Treponema utilizes products of cellulose fermentation, therefore as cellulose is continuously degraded through the hindgut, *Treponema* increase as well. (Stanton and Canale-Parola, 1980; Paster and Canale-Parola, 1982).

Alpha diversity is used to evaluate microbial diversity within samples. Similar to results from the current study where alpha diversity was greater in fecal compared to cecal samples, Dougal et al. (2012) noted increased Shannon diversity in fecal samples compared to cecal samples. Diversity typically decreases as soluble carbohydrate inclusion increases, which is likely a result of decreased pH brought on by increased VFA concentrations which ultimately inhibits some bacteria while promoting other bacteria. Warzecha et al. (2017) reported decreased Shannon index values after horses were fed a high starch concentrate (up to 1.8 g NSC/kg BW, as fed).

Beta diversity measures differences in diversity between hay type and location. Fernandes et al. (2014) reported no detectable difference in beta diversity at the genus level of horses adapted to a commercial ensiled conserved forage-grain diet or ad libitum ryegrass-clover pasture. Costa et al. (2015) observed diversity of compartments in the hindgut (cecum, pelvic flexure, small colon, and rectum) of horses were similar in euthanized horses of various ages and breeds fed grass hay and dietary concentrate. Costa et al. (2015) reported the similarity in diversity and bacterial communities at higher taxonomic levels between fecal, cecal, and large colon samples allow for fecal samples to be used as an adequate representation of the main fermentation chambers in the horses. Based on the current study, dissimilarity in microbial community structure was found between all hay by location interactions, therefore fecal samples were not representative of the cecal microbial environment.

Conclusion

In all, hay type impacts pH, VFA concentrations, and the gastrointestinal microbiome of horses. Microbial fermentation in alfalfa-fed horses produces the greatest VFA concentration in the cecum due to more readily available structural carbohydrates for microbial fermentation. Alpha diversity measures were greater in fecal samples compared to cecal samples due to the increase of VFA concentration in the cecum compared to rectum, most notably in alfalfa-fed horses. Fecal samples differed in abundance of taxa and diversity measures when compared to cecal samples, therefore fecal samples are not representative of microbiota shifts occurring in the cecum.

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Table and Figures

Table 2.1. Diet composition (dry matter basis) of hay[1]

Item	Brome[2]	Alfalfa
Dry matter, %	93.90	90.30
Crude protein, %	7.10	19.30
Crude fat, %	3.60	2.30
Neutral detergent fiber, %	62.30	47.20
Acid detergent fiber, %	38.90	38.70
Digestible energy, Mcal/kg	2.18	2.07
Calcium, %	0.40	1.63
Phosphorus, %	0.10	0.29
Magnesium, %	0.12	0.22
Potassium, %	1.43	2.39
Sodium, %	0.01	0.02
Iron, mg/kg	147.00	730.00
Zinc, mg/kg	11.00	24.00
Copper, mg/kg	5.00	10.00
Manganese, mg/kg	55.00	41.00

[1]Fed *ad libitum* to horses

[2]Smooth bromegrass

Table 2.2. Effect of hay type on cecal and rectal pH and VFA concentrations in horses[1]

		Brome		Alfalfa			
Item	**Time[2]**	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[3]**
pH	0	6.86^{ac}	6.46^{b}	6.79^{ac}	6.98^{c}		
	3	6.84^{a}	6.57^{a}	6.72^{a}	7.11^{b}		
	6	6.79^{a}	6.62^{a}	6.66^{a}	7.07^{b}		
	9	6.89^{ac}	6.56^{b}	6.69^{b}	7.10^{c}		
	12	6.82^{a}	6.54^{b}	6.80^{a}	6.98^{a}	0.12	F, F*L
	15	6.83^{a}	6.54^{b}	6.72^{ab}	7.08^{c}		
	18	6.80^{ab}	6.60^{a}	6.66^{a}	7.03^{b}		
	21	6.91^{a}	6.60^{b}	6.65^{b}	6.95^{ab}		
	24	6.88^{a}	6.61^{b}	6.77^{ab}	7.00^{a}		
Acetate, mM	0	36.58^{a}	23.14^{a}	72.34^{b}	40.96^{a}		
	3	28.03^{a}	29.80^{a}	61.30^{b}	43.03^{ab}		
	6	32.62^{a}	26.69^{a}	58.50^{b}	43.46^{ab}		
	9	46.20^{a}	33.50^{a}	79.39^{b}	42.29^{a}		F, H, L,
	12	39.18^{a}	33.35^{a}	84.85^{b}	42.58^{a}	8.74	F*L,
	15	36.66^{a}	47.29^{a}	76.05^{b}	46.38^{a}		F*H,
	18	35.98^{a}	26.97^{a}	77.44^{b}	76.42^{b}		F*H*L
	21	37.13^{a}	29.30^{a}	73.75^{b}	40.88^{a}		
	24	32.12^{a}	33.55^{a}	64.32^{b}	43.98^{a}		
Propionate, mM	0	9.40^{a}	5.49^{a}	12.70^{b}	8.78^{ab}		
	3	6.89^{a}	7.36^{ab}	9.89^{b}	9.21^{ab}		
	6	7.57	6.34	9.47	9.19		
	9	10.96	8.05	13.34	9.43		F, H,
	12	10.10^{a}	8.31^{a}	15.01^{b}	8.79^{a}	1.84	F*H,
	15	9.23^{a}	10.99^{ab}	12.77^{b}	9.08^{ab}		F*H*L
	18	8.59^{a}	6.20^{a}	12.49^{b}	15.71^{b}		
	21	8.60^{a}	6.96^{a}	12.01^{b}	8.23^{ab}		
	24	7.73^{a}	7.60^{a}	10.70^{b}	9.38^{ab}		
Acetate:Propionate, mM	0	4.11^{a}	4.36^{a}	5.91^{b}	4.90^{ab}		
	3	4.23^{a}	4.16^{a}	6.38^{b}	5.01^{a}		
	6	4.43^{a}	4.49^{a}	6.21^{b}	4.72^{a}		
	9	4.12^{a}	4.42^{a}	5.98^{b}	4.71^{a}		
	12	3.92^{a}	4.21^{a}	5.66^{b}	4.75^{ab}	0.42	F, F*L
	15	4.01^{a}	4.52^{ac}	5.95^{b}	5.20^{bc}		
	18	4.20^{a}	4.79^{a}	6.26^{b}	4.93^{a}		
	21	4.31^{a}	4.55^{a}	6.18^{b}	4.95^{a}		
	24	4.12^{a}	4.39^{a}	6.11^{b}	4.65^{a}		
Butyrate, mM	0	2.92^{a}	2.27^{a}	5.47^{b}	3.75^{ab}		
	3	2.13^{a}	3.17^{ab}	4.16^{b}	4.25^{b}		F, H, l†,
	6	2.71^{a}	2.56^{a}	4.40^{b}	3.93^{ab}	0.85	F*H,
	9	4.78^{a}	3.19^{a}	6.26^{b}	4.05^{a}		F*H*L

	12	3.58[a]	3.48[a]	7.35[b]	3.62[a]		
	15	3.62[a]	4.08[a]	6.09[b]	4.21[a]		
	18	3.58[a]	2.38[a]	6.10[b]	7.16[b]		
	21	3.57[a]	2.72[a]	5.69[b]	3.83[a]		
	24	2.93[a]	3.02[a]	4.94[b]	4.25[ab]		
Total VFA, mM	0	48.90[a]	32.48[a]	90.50[b]	57.90[a]		
	3	37.05[a]	41.92[a]	75.35[b]	61.68[ab]		
	6	42.91[a]	37.21[a]	72.38[b]	61.71[ab]		
	9	61.94[a]	47.23[a]	99.00[b]	61.49[a]		F, H, L,
	12	52.86[a]	47.33[a]	107.21[b]	60.19[a]	11.92	F*H,
	15	49.50[a]	65.72[a]	94.91[b]	65.36[a]		F*H*L
	18	48.15[a]	37.32[a]	96.03[b]	109.45[b]		
	21	49.30[a]	40.71[a]	91.45[b]	58.29[a]		
	24	42.78[a]	47.23[a]	79.95[b]	62.37[ab]		

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations include the cecum and rectum

[2]Time, h: 0 = 0600 (baseline), 3 = 0900, 6 = 1200, 9 = 1500, 12 = 1800, 15 = 2100, 18 = 2400, 21 = 0300, 24 = 0600 (final)

[3]F = main effect of hay type (alfalfa and brome); L = main effect of location (cecum and rectum); H = main effect of hour; F*L = interaction between hay and location; F*H = interaction between hay and hour; F*H*L = 3-way interaction effect of hay, hour, and location; $P \leq 0.05$

†l = main effect of location (cecum and rectum); $0.05 < P \leq 0.10$

[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Table 2.3. Effect of hay type and sampling location on bacterial phyla detected in horses[1]

	Brome		Alfalfa			
Phylum, %	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[2]**
Bacteroidetes	52.00[a]	36.61[b]	51.03[a]	18.45[c]	2.93	F, L, F*L
Firmicutes	39.73[ab]	44.17[a]	35.24[b]	62.83[c]	2.12	F, L, F*L
Spirochaetes	2.38[a]	8.47[b]	2.32[a]	2.90[a]	1.09	F, L, F*L
Fibrobacteres	1.52[a]	1.27[a]	0.23[b]	0.30[b]	0.30	F
Unassigned	1.26[a]	1.21[a]	8.61[b]	2.43[a]	1.02	F, L, F*L
Tenericutes	1.19[a]	1.10[a]	1.47[a]	2.32[b]	0.15	F, L, F*L
Euryarchaeota	0.16[a]	0.55[ab]	0.21[a]	2.29[b]	0.67	f†, L
Verrucomicrobia	0.43[a]	5.40[b]	0.13[c]	6.46[b]	0.68	L
Proteobacteria	0.72	0.44	0.54	0.77	0.12	f*l†
Actinobacteria	0.17[a]	0.37[b]	0.10[a]	0.67[c]	0.07	F, L, F*L
Cyanobacteria	0.33[a]	0.10[b]	0.04[b]	0.13[b]	0.06	F, F*L
Firmicutes:Bacteroidetes	0.78[a]	1.25[a]	0.71[a]	4.41[b]	0.59	F, L, F*L

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations included the cecum and rectum

[2]F = main effect of hay type (alfalfa and brome); L = main effect of location (cecum and rectum); F*L = interaction between hay and location; $P \leq 0.05$

†f = main effect of hay (brome and alfalfa); f*l = interaction between hay and location; $0.05 < P \leq 0.10$

[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Table 2.4. Effect of hay type and sampling location on bacterial families that comprise ≥ 1% relative abundance[1]

	Brome		Alfalfa			
Family, %	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[2]**
Unassigned (Bacteroidales)[3]	38.33[a]	29.90[b]	37.20[a]	13.45[c]	4.27	F, L, F*L
Lachnospiraceae	13.43[ab]	12.48[a]	12.34[a]	16.94[b]	1.39	F*L
Ruminococcaceae	10.08[a]	12.85[ab]	9.59[a]	18.48[b]	2.39	L, f*l†
Unassigned (Clostridiales)[3]	10.42[a]	11.38[a]	6.97[b]	10.72[a]	0.80	F, L, F*L
Paraprevotellaceae	10.30[a]	4.43[b]	10.03[a]	1.98[c]	0.81	f†, L
Spirochaetaceae	2.38[a]	8.46[b]	2.32[ab]	2.90[a]	1.11	F, L, F*L
Unassigned	1.27[a]	1.21[a]	8.61[b]	2.43[a]	1.02	F, L, F*L
RFP12	0.33[a]	5.39[b]	0.08[c]	6.36[b]	0.85	L
Erysipelotrichaceae	1.30[a]	1.83[a]	0.73[a]	5.79[b]	0.96	F, L, F*L
Prevotellaceae	2.63	1.14	3.52	1.82	0.91	L
Lactobacillaceae	1.28[ab]	0.55[a]	3.08[c]	1.13[b]	0.44	F, L, f*l†
Unassigned (RF39)[3]	1.13[a]	0.88[a]	1.39[a]	2.04[b]	0.19	F, F*L
Veillonellaceae	1.02	1.53	1.17	1.40	0.22	l†
Mogibacteriaceae	0.23[a]	1.38[b]	0.21[a]	2.67[c]	0.31	F, L, F*L
Clostridiaceae	0.96[a]	1.36[a]	0.30[b]	1.55[c]	0.22	L, F*L
Streptococcaceae	0.33[a]	0.21[a]	0.35[a]	2.95[b]	0.81	F, L, F*L
Fibrobacteraceae	1.52[a]	1.27[a]	0.22[b]	0.30[b]	0.32	F
Methanobacteriaceae	0.14	0.43	0.02	1.93	0.91	NS[4]

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations included the cecum and rectum

[2]F = main effect of hay type (alfalfa and brome); L = main effect of location (cecum and rectum); F*L = interaction between hay and location; $P \leq 0.05$

[3]Bacterial families unidentified at the family level but are within the bacterial order listed between parentheses

[4]NS = no difference detected; $P > 0.10$

†f*l = interaction between hay and location; f = main effect of hay type (alfalfa and brome); $0.05 < P \leq 0.10$

[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Table 2.5. Effect of hay type and sampling location on bacterial genera identified to represent ≥ 1% relative abundance[1]

	Brome		Alfalfa			
Genus, %	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[2]**
Treponema	2.37[a]	5.66[b]	2.32[a]	5.71[b]	0.91	L
Ruminococcus	3.65	3.47	2.64	3.89	0.65	NS[3]
Unassigned	1.25[a]	1.71[a]	8.60[b]	1.91[a]	0.82	F, L, F*L
CF231	4.75[a]	0.99[b]	3.81[a]	1.12[b]	0.48	L
YRC22	2.43[a]	1.16[b]	4.53[a]	1.23[b]	0.50	F, L, F*L
Prevotella	2.62	1.49	3.53	1.46	1.33	L
Coprococcus	1.88	1.64	1.60	1.45	0.22	NS[3]
Lactobacillus	1.26[a]	0.68[a]	3.08[b]	1.01[a]	0.37	F, L, F*L
Blautia	1.90[a]	0.86[b]	1.27[ab]	0.70[b]	0.34	L
Phascolarctobacterium	0.93	1.06	1.11	1.45	0.21	F
Streptococcus	0.32[a]	2.31[b]	0.37[a]	0.86[ab]	0.75	L
Fibrobacter	1.52[a]	0.88	0.22[b]	0.66	0.31	F, F*L
[Prevotella]	1.22[a]	0.32[b]	1.33[a]	0.36[b]	0.10	L
Clostridium	0.70[a]	0.86[a]	0.16[b]	0.83[a]	0.14	F, L, F*L
Methanobrevibacter	0.14[a]	1.73[b]	0.01	0.61	0.53	L
Pseudobutyrivibrio	0.17[a]	0.84[bc]	0.44[b]	0.99[c]	0.21	L

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations included the cecum and rectum

[2]F = main effect of hay type (alfalfa and brome); L = main effect of location (cecum and rectum); F*L = interaction between hay and location; $P \leq 0.05$

[3]NS = no difference detected; $P > 0.10$

[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Table 2.6. Effect of hay type and sampling location on identified bacterial species detected in ≥ 1% relative abundance[1]

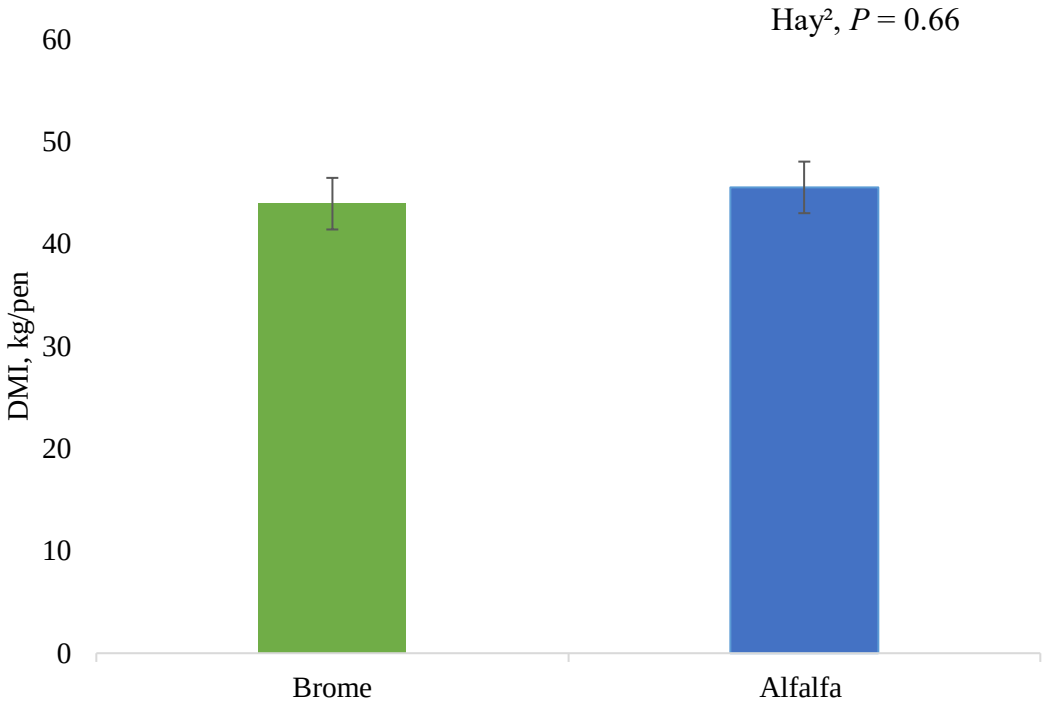
	Brome		Alfalfa			
Species, %	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[2]**
Fibrobacter succinogenes	1.52[a]	1.26[a]	0.22[b]	0.28[b]	0.30	F
Ruminococcus flavefaciens	1.04[a]	0.48[bc]	0.26[b]	0.82[ac]	0.13	F, F*L

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations included the cecum and rectum

[2]F = main effect of hay type (alfalfa and brome); F*L = interaction between hay and location; $P \leq 0.05$

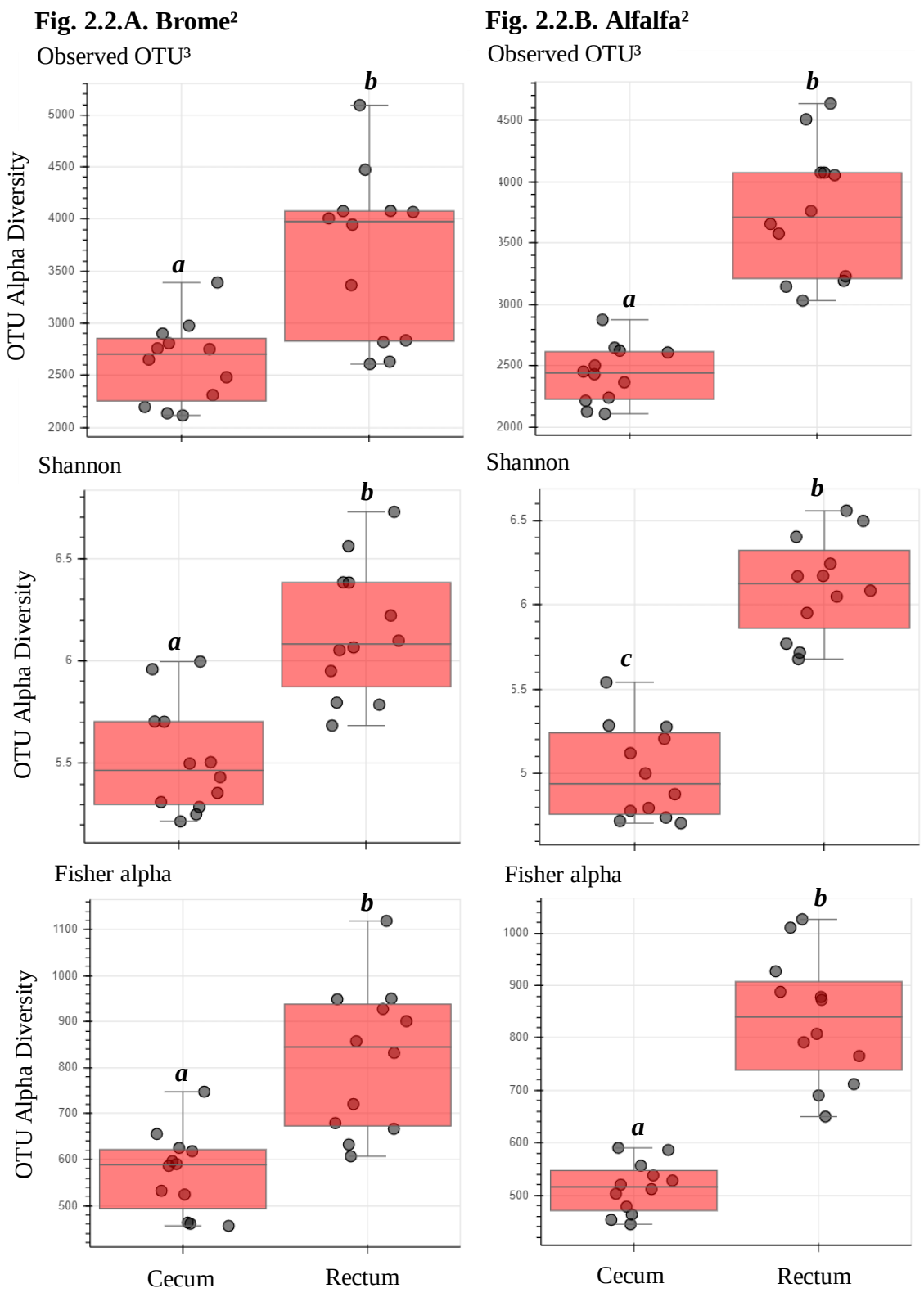
[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Figure 2.1. Effect of hay type on dry matter intake (DMI)[1]



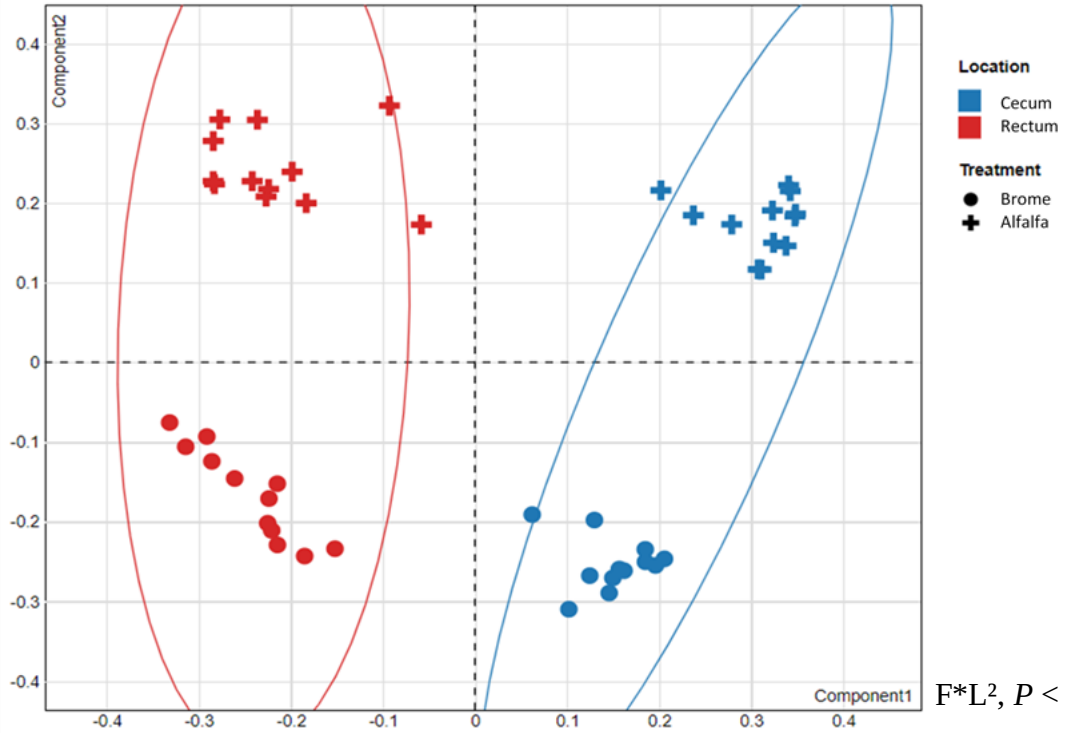
[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses
[2]Hay = main effect of hay type

Figure 2.2. Effect of hay type and sampling location on alpha diversity measures[1]



[1]Alpha diversity was measured using Observed OTU, Shannon, and Fisher alpha indices
[2]Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations included the cecum and rectum
[3]OTU = operational taxonomic unit
[abc]Means within the same row with a different letter are different ($P \leq 0.05$)

Figure 2.3. Effect of hay type and sampling location on beta diversity[1]



F*L[2], $P < 0.05$

[1]Beta diversity was analyzed using a Bray-Curtis distance matrix, visualized on a PCoA plot, and comparisons were made using PERMutational Multivariate Analysis of Variance. Hay type [smooth bromegrass (brome) or alfalfa] was fed *ad libitum* to horses; sampling locations included the cecum and rectum;
[2]F*L = interaction effect of hay by location

Chapter 3 - Efficacy of corn dried distiller's grains with solubles (DDGS) as a replacement for soybean meal in Boer-type goat finishing diets[1]

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Abstract

Due to increased use of dried distillers grains with solubles (DDGS) in animal feed and accessibility of ethanol plants in the Midwest, the effect of feeding DDGS in place of soybean meal (SBM) on growth, economics, carcass characteristics, backfat fatty acid profiles, and fecal microbiome of Boer-type goats was evaluated. Forty-eight Boer-type goats of mixed genetics (approximately 70 d of age; 28.21 ± 0.96 kg) were blocked by BW and assigned to 1 of 4 dietary treatments in a completely randomized design. Treatments were 0%, 33%, 66%, or 100% DDGS replacing SBM, equating to the inclusion of 0% (**0DDGS**), 10.3% (**10DDGS**), 20.5% (**20DDGS**), or 31.1% (**30DDGS**) DDGS in the total diet. The levels of corn and soybean hulls varied to maintain isocaloric and isonitrogenous diets. Goats were provided *ad libitum* access to feed and water for 47 d. There were 3 goats/pen and 4 pens/treatment. The inclusion of DDGS linearly improved ($P = 0.02$) ADG, driven by improved ($P = 0.001$) G:F, without affecting ADFI ($P > 0.10$). Feed cost/kg gain decreased ($P < 0.0001$) with increased DDGS inclusion, which led value of gain increasing linearly ($P = 0.02$) with increased DDGS inclusion. Fecal genera with individual relative abundances greater than 1% that were impacted by DDGS inclusion included increased *Ruminococcus* and *Methanobrevibacter* ($P < 0.01$) and decreased *Lachnoclostridium* ($P = 0.02$). *Ruminococcus* and *Methanobrevibacter* most likely increased in 30DDGS due to greater amounts of soluble fiber passing through the rumen, thus being fermented in the hindgut. There was no detected difference in overall percentage of phyla Bacteroidetes ($P = 0.36$) and Firmicutes ($P = 0.12$) among treatments; however, Firmicutes to Bacteroidetes was higher ($P = 0.05$) in goats fed 30DDGS than those fed lower levels of DDGS. There was no observed impact of treatment on ($P = 0.47$) β-diversity, although species richness and evenness tended to increase ($P = 0.09$) in goats fed levels of DDGS possibly because more soluble fiber available for

fermentation in the hindgut. Polyunsaturated fatty acids tended to quadratically increase (P = 0.06) with increased DDGS inclusion, yet there was no observed difference in the SFA to USFA ratio (P = 0.93) or iodine value (P = 0.36) with increased levels of DDGS. In summary, up to 100% of the SBM in a Boer-type finishing goat ration can be replaced by corn DDGS with no detected difference in growth performance, fecal microbial populations, carcass characteristics, or fatty acid profile.

Key words: byproducts, dried distillers grains, finishing, goat, microbiome, soybean meal

Introduction

The United States goat population has more than doubled in the past 16 years (1.25 to 2.62 million head; NASS, 2002 and 2018). As goat production has increased, so has the demand for economical feedstuffs. During the same time period, an increase in the number of corn ethanol facilities has led to large availability of corn dried distillers grains with solubles (DDGS). Previous studies recorded that DDGS can completely replace soybean meal (SBM) in growing lamb diets (Huls et al., 2006), and lamb feedlot rations (Crane et al., 2017). Similarly, replacing dietary DDGS for SBM in Boer-cross kids does not impact average daily gain (ADG), average daily feed intake (ADFI), or carcass yield (Gurung et al., 2009; Hutchens et al., 2012; Maynard, 2015).

While the addition of dietary DDGS has minimal effects in ruminant carcasses, fatty acid profiles often differ when compared to animals consuming SBM (Schingoethe et al., 2009; Xu et al., 2010; Williams et al., 2016) due to the increased concentration of unsaturated fatty acids (USFA) in DDGS. Although USFA can undergo biohydrogenation in the rumen (Beam et al., 2000), Camareno et al. (2016) demonstrated that dietary de-oiled DDGS led to increased concentrations of USFA in subcutaneous adipose tissue of meat goats. Accordingly, the short

chain to long chain fatty acid ratio decreases as percentage of dietary DDGS increase (Williams et al., 2016), which may cause alterations in goat fat quality.

Reports recording the effects of DDGS on the ruminal microbiome are conflicting. Rice et al. (2012) reported a greater relative abundance of Firmicutes compared to Bacteroidetes with the inclusion of DDGS in cattle, while Callaway et al. (2010) reported no differences. While ruminal microbial community shifts as a result of grain inclusion in goats have been characterized (Mao et al., 2016), no research reports exist evaluating the effect of DDGS. Therefore, our objective was to evaluate the effect of feeding corn DDGS in place of soybean meal on growth, economics, carcass characteristics, 12^{th} rib fatty acid profiles, and fecal microbiome of Boer-type finishing goats.

Materials and Methods

Animals and Diets

The Kansas State University Institutional Animal Care and Use Committee approved all animal protocols used in this experiment. Forty-eight Boer-cross goat kids (approximately 70 d of age; 28.2 ± 0.96 kg) were housed in 3 m × 1.5 m pens at the Kansas State University Sheep and Meat Goat Center, with 4 pens/treatment and 3 kids/pen. Pens were equipped with individual waterers and free choice feeders.

On d 0, goats were randomly allotted to pens, and pens randomly allotted to 1 of 4 treatment diets in a completely randomized design. Dietary treatments included: 0% SBM replaced by DDGS (**0DDGS**), 33% SBM replaced by DDGS (**10DDGS**), 66% SBM replaced by DDGS (**20DDGS**), and 100% SBM replaced by DDGS (**30DDGS**). Diets were isocaloric and isonitrogenous (Table 3.1). Diets were pelleted at the Kansas State University O.H. Kruse Feed Technology Innovation Center. Goats were allowed *ad libitum* access to their respective diets

and no supplemental forage was provided. Goats and feeders were weighed weekly to determine average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F). Goats were weighed on d 0 and 1, as well as on d 46 and 47, with the average weight per pen used to determine the initial and final body weight, respectively.

Economics

Economic analyses were performed to determine financial impact of dietary treatments. The total cost of diets 0DDGS, 10DDGS, 20DDGS, and 30DDGS were $162.00, $152.62, $143.14, and $134.73, respectively (Table A.3). Total feed cost per goat was calculated as: ADFI × feed cost per kg × 47 days on feed. Feed cost per kg of gain was calculated as: total feed cost per goat ÷ total gain per goat from d 0 to d 47. Value of gain (VOG; $) was calculated as: [(ending weight × $221 cwt) – (beginning weight × $200 cwt)] ÷ overall gain per goat (USDA AMS, 2018; Rasby et al, 2015).

Microbiome

Fecal pellets were collected via rectal grab on d 47 from goats fed treatment 0DDGS and 30DDGS. Samples were placed into individual vials and stored at -80° C until DNA isolation. Genomic DNA was isolated from approximately 200 mg of feces using the Powersoil DNA Isolation Kit (Qiagen Inc., Valencia, CA) at a commercial laboratory (MR DNA, Shallowater, TX). DNA was stored at -20°C until PCR amplification. The 16S universal Eubacterial primers 515F (5'-GTGYCAGCMGCCGCGGTAA) and 806R (5'-GGACTACNGGGTWTCTAAT) were used to amplify the 16S gene of DNA samples on the Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) platform via the bTEFAP DNA analysis service originally described by Dowd et al. (2008). All samples underwent a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen Inc, Valencia, CA) under the following conditions: 94° C for 3 min, followed

by 28 cycles of 94° C for 30 s; 53° C for 40 s and 72° C for 1 min; followed by the final elongation step at 72° C for 5 min. The PCR products were checked for relative intensity of bands and success of amplification in 2% agarose gel before being pooled together in equal proportions based on molecular weight and DNA concentration. Pooled samples were purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, Beverly, MA) and pyrosequencing was performed by MR DNA (MR DNA, Shallowater, TX) using Illumina HiSeq (Illumina Inc., San Diego, CA) chemistry following manufacturer's guidelines. All microbiome data were processed through a proprietary analysis pipeline (www.mrdnalab.com; MR DNA, Shallowater, TX). Final microbial analyses were performed on 1,448,047 sequences identified in the bacteria and archaea domains. Alpha and beta diversity analyses were performed on samples and sequences were rarefied to 30,000 sequences and bootstrapped at 20,000 sequences.

Carcass Characteristics

At the end of the experiment, the lightest and heaviest goat per pen were harvested (Paradise Locker, Inc., Trimble, MO) to determine hot carcass weight (HCW), carcass yield, loin eye area (LEA), fat depth at the 12^{th} rib, and body wall thickness at the 12th rib. Fat samples were collected over the 12^{th} and 13^{th} ribs and stored at -80° C until analysis of fatty acid profiles via AOAC Method 996.06 by a commercial laboratory (Barrow-Agee Laboratories, LLC, Memphis, TN).

Statistical analyses

Data were analyzed using the GLIMMIX procedure of SAS Studio (Version 9.4; SAS Inst., Cary, NC) with Tukey's test for post hoc pairwise comparisons. Pen served as the experimental unit for growth data, while individual goat served as the experimental unit for economic analyses, microbiome, carcass characteristics, and backfat fatty acid profile. Pre-

planned contrast statements were used to evaluate linear and quadratic effects, as well as DDGS vs. none. Analyses for alpha and beta diversity were conducted as described by Dowd et al. (2008) using Qiime (www.qiime.org). Significance was determined at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

Results and Discussion

Growth performance and economic value

There was no evidence ($P > 0.10$) of dietary treatment on final body weight (Table 3.2). Increasing levels of DDGS increased ($P = 0.02$) ADG in a linear manner. Previous reports regarding the effect of DDGS on ADG of small ruminants are inconsistent. Castro-Pérez et al. (2014) reported that lambs fed a finishing diet containing DDGS in place of SBM had improved ADG. Meanwhile, Gurung et al. (2009) reported no detected difference in ADG when Kiko × Spanish male goats were fed isonitrogenous diets containing either DDGS or SBM. Maynard (2015) reported a tendency for ADG to decrease in Savannah- and Boer-cross goats fed diets with DDGS replacing 45% of SBM, but these diets were not isocaloric or isonitrogenous, which likely impacted their observations. While there was no evidence ($P > 0.10$) of dietary treatment affecting ADFI or dry matter intake (DMI) in the current study, increasing levels of DDGS increased ($P = 0.0002$) G:F in a linear manner. Specifically, goats fed 20DDGS or 30DDGS had greater ($P < 0.05$) G:F than those fed 0DDGS or 10DDGS. Similar effects have been noted in cattle finishing diets containing up to 40% DDGS in feedlot steers producing quadratic increases in G:F with increased DDGS inclusion rates (Walter et al., 2010; Klopfenstein et al., 2007). Walter et al., (2010) attributed this increased efficiency to the fact that DDGS increased NEg of the diet coupled with a reduced DMI. However, neither NEg nor DMI differed between diets in the current study. Schingoethe et al. (2009) demonstrated substituting SBM with corn DDGS

increases the percentage of ruminal undegradable protein in dairy cattle. Therefore, more protein would likely bypass the rumen and be available for digestion and absorption in the small intestine when DDGS is used to replace SBM in the diet of a ruminant animal. In the current study, diets were formulated to be isonitrogenous; however, protein solubility was not taken into account.

Feed cost per goat tended to decrease linearly ($P = 0.06$) with increasing levels of DDGS (Table 3.2). This led to a linear increase in savings ($P < 0.0001$) in feed cost/kg of gain with increasing levels of DDGS. Specifically, goats fed 30DDGS had a feed cost/kg gain of $0.32 or $0.39 less ($P < 0.05$) than those fed 10DDGS or 0DDGS, respectively. Similarly, the VOG increased linearly ($P = 0.02$) with increasing levels of DDGS included in the diet. Prices used in the current study were based on commodity prices in February 2018 and may not be representative of current prices.

Microbial populations

When DDGS completely replaced SBM, 1 bacterial phylum and 12 bacterial genera were influenced. The phylum Euyarchaeota, within the domain Archea, increased ($P = 0.01$; Fig. 3.1.) in goats fed 30DDGS compared to 0DDGS, largely driven by an increase ($P = 0.009$) in the genus *Methanobrevibacter* (Table 3.3). These shifts may be a result of greater post ruminal fiber fermentation. Although treatment diets in the current study had similar neutral detergent fiber (NDF) values, Firkins et al. (1986) found that diets containing 20% DDGS (DM basis) had greater post-ruminal NDF digestion when compared to a control containing 17% dry corn gluten feed. Increased post ruminal fiber digestion would have yielded increased production of H+ and CO_2, the preferred substrates of *Methanobrevibacter* (Morgavi et al., 2010).

Bacteroidetes and Firmicutes were the most abundant phyla in goats consuming both treatment diets. Similar results were reported in both goats (Grilli et al., 2016; Mao et al., 2016; Tao et al., 2017) and cattle (Callaway et al., 2010; Castillo-Lopez et al. 2014) where Bacteroidetes and Firmicutes were the predominant ruminal phyla. In the current study, DDGS had no detected difference on the relative populations of Bacteroidetes ($P = 0.36$) and Firmicutes ($P = 0.12$) compared to goats fed SBM (Table 3.3). But, Firmicutes:Bacteroidetes was greater ($P = 0.05$) in goats consuming the 30DDGS diet due to a numerical increase in Firmicutes and concurrent decrease of Bacteroidetes. This contradicts reports by both Castillo-Lopez et al. (2014) and Callaway et al., (2010) where DDGS had no detected difference on Firmicutes:Bacteroidetes, but treatment diets in both studies varied in crude protein and energy.

While no differences were detected in Firmicutes between diets, 9 genera within Firmicutes were influenced by the inclusion of DDGS. *Acetitomaculum* ($P = 0.03$), *Bulleidia* ($P = 0.008$), and *Pseudoramibacter* ($P = 0.04$) and *Ethanoligenens* ($P = 0.01$) increased in relative abundance while *Papillibacter* ($P = 0.005$), *Desulfotomaculum* ($P = 0.05$), and *Eisenbergiella* ($P = 0.03$) decreased in goats fed diets 30DDGS compared to 0DDGS. *Ruminococcus* increased ($P = 0.01$; Table 3.3; Fig. 3.2) in goats fed 30DDGS and this shift continued into the species level whereby *Ruminococcus bromii* was greater ($P = 0.001$) in 30DDGS-fed goats. Rice et al. (2012) also observed an increase in *Ruminococcus* and *R. bromii* from fecal samples of beef steers fed diets containing 10% corn distillers grains (DM basis). *Ruminoccocus bromii* is an amylolytic bacteria capable of degrading resistant starches that bypass the upper gastrointestinal tract (Ze et al., 2015*). Ruminococcus bromii* may play a role in fermenting DDGS in the large intestine. In addition, feces from DDGS-fed goats contained fewer *Lachnoclostridium* ($P = 0.02$), a bacteria reported to ferment mono- and disaccharides into acetate (Yutin and Galeperin, 2013). Volatile

fatty acid analysis was not performed on fecal samples in the current study, so the concentration of acetate is unknown leaving uncertainty in how DDGS influence fermentation end products. Previous studies in cattle (Walter et al., 2012) and feedlot lambs (Crane et al., 2017) have reported no difference in acetate concentrations of ruminal fluid with titrating DDGS in the diets.

Of the 12 genera influenced by diet, only the previously discussed *Ruminococcus*, *Methanobrevibacter*, and *Lachnoclostridium* individually contributed ≥ 1% relative abundance in the total bacterial population. The remaining 9 genera collectively totaled ≤ 1.0% of the total population. *Atopobium*, within the phylum Actinobacteria, increased ($P = 0.05$) and *Prolixibacter*, from the Cytophaga-Flavobacterium-Bacteroides phylum, decreased ($P = 0.03$) when DDGS replaced SBM in diets. Genera within the phylum Actinobacteria have been found to aid in digestion of complex carbohydrates (Lewin et al., 2017) and *Prolixibacter* may be of importance in fermenting sugars (Holmes et al., 2007). However, few publications have described the impact of *Atopobium* or *Prolixibacter* on the mammalian gastrointestinal tract.

Alpha diversity is used to quantify the number of different microbial species within each fecal sample. Species richness, as indicated by the total number of operational taxonomic units (OTU), increased ($P = 0.04$) in goats fed 30DDGS compared to 0DDGS (Table 3.4). Similar findings were reported by Castillo-Lopez et al. (2014) who found more OTU in ruminal and duodenal digesta from feedlot steers fed DDGS in place of corn bran. The Shannon index provides more in depth information on the community structure than species richness alone because it also takes into account relative abundance. Utilizing this index, species richness and evenness tended to increase ($P = 0.09$) when DDGS completely replaced SBM. This may be due to more fermentable substrate passing into the hindgut.

To further characterize the microbial ecology, beta diversity was measured in order to compare differences in microbial community structure between samples. Based on the principle coordinate plot (Fig. 3.3), no apparent clustering occurred between diets 0DDGS and 30DDGS ($P = 0.43$) reflecting minimal differences between taxonomic abundances in fecal samples.

Despite increases in alpha diversity, inclusion of DDGS in Boer-type goat diets only created a 2.3% difference in the relative abundance of genera that differed statistically. Since nutrient digestibility and fermentative end products were not evaluated, it is unclear if these microbial shifts directly impacted the improved growth and efficiency observed in DDGS fed goats.

Carcass characteristics

Inclusion of DDGS had no observed impact ($P > 0.10$) on any carcass characteristics measured (Table 3.5). Others have reported no changes in HCW, fat depth, or ribeye area of feedlot lambs fed varying levels of DDGS up to 60% replacing cereal grains (Schauer et al., 2008; Van Emon et al., 2013). Felix et al. (2012) replaced corn with DDGS in lamb finisher rations up to 60% (DM basis) and found a positive quadratic response in HCW to DDGS inclusion. Huls et al. (2006) replaced SBM completely with DDGS in finishing lamb diets, and reported that HCW was unchanged by diet; however, backfat thickness increased by approximately 17%, possibly due to increased energy in the DDGS diet.

Fatty acid profiles of rib fat were largely unaffected by treatment ($P > 0.05$; Table 3.6). Palmitoleic acid and linolenic acid had a quadratic relationship ($P = 0.03$) with DDGS inclusion. Furthermore, margaric acid and gadoleic acid tended to increase quadratically ($P = 0.09$ and $P = 0.06$, respectively) with increased DDGS inclusion. Similarly, Williams et al. (2016) reported increased concentrations of linolenic and gadoleic acid as well as stearic acid in the milk of goats

fed DDGS in place of SBM. Camareno et al. (2016) also found greater concentrations of gadoleic acid in addition to linoleic and oleic acids in subcutaneous adipose tissue in goats fed 30% de-oiled DDGS. Capric acid had a tendency ($P = 0.09$) to be greater in goats fed 0DDGS compared to all other levels. Variations reported between the current study and those previous may be due to dietary levels of DDGS whereby levels were titrated up to 30% and 59% by Camareno et al. (2016) and Williams et al. (2016), respectively. As DDGS replaced SBM in feedlot goat diets, n-3 fatty acids, n-6 fatty acids, and polyunsaturated fatty acids (PUSFA) of fat taken over the 12th rib tended to increase quadratically ($P = 0.06$). An increase in PUSFA was also detected by Camereno et al. (2016), likely explained by the fact that DDGS have nearly 3-fold greater fat content (DM basis) than corn (Klopfenstein et al., 2008). Quadratic increases in PUSFA with DDGS inclusion were also reported in steaks harvested from steers fed DDGS compared to SBM (Segers et al., 2011). Similarly, the PUSFA:USFA tended to increase in steaks from steers fed DDGS. There were no differences in the saturated fatty acid (SFA) to USFA ratio ($P = 0.93$) or in iodine value ($P = 0.36$), an indicator of unsaturation, in the current study. Unsaturated fatty acids in the rumen undergo biohydrogenation by ruminal bacteria after hydrolysis, passing more SFA into the lower intestinal tract (Harfoot and Hazlewood, 1997). Castillo-Lopez et al. (2014) found that the increase of USFA passing into the rumen of steers fed DDGS increased SFA flow into the duodenum because of increased biohydrogenation. However, no differences were detected ($P = 0.97$) in SFA deposited into fat in the current study.

Summary

In summary, replacing SBM with corn DDGS in a Boer-type goat finishing ration improved ADG and G:F without negatively impacting carcass characteristics. Additionally, DDGS inclusion resulted in minimal shifts in the fecal microbiome. When combined with a

greater value of gain based on commodity prices during this study, this byproduct appears to be a viable alternative to SBM in meat goats.

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Tables and Figures

Table 3.1. Dietary composition (dry matter basis)[1]

	Treatment[2]			
Item	**0DDGS**	**10DDGS**	**20DDGS**	**30DDGS**
Diet composition, %				
Corn DDGS[3]	-	10.30	20.50	31.05
Soybean meal, 48%	15.45	10.26	5.12	-
Corn	52.75	51.17	49.61	48.31
Soybean hulls	25.93	22.61	19.31	15.04
Vitamin/mineral pack[4]	1.27	1.06	0.86	0.80
Nutrient composition				
Crude protein, %	17.90	17.50	17.30	17.40
Crude fiber, %	10.90	10.90	9.50	9.20
Fat, %	2.70	3.60	4.20	5.20
Total digestible nutrients[5], %	72.30	73.20	74.50	75.30
Net energy for gain, Mcal/kg	1.20	1.19	1.20	1.21
Neutral detergent fiber, %	22.30	25.20	23.00	22.50
Acid detergent fiber, %	16.00	15.90	14.40	13.30
Sulfur, %	0.19	0.20	0.20	0.23
Calcium, %	0.98	1.02	0.84	0.94
Phosphorus, %	0.38	0.41	0.37	0.42

[1]Diets were balanced to be ≥ CP and DE requirements (DM basis) for 25 kg Boer doelings and male castrates gaining 100 to 150 $g \cdot head^{-1} \cdot day^{-1}$ (NRC, 2007)
[2]Treatment: Complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM);10DDGS = 33 % DDGS in place of SBM; 20DDGS = 66 % DDGS in place of SBM; 30DDGS = 100% DDGS in place of SBM
[3]DDGS = Dried distillers grains with solubles
[4]Pack contains: AmCl, Cu Sulfate, Zn Oxide, Monocalcium Phosphate, Se Selenite, Vit. A 30,000, Vit. D 30,000, and Vit. E 30,000.
[5]Calculated

Table 3.2. Impact of dried distillers grains with solubles (DDGS) in place of soybean meal (SBM) on Boer goat performance and economics

Item	Treatment[1]					*P*-value			
							DDGS vs.		
	0DDGS	10DDGS	20DDGS	30DDGS	SEM	Treatment[2]	none[3]	Linear	Quadratic
Body weight, kg									
d 0-1[2]	28.22	28.16	28.16	28.31	0.96	0.99	0.99	0.95	0.92
d 46-47[2]	37.08	38.39	38.80	40.39	1.39	0.44	0.21	0.12	0.92
Average daily gain, kg/d	0.06	0.07	0.08	0.08	0.006	0.09	0.03	0.02	0.56
Average daily feed intake, kg/d	1.08	1.26	1.06	1.11	0.08	0.35	0.54	0.74	0.44
Dry matter intake, kg/d	0.96	1.12	0.94	0.98	0.07	0.35	0.53	0.74	0.45
Gain:Feed	0.06[a]	0.06[a]	0.08[b]	0.09[b]	0.005	0.001	0.01	0.0002	0.82
Feed cost, $/goat[5]	9.08	9.97	7.85	7.73	0.66	0.10	0.47	0.06	0.46
Feed cost, $/kg gain[6]	1.04[a]	0.97[a]	0.74[ab]	0.65[b]	0.04	< 0.0001	0.0001	<0.0001	0.69
Value of gain, $/gain[7]	6.47	6.21	6.16	6.02	0.11	0.09	0.02	0.02	0.59

[1]Treatment: Complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM); 10DDGS = 33% DDGS in place of SBM; 20DDGS = 66% DDGS in place of SBM; 30DDGS = 100% DDGS in place of SBM

[2]Main effect of treatment

[3]Pre-planned contrast: 0DDGS vs all levels of DDGS

[4]Two-day weights were taken to determine initial and final body weight

[ab]Means within a row with different subscripts differ ($P < 0.05$)

[5]Based on local ingredient prices in January 2018

[6]Feed cost/kg gain = total feed cost / total gain per goat

[7]Value of Gain (income/gain) = [(Ending weight*$4.88) – (beginning weight*4.40)] / (ending weight – beginning weight)

Table 3.3. Effect of dried distillers grain with solubles (DDGS) on the most common fecal genera and most abundant phyla of Boer goats[1]

	Treatment[2]			
Item, %	**0DDGS**	**30DDGS**	**SEM**	***P*-value**
Treponema	19.922	17.866	3.12	0.65
Clostridium	8.506	9.665	1.04	0.44
Cytophaga	8.162	7.737	3.38	0.93
Prevotella	5.964	7.464	1.23	0.40
Bacteroides	6.026	5.881	0.79	0.89
Ruminococcus	4.595	7.181	0.69	0.01
Turicibacter	4.773	5.390	0.89	0.63
Eubacterium	4.351	4.585	0.53	0.76
Paludibacter	4.304	3.043	0.45	0.06
Barnesiella	2.871	3.749	0.82	0.46
Rikenella	5.018	1.489	1.61	0.13
Methanobrevibacter	1.884	3.596	0.42	0.009
Lachnoclostridium	3.329	1.630	0.48	0.02
Coprobacter	1.998	1.781	0.55	0.78
Alistipes	1.712	1.738	0.57	0.97
Oscillospira	1.331	1.800	0.19	0.10
Parabacteroides	1.584	1.146	0.24	0.22
Ruminiclostridium	0.937	1.501	0.50	0.43
Spirochaeta	1.242	0.751	0.21	0.12
*Papillibacter**	0.444	0.223	0.05	0.005
*Desulfotomaculum**	0.104	0.050	0.02	0.05
*Eisenbergiella**	0.068	0.006	0.02	0.03
*Acetitomaculum**	0.010	0.054	0.01	0.03
*Bulleidia**	0.0023	0.011	0.03	0.008
*Pseudoramibacter**	0.0029	0.0061	0.001	0.04
*Atopobium**	0.0016	0.0056	0.001	0.05
*Prolixibacter**	0.0048	0.0012	0.001	0.03
*Ethanoligenens**	0.00081	0.0050	0.001	0.01
Firmicutes	34.46	39.94	2.42	0.12
Bacteroidetes	39.37	35.37	2.99	0.35
Firmicutes:Bacteroidetes	0.90	1.30	0.14	0.05

[1]Sequencing was performed on the Illumina HiSeq (Illumina Inc., San Diego, CA) platform by MR DNA (MR DNA, Shallowater, TX). Genera are ordered by most abundant sequences.

[2]Treatment: Complete pelleted diets with 0 = 0% dried distillers grains with solubles (DDGS) in place of (SBM); 30 = 100% DDGS in place of SBM

*Genera that are ≤ 1% of relative population but differ between treatment diets ($P < 0.05$)

Table 3.4. Impact of dried distillers grain with solubles (DDGS) in place of soybean meal (SBM) on alpha diversity measures

Item	0DDGS[1]	SEM	30DDGS[1]	SEM	*P*-value
Observed OTU[2] index	1300.50	4.48	1401.00	4.01	0.04
Shannon index[3]	6.59	0.016	6.84	0.018	0.09

[1]Treatment: Complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM); 30DDGS = 100% DDGS in place of SBM

[2]Operational Taxonomic Units

[3]Diversity index accounting for both evenness and richness

Table 3.5. Impact of dried distillers grains with solubles (DDGS) in place of soybean meal (SBM) on Boer goat carcass characteristics

	Treatment[1]					*P*-value			
Item	0DDGS	10DDGS	20DDGS	30DDGS	SEM	Treatment[2]	DDGS vs. none[3]	Linear	Quadratic
Hot carcass weight, kg	18.40	18.40	19.60	19.80	1.03	0.67	0.48	0.26	0.94
Carcass yield[4], %	49.40	48.90	50.30	48.60	0.79	0.48	0.88	0.74	0.44
Loin eye area[5], cm²	11.60	13.10	13.30	13.50	0.95	0.51	0.14	0.18	0.50
Loin eye depth[5], cm	2.90	2.80	3.00	2.70	0.14	0.50	0.85	0.76	0.50
Backfat depth[6], mm	1.40	1.40	1.40	1.60	0.24	0.98	0.88	0.73	0.80
Body wall thickness, cm	1.52	1.71	1.68	1.78	0.15	0.66	0.25	0.28	0.75

[1]Treatment: Complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM); 10DDGS = 33% DDGS in place of SBM; 20DDGS = 66% DDGS in place of SBM; 30DDGS = 100% DDGS in place of SBM

[2]Main effect of treatment

[3]Pre-planned contrast: 0DDGS vs all levels of DDGS

[4]Carcass yield, %, calculated by dividing hot carcass weight by live weight recorded before transport to packing plant

[5]*Longissimus dorsi*

[6]Subcutaneous fat depth measured over the 12th rib

Table 3.6. Impact of dried distillers grains with solubles (DDGS) in place of soybean meal (SBM) on Boer goat fatty acid profiles at the 12th rib

	Treatment[1]					*P*-value			
							DDGS vs.		
Fatty acid, % weight	0DDGS	10DDGS	20DDGS	30DDGS	SEM	Treatment[2]	None[3]	Linear	Quadratic
Capric	0.01	0.00	0.00	0.00	0.005	0.41	0.09	0.19	0.32
Lauric	0.029	0.059	0.015	0.00	0.032	0.61	0.91	0.37	0.49
Dodecenoic	0.00	0.00	0.016	0.00	0.008	0.41	0.57	0.66	0.33
Myristic	2.48	2.44	2.26	2.41	0.35	0.97	0.79	0.81	0.79
Myristoleic	0.76	0.55	0.67	0.77	0.12	0.55	0.51	0.76	0.21
Pentadecanoic	1.23	1.13	1.31	1.12	0.078	0.31	0.62	0.68	0.61
Pentadecenoic	0.28	0.26	0.39	0.15	0.11	0.51	0.93	0.61	0.33
Palmitic	20.00	19.85	18.88	20.44	1.032	0.75	0.82	0.94	0.42
Palmitoleic	3.93	3.29	3.28	3.99	0.3	0.19	0.24	0.91	0.03
Margaric	2.9	2.12	3.44	2.6	0.302	0.27	0.67	0.66	0.09
Margaroleic	4.21	3.74	4.02	3.7	0.39	0.81	0.43	0.57	0.77
Stearic	7.88	8.00	8.35	6.96	1.00	0.79	0.92	0.59	0.46
Oleic	46.25	47.28	44.9	47.21	0.83	0.17	0.83	0.89	0.45
Linoleic	3.46	3.85	4.33	3.73	0.3	0.24	0.15	0.34	0.11
Linolenic	0.39	0.62	0.57	0.42	0.08	0.16	0.13	0.91	0.03
Nonadecenoic	0.019	0.021	0.00	0.016	0.016	0.79	0.74	0.70	0.68
Gadoleic	0.045	0.13	0.093	0.014	0.04	0.22	0.49	0.48	0.06
Eicosadienoic	0.014	0.043	0.019	0.00	0.018	0.43	0.75	0.43	0.20
Arachidonic	0.12	0.049	0.15	0.11	0.028	0.11	0.66	0.53	0.59
Other	5.99	5.57	7.32	5.89	1.28	0.78	0.86	0.81	0.70
n-3[4]	0.508	0.67	0.71	0.53	0.086	0.27	0.20	0.75	0.06
n-6[5]	3.98	4.56	5.07	4.27	0.35	0.18	0.12	0.39	0.06
n-3:n-6[6]	0.12	0.14	0.15	0.13	0.015	0.64	0.34	0.68	0.23
Saturated[7]	34.53	34.6	34.24	33.53	1.75	0.97	0.84	0.67	0.82
Unsaturated[8]	59.48	59.82	58.43	60.21	0.92	0.56	0.10	0.85	0.44
Saturated:Unsaturated[9]	0.58	0.59	0.59	0.56	0.04	0.93	0.88	0.65	0.67
Polyunsaturated[10]	3.98	4.56	5.07	4.27	0.35	0.18	0.12	0.39	0.06
Monounsaturated[11]	55.5	55.26	53.38	55.94	0.96	0.27	0.57	0.90	0.16

Iodine Value[12]	50.56	52.17	50.79	51.97	0.77	0.36	0.24	0.42	0.79

[1]Treatment: Complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM); 10DDGS = 33% DDGS in place of SBM; 20DDGS = 66% DDGS in place of SBM; 30DDGS = 100% DDGS in place of SBM

[2]Main effects of treatment

[3]Pre-planned contrast: 0DDGS vs all levels of DDGS

[4]n-3 = C18:3 + C20:4

[5]n-6 = C18:2 + C18:3 + C20:2 + C20:4

[6]n-3:n-6 = (C18:3 + C20:4) / (C18:2 + C18:3 + C20:2 + C20:4)

[7]Saturated = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0

[8]Unsaturated = C12:1 + C14:1 + C15:1 + C16:1 + C17:1 + C18:1 +C18:2 + C18:3 + C19:1 + C20:1 + C20:2 + C20:4

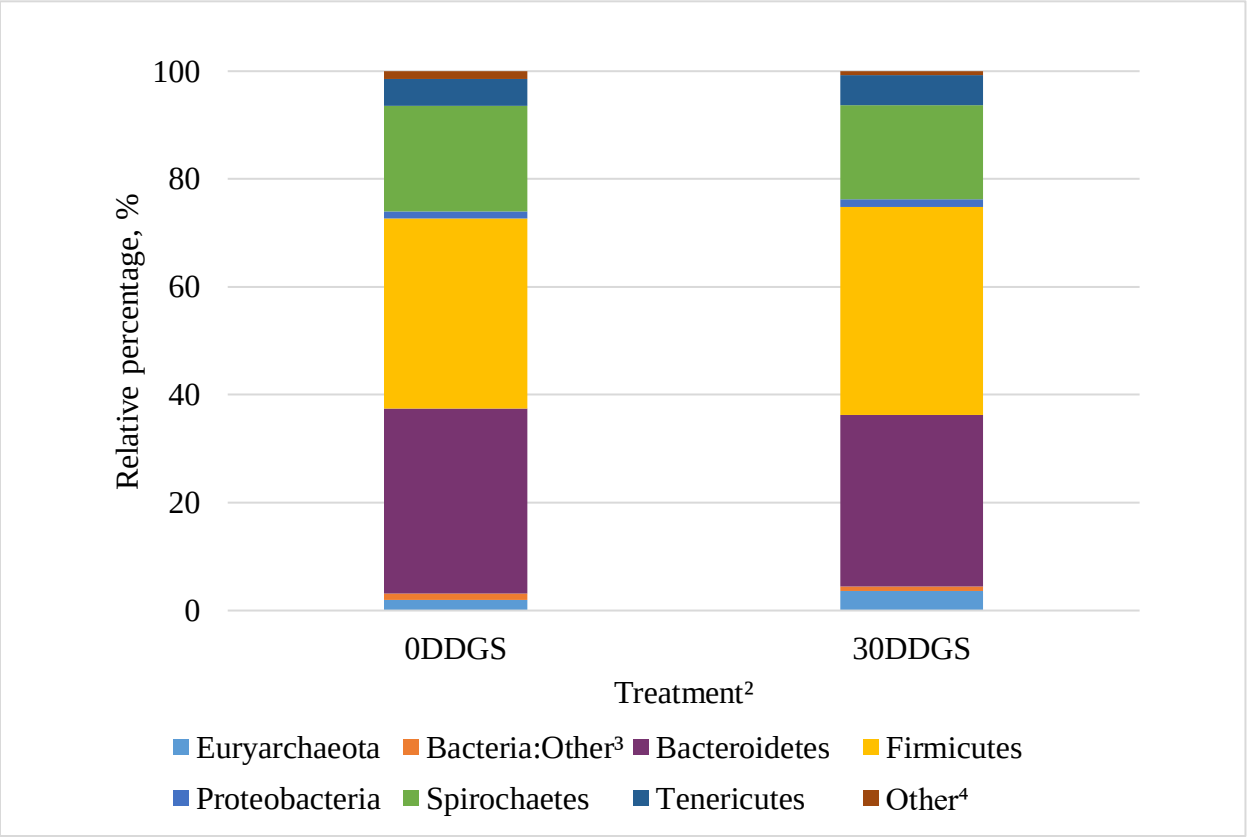
[9]Saturated:Unsaturated = (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0) / (C12:1 + C14:1 + C15:1 + C16:1 + C17:1 + C18:1 +C18:2 + C18:3 + C19:1 + C20:1 + C20:2 + C20:4)

[10]Polyunsaturated = C18:2 + C18:3 + C20:2 = C20:4

[11]Monounsaturated = C12:1 + C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C19:1 + C20:1

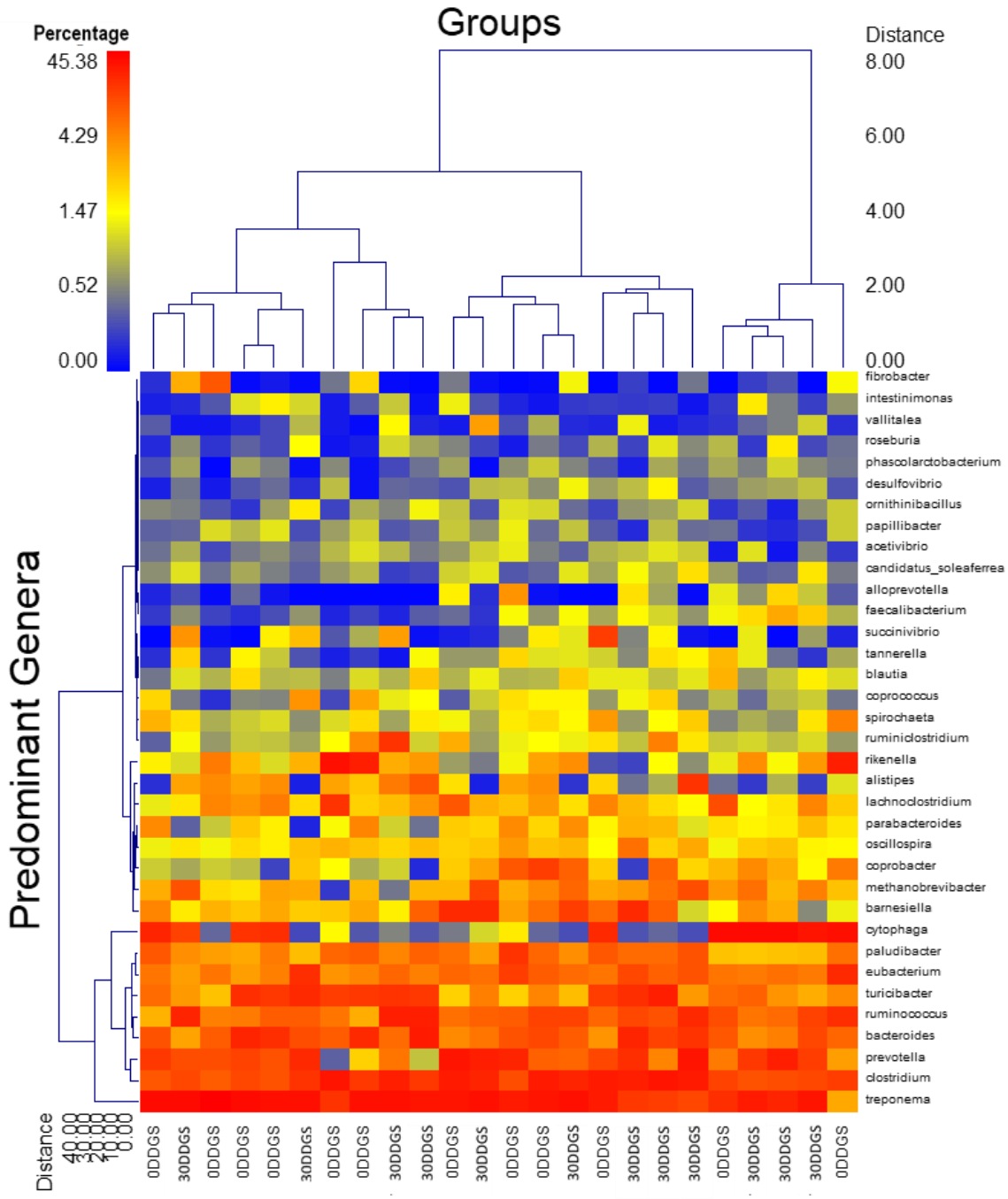
[12]Iodine Value = (C16:1*0.95) + (C18:1*0.86) + (C18:2*1.732) + (C18:3*2.616) + (C20:1*0.785)

Figure 3.1. Effect of replacing soybean meal (SBM) with dried distillers grains with solubles (DDGS) in Boer-type goat rations on bacterial phyla in fecal samples[1]



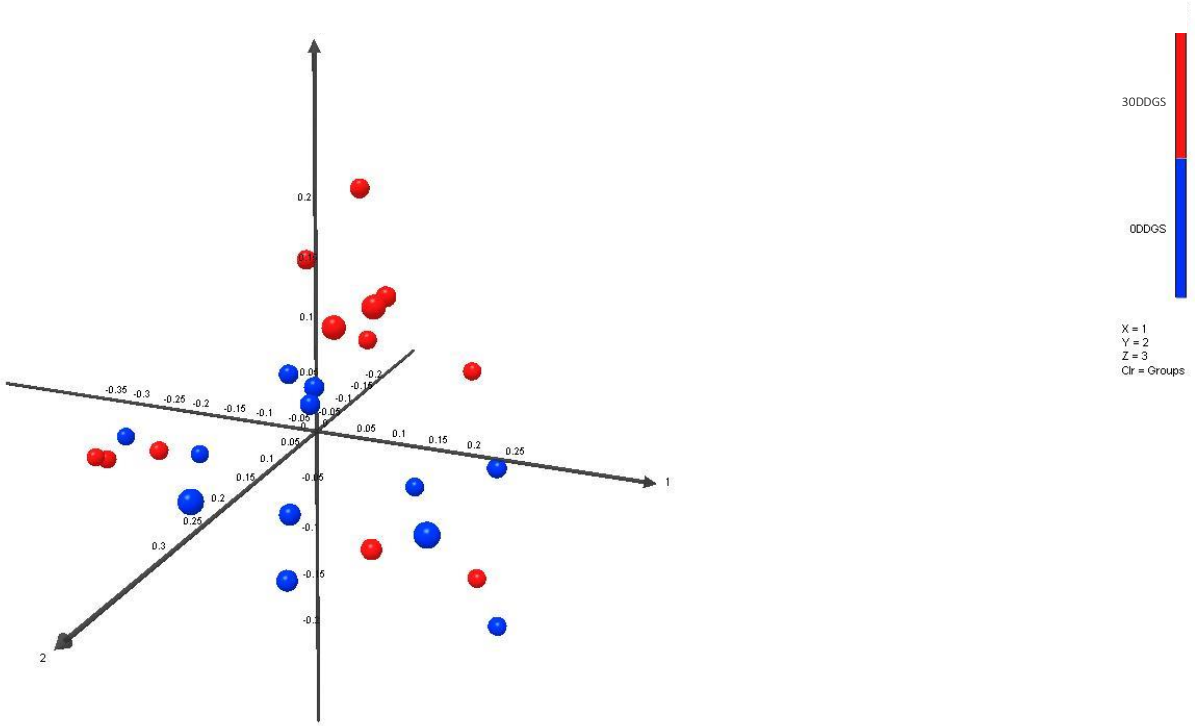
[1]The most abundant phyla consisted of Bacteroidetes, Firmicutes, and Spirochaetes; however, only Acidobacteria (not shown in figure; < 1% relative abundance in sample population) and Euyarchaeota increased (P = 0.08 and P = 0.01, respectively) from ODDGS to 30DDGS.
[2]Treatments were complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of SBM; 30DDGS = 100% DDGS in place of SBM.
[3]Bacteria unidentified at the phylum level
[4]Bacterial phyla that make up < 1% relative abundance in sample populations.

Figure 3.2. Effect of replacing soybean meal (SBM) with dried distillers grains with solubles (DDGS) in Boer goat rations on relative abundance of genera in fecal samples



[1]Samples, labeled on the X-axis, with more genera are clustered closer together and the predominant genera are represented along the Y-axis. Treatments were complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM); 30DDGS = 100% DDGS in place of SBM.

Figure 3.3. Effect of replacing soybean meal (SBM) with dried distillers grains with solubles (DDGS) on beta diveristy in Boer goat fecal samples[1]



[1]Based on the figure, no apparent phylogenic assemblage appears to be different between the two treatments. This is confirmed based on an analysis of similarities with $P = 0.43$. 30DDGS = red, 0DDGS = blue. Treatments were complete pelleted diets with 0DDGS = 0% dried distillers grains with solubles (DDGS) in place of soybean meal (SBM); 30DDGS = 100% DDGS in place of SBM.

Chapter 4 - Impact of varying protein and chloride sources on feedlot goat performance and fecal microbiome

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Abstract

Increasing rumen undegradable protein (RUP) in feedlot lamb diets has been shown to increase average daily gain (ADG) and feed efficiency. Thus, the effect of replacing soybean meal (SBM) with corn dried distillers grains with solubles (DDGS) or SoyPlus (Dairy Nutrition Plus, Ames, IA) and AmCl was replaced with SoyChlor (Dairy Nutrition Plus, Ames, IA) on growth, economics, carcass characteristics, and fecal microbiome of feedlot goats was evaluated in this study. Seventy-five Boer-type goats of mixed genetics (approximately 75 d of age; 23.53 ± 1.07 kg) were blocked by body weight and randomly assigned to 1 of 5 dietary treatments in a completely randomized design. Treatments were isocaloric and isonitrogenous and consisted of SBM with AmCl (SBM+AmCl), DDGS with AmCl (DDGS+AmCl), SoyPlus (Dairy Nutrition Plus, Ames, IA) with AmCl (SoyPlus+AmCl), SBM with SoyChlor (Dairy Nutrition Plus Ames, IA) (SBM+SoyChlor), and SoyPlus with SoyChlor (SoyPlus+SoyChlor). Goats were provided ad libitum access to feed and water for 42 d with 3 goats/pen and 5 pens/treatment. Data were analyzed using the Glimmix procedure of SAS with pen as the experimental unit for growth data and goat as the experimental unit for economics, carcass, and microbiome data. Alpha and beta diversity were analyzed using Qiime 2. Goats consuming SoyChlor had improved ADG (P = 0.01), a tendency for increased dry matter intake (DMI; P = 0.06), increased gain:feed (P = 0.04) and greater value of gain (P = 0.01) than goats consuming AmCl. SoyPlus had no effect on ADG (P > 0.10), but tended to decrease DMI (P = 0.06) when compared to SBM. Goats consuming DDGS had decreased feed cost/kg gain than those fed SBM (P = 0.02) and SoyPlus (P = 0.01). Protein source did not alter Firmicutes:Bacteroidetes (P > 0.10), despite goats fed SBM+AmCl having greater abundance of Bacteroidetes (P = 0.04) than DDGS+AmCl-fed goats. Of genera making up ≥ 1% relative abundance, *Clostridium*, *Tannerella*, *Paludibacter*, and

Methanobrevibacter were greater ($P \leq 0.05$) with SBM+AmCl than DDGS+AmCl and SoyPlus+AmCl. *Prevotella* was greater ($P = 0.02$) in goats fed SoyPlus+AmCl than DDGS+AmCl. No differences were found in alpha and beta diversity measures ($P > 0.10$, $r \approx 0$, respectively). Loin eye area was greater in goats fed SBM than those fed SoyPlus ($P = 0.05$) or DDGS ($P = 0.04$), regardless of chloride source. In summary, feedlot goat diets with increased RUP improved feed cost/kg gain and decreased DMI without negatively affecting ADG without influencing fecal microbial community structure or carcass parameters.

Key words: dried distillers grains, finishing, goat, microbiome, rumen undegradable protein, soybean meal

Introduction

An increase in goat populations across the Midwest has increased the demand for low-cost feedstuffs. While soybean meal (SBM) is a valuable protein source, it can be more costly than by-products that may be used in place of SBM. Corn dried distillers grain with solubles (DDGS), containing a 3-fold increase in protein, fat, fiber, and P concentrations (Klopfenstein et al., 2007) compared to corn, is a readily available and economical option in the Midwest due to the presence of ethanol plants. Furthermore, DDGS has approximately 70% rumen undegradable protein (RUP; NRC, 2016) compared to the 30% RUP of SBM (NRC, 2016). Previous research has reported greater concentrations of RUP improves ADG and gain:feed (G:F) in lambs fed isonitrogenous diets containing wheat straw, corn, and soybeans (Haddad et al., 2005). Tufarelli et al. (2009) reported that including corn gluten meal at a rate of 8.5% in a diet with soybean meal improves G:F with no difference in ADG in lambs versus those fed a diet with SBM.

Much of the literature over the goat's microbiome as it relates to nutrition has centered around the impact of altering the concentrate:forage ratio (Mao et al., 2014; Grilli et al., 2016,

Tao et al., 2017). While shifts in microbiota in the rumen of cattle have been extensively explored, relatively few studies have evaluated the effect of various protein and chloride sources on the microbiome of the goat gastrointestinal tract. Therefore, our objective was to evaluate the effect of feeding proteins sources with varying concentrations of RUP in place of soybean meal and SoyChlor in place of ammonium chloride (AmCl) on growth, economics, carcass characteristics, and fecal microbiome of Boer-type feedlot goats.

Materials and Methods

Animals

The Kansas State University Institutional Animal Care and Use Committee approved all animal protocols used in this experiment. Seventy-five Boer-cross goat kids (approximately 75 d of age; 23.53 ± 1.07 kg) were housed in 3 m × 1.5 m pens at the Kansas State University Sheep and Meat Goat Center, with 5 pens/treatment and 3 kids/pen. Of the 75 kids, 15 were wethers and 60 were doelings. Pens were equipped with individual waterers and free choice feeders.

Dietary treatments

Goats were randomly allotted to pens, and pens were randomly allotted to 1 of 5 treatment diets. Dietary treatments included soybean meal (SBM) with AmCl (SBM+AmCl), corn dried distillers grains with solubles (DDGS) with AmCl (DDGS+AmCl), SoyPlus (Dairy Nutrition Plus, Ames, IA) with AmCl (SoyPlus+AmCl), SBM with SoyChlor (Dairy Nutrition Plus Ames, IA; SBM+SoyChlor), and SoyPlus with SoyChlor (SoyPlus+SoyChlor) and were fed over 42 d. The inclusion levels of soybean hulls and corn were varied to maintain isocaloric and isonitrogenous diets (Table 4.1). Diets were pelleted at Kansas State University O.H. Kruse Feed Technology Innovation Center. Goats were transitioned onto their respective diets over 14 d

prior to d 0 by offering all pens *ad libitum* access to SBM+AmCl and slowly transitioning to their respective diets. No supplemental forage was provided throughout the study.

On d 14, goats were weighed and orally drenched with Ivermectin (200 μg Ivermectin per kg of body weight; Ivermectin Sheep Drench, Durvet Inc., Blue Springs, MO). Goats and feeders were weighed weekly to determine average daily gain (ADG), average daily feed intake (ADFI), dry matter intake (DMI), and gain:feed (G:F). Goats were weighed on d 42 to determine final body weight.

All goats were treated for coccidiosis on d 21 to 26 via Corid (Merial Ltd., Duluth, GA) administered though drinking water (16 oz. Corid/100 gal water). One goat from the DDGS+AmCl treatment group was removed from the study after not responding to treatment for coccidiosis.

Urinalysis

Due to unforeseen difficulties collecting urine samples from all goats, urine pH was not measured and urinalysis to evaluate leucocytes, nitrites, proteins, red blood cells, and microscopic crystals was unsuccessful. Therefore, data is unavailable regarding the effect of alternating chloride source on goat urinalysis.

Economic analyses

Economic analyses was performed to assess financial impact of dietary treatments. Total cost of SBM+AmCl, DDGS+AmCl, SoyPlus+AmCl, SBM+SoyChlor, and SoyPlus+SoyChlor were \$150.67, \$125.04, \$162.76, \$172.76, and \$178.25/ton, respectively (Table A.4). Total feed cost per goat was calculated as: ADFI × feed cost/kg × 42 d. Feed cost per kg of gain was calculated as: total feed cost/goat ÷ overall gain per goat from d 0 to 42. Value of gain (VOG) was calculated as: [(ending weight × \$221 cwt) – (beginning weight × \$200 cwt)] ÷ overall gain

per goat (USDA AMS, 2018; Rasby et al, 2015). Prices used in the current study were based on commodity prices in August 2018 and may not be representative of current prices (Table A.4)

Microbiome analyses

Fecal pellets were collected via rectal grab on d 42 from goats fed treatments SBM+AmCl, DDGS+AmCl, and SoyPlus+AmCl to evaluate the impact of protein source on feedlot goat fecal microbiome. Samples were placed into individual vials and stored at -80° C until microbiome sequencing could be performed. Genomic deoxyribonucleic acid (DNA) was isolated from approximately 250 mg of feces using the Powersoil DNA Isolation Kit (Qiagen Inc., Valencia, CA) by a commercial laboratory (MR DNA, Shallowater, TX) following manufacturer instructions. Samples were then stored at -20° C until polymerase chain reaction (PCR) amplification. The 16S universal Eubacterial primers 515F (5'-GTGYCAGCMGCCGCGGTAA) and 806R (5'-GGACTACNGGGTWTCTAAT) were used to evaluate microbial ecology of samples on the Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) platform via the bTEFAP DNA analysis service originally described by Dowd et al. (2008). All samples underwent a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen Inc, Valencia, CA) under the following conditions: 94° C for 3 min; followed by 28 cycles of 94° C for 30 s, 53° C for 40 s and 72° C for 1 min; followed by the final elongation step at 72° C for 5 min. The PCR products were checked for relative intensity of bands and success of amplification in 2% agarose gel before being pooled together in equal proportions based on molecular weight and DNA concentration. Pooled samples were purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, Beverly, MA) and pyrosequencing was performed using Illumina HiSeq (Illumina Inc., San Diego, CA) chemistry following manufacturer's guidelines by MR DNA (MR DNA, Shallowater, TX). All microbiome

data were processed through a proprietary analysis pipeline (www.mrdnalab.com; MR DNA, Shallowater, TX). Final microbial analyses was performed on 6,781,640 sequences identified in the bacterial and archaeal domains and an average of 154,128 reads were observed per sample. Samples for alpha and beta diversity analysis were rarefied to 20,000 sequences.

Carcass analyses

At the end of the experiment, the lightest and heaviest goat per pen were harvested (Paradise Locker, Inc., Trimble, MO) to determine hot carcass weight (HCW), carcass yield, loin eye area (LEA), fat depth at the 12th rib, and body wall thickness (BWT) at the 12th rib.

Statistical analyses

Data were analyzed using the GLIMMIX procedure of SAS Studio (Version 9.4; SAS Inst., Cary, NC) with Tukey's test for post hoc pairwise comparisons. Pen served as the experimental unit for growth data, while individual goat served as the experimental unit for economic, microbiome, and carcass data. Pre-planned contrast statements were used to evaluate SBM vs DDGS, SBM vs SoyPlus, DDGS vs SoyPlus, and AmCl vs SoyChlor. Analyses for alpha and beta diversity were conducted as described by Dowd et al. (2008) using Qiime (www.qiime.org; Bolyen et al., 2018). Statistical comparisons for alpha diversity used Kruskal-Wallis pairwise comparisons to compare observed OTUs and the Shannon Diversity index. Beta diversity was analyzed using a weighted UniFrac distance matrix with pairwise analysis of similarities (ANOSIM) to determine correlation (r) between samples. A greater r (±1) indicated increased similarity between samples within the same treatment group whereas 0 indicated no relationship. Significance was determined at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Results

Growth performance and economic value

Using these dietary treatments, varying protein and chloride sources had no observed impact on final body weight ($P > 0.10$; Table 4.2). Goats consuming SoyChlor had greater ($P = 0.01$) ADG and tended to have increased DMI and ADFI ($P = 0.06$ and $P = 0.08$, respectively) leading to greater G:F ($P = 0.04$) than AmCl-fed goats. Goats consuming SoyPlus, regardless of chloride source, tended to have decreased DMI and ADFI ($P = 0.06$) than goats fed SBM.

Feed cost per goat was decreased with diets containing DDGS rather than SBM ($P = 0.001$) or SoyPlus ($P = 0.02$; Table 4.2). Diets containing AmCl were cheaper ($P < 0.0001$) than those with SoyChlor. Feed cost per kg gain with diets containing DDGS were less than those with SBM ($P = 0.02$) and SoyPlus ($P = 0.01$). SoyChlor produced greater VOG ($P = 0.01$) compared to AmCl diets.

Microbial populations and diversity

Taxonomic classification of feces from goats fed varying protein sources yielded 24 bacterial phyla, 256 genera, and 531 species. Bacteroidetes and Firmicutes were the most abundant phyla in fecal samples of goats fed SBM+AmCl, DDGS+AmCl, and SoyPlus+AmCl (Table 4.3). No difference in Firmicutes:Bacteroidetes was observed between treatments ($P = 0.63$). Spirochaetes and Proteobacteria were the third and fourth most abundant phylum, respectively, in goats fed SBM+AmCl, DDGS+AmCl, and SoyPlus+AmCl.

Few differences ($P \geq 0.11$) were found in the abundance phyla between treatment groups in goat fecal samples (Table 4.3). Goats consuming SBM+AmCl had a greater ($P = 0.04$) relative abundance of Bacteroidetes than those fed DDGS+AmCl. The relative abundance of Euryarchaeota was increased in goats consuming SBM+AmCl compared to those fed

DDGS+AmCl (P = 0.003) and SoyPlus+AmCl (P = 0.0001), which were not different from each other (P = 0.34). Fibrobacteres tended to increase (P = 0.06) in DDGS+AmCl-fed goats compared to SBM+AmCl-fed goats. Actinobacteria and Verrucomicobia tended to be greater (P = 0.09) in goats fed SoyPlus+AmCl compared to those fed DDGS+AmCl.

Twenty-one genera each individually constituted ≥ 1% relative abundance and account for the majority of genera identified in the feces of goats fed SBM+AmCl (82.46%), DDGS+AmCl (81.38%), and SoyPlus+AmCl (82.65%; Table 4.4; Figure 4.1). The genera *Clostridium*, *Paludibacter*, *Tannerella*, and *Methanobrevibacter* were in greater abundances in goats consuming SBM+AmCl compared to DDGS+AmCl (P = 0.01, P = 0.04, P = 0.005, and P = 0.002, respectively) and SoyPlus+AmCl (P = 0.001, P = 0.0001, P = 0.01, and P = 0.0001, respectively). In addition, DDGS+AmCl-fed goats had a greater abundance of *Paludibacter* (P = 0.04) than SoyPlus+AmCl-fed goats.

Prevotella was found in a greater abundance (P = 0.02) in SoyPlus+AmCl-fed goats compared to those consuming DDGS+AmCl. *Coprobacter* was in a greater abundance (P = 0.04) in goats fed SBM+AmCl compared to SoyPlus+AmCl and tended to be greater (P = 0.07) in goats fed SBM+AmCl compared to DDGS+AmCl. *Lachnoclostridium* was greater (P = 0.02) in SBM+AmCl-fed goats compared to those fed SoyPlus+AmCl.

Ruminococcus bromii increased in goats fed DDGS+AmCl (P = 0.05) and tended to increase in goats fed SoyPlus+AmCl (P = 0.09) in place of SBM+AmCl. Goats consuming SoyPlus+AmCl had greater (P = 0.03) concentrations of *Prevotella ruminicola* compared to those fed DDGS+AmCl. Furthermore, *Bifidobacterium merycicum* tended to increase in SoyPlus+AmCl compared to goats consuming DDGS+AmCl (P = 0.09) or SBM+AmCl (P = 0.08).

When utilizing observed OTUs and Shannon indices as alpha diversity metrics, the microbial diversity did not differ between treatments ($P > 0.10$; Table 4.5). Based on pairwise ANOSIM and a weighted UniFrac distance matrix (Figure 4.2), no relationship between samples was evident between goats fed SBM+AmCl and DDGS+AmCl (r = 0.09), SBM+AmCl and SoyPlus+AmCl (r = 0.05), or DDGS+AmCl and SoyPlus+AmCl (r = -0.003).

Carcass characteristics

When protein and chloride source varied, difference in carcass yield, backfat depth, and BWT were not detected ($P > 0.10$; Table 4.6). Loin eye area increased in goats fed SBM compared to DDGS ($P = 0.04$) and SoyPlus ($P = 0.005$). Goats consuming SBM diets also had a tendency for greater HCW ($P = 0.06$) and greater LED ($P = 0.02$) than goats fed SoyPlus diets, regardless of chloride source.

Discussion

Growth performance and economic value

Protein source had no observed effect on ADG when fed at these levels, possibly due to all goats consuming more crude protein (CP; 151.87 g/d, 164.37 g/d, 137.12 g/d, 165.47, and 147.23g/d for SBM+AmCl, DDGS+AmCl, SoyPlus+AmCl, SBM+SoyChlor, and SoyPlus+SoyChlor, respectively) than the daily requirement set by the NRC (2007) for 25 kg growing doelings and males castrates gaining 100 to 150 g/d. Gurung et al. (2009) also found no difference in ADG between Kiko × Spanish goats fed diets were DDGS replaced corn and SBM. Maynard et al. (2015) found a tendency for decreased ADG with DDGS inclusion in place of SBM in goats; however, diets were not isonitrogenous or isocaloric. Performance studies in sheep have also shown no effect of DDGS when SBM was replaced in isonitrogenous diets (Huls et al., 2006; Crane et al., 2017). Others have reported substituting DDGS for SBM and barley

(Shauer et al., 2008) or dry-rolled corn (Castro-Pérez et al., 2014) increased ADG in sheep. Similar improvements have been reported in cattle when DDGS replaced dry-rolled corn (Buckner et al., 2007). Based on commodity prices in August 2018 for Kansas, goats consuming DDGS had a lower feed cost per goat when compared to those consuming SoyPlus or SBM. When analyzed on a $/kg CP basis, DDGS was lower when compared to SBM or SoyPlus ($0.73/kg CP, $0.53/kg CP, and $1.76/kg CP for SBM, DDGS, and SoyPlus, respectively).

In the current study, goats fed SBM or SoyPlus in conjunction with SoyChlor had greater ADG than those fed SBM or SoyPlus with AmCl. This is likely attributed to the fact that goats fed diets containing SoyChlor consumed more crude protein (156.35 g/d vs 151.12 g/d) and net energy for gain (1.15 Mcal/d vs 1.08 Mcal/d) than those consuming diets containing AmCl. The increased ADG found in the current study may also be contributed to the fact that diets containing SoyChlor had an average of 71% RUP as a percent of CP, compared to diets containing AmCl, which had an average of 67% RUP as a percent of CP. Haddad et al. (2005) reported increasing RUP improved ADG and feed efficiency (FE) in lambs fed isonitrogenous diets of wheat straw, corn, and soybeans. Furthermore, Tufarelli et al. (2009) reported that including 8.5% corn gluten meal in a diet at the expense of SBM improves FE in lambs. Corn gluten meal is similar to DDGS as it contains approximately 60% RUP as a percent of CP (NRC, 2016). In the current study, goats consuming AmCl diets had a lower feed cost/goat; however, the increased ADG in goats fed SoyChlor diets is reflective of feed cost/kg gain being similar.

Microbial populations and diversity

Others have evaluated the goat ruminal microbiome on varying concentrate:forage diets have also reported Firmicutes and Bacteroidetes as the most common phyla in goats (Mao et al., 2014; Grilli et al., 2016; Tao et al., 2017). Similar to no differences in Firmicutes:Bacteroidetes

across treatments in the current study, Castillo-Lopez et al. (2013) found no difference in Firmicutes:Bacteroidetes in duodenal samples of steers fed DDGS in place of corn bran. However, Callaway et al. (2010) reported that Firmicutes:Bacteroidetes tended ($P \leq 0.10$) to decrease in fecal samples from steers consuming DDGS in place of a commercial grain supplement.

Spirochaetes, the third most abundant phylum in goats regardless of diet, contains microbes capable of fermenting xylan, pectin, arabinogalactan, cellobiose, and glucose (Stanton and Canale-Parola, 1980; Paster and Canale-Parola, 1982). Others have reported decreased abundances of *Treponema* in fecal samples collected from cattle consuming diets of 10% wet corn distillers grains (Rice et al., 2012) and 50% DDGS (Callaway et al., 2010) in place of corn compared to the current study. Stanton and Canale-Parola (1980) reported that *T. bryantii* grew abundantly when co-cultured with the cellulolytic bacteria *Bacteroides succinogenes* and *Ruminococcus albus*. Spirochaetes do not ferment cellulose, but they utilize the end products of cellulose hydrolysis; therefore, cellulolytic bacteria may promote a larger Spirochaete population (Paster and Canale-Parola, 1982). Arieli and Sklan (1985) reported approximately 30% of total cellulose degradation occurs in the cecum and large intestine of sheep fed a pelleted ration containing 18% CP and 8.3% cellulose. Firkens et al. (1986) reported greater post-ruminal NDF digestion with diets containing 20% DDGS (DM basis) compared to a 17% dry corn gluten feed. While the cellulolytic bacteria *R. flavefaciens* tended to be greater in goats fed SBM compared to DDGS, another cellulolytic bacterium, *F. succinogenes*, tended to be greater in DDGS-fed goats compared to SBM-fed goats. If more soluble fiber is available for fermentation in the hindgut when DDGS are fed, the tendency for an increase in *T. bryantii* in goats fed DDGS+AmCl compared to those consuming SBM+AmCl may be partially explained.

The relative abundance of *Clostridium*, *Prevotella* and *Bacteroides* in the current study are similar to those reported by Rice et al. (2012) when cattle were fed diets containing 10% wet corn distillers grain. The current study's results are consistent with Callaway et al. (2010) in that DDGS did not alter the abundance of *Ruminococcus* in cattle feces. *Ruminococcus bromii* increased in goats fed DDGS+AmCl or SoyPlus+AmCl in place of SBM+AmCl. *Ruminococcus bromii* is a specialized amylolytic bacteria that can degrade resistant starches (Ze et al., 2015) and has been shown to increase in cattle fed diets containing 10% DDGS (Rice et al., 2012). *Prevotella ruminicola* aides in the degradation of hemicellulose (Nagaraja, 2016) and peptides (Wallace et al., 1997) within the rumen. Goats consuming SoyPlus+AmCl had greater concentrations of *Prevotella ruminicola* compared to those fed DDGS. Therefore, greater hemicellulose and peptide degradation within the hindgut of SBM+AmCl-fed goats may have occured. *Bifidobacterium merycicum*, capable of degrading monosaccharides (Biavati and Mattarelli, 1991), tended to increase in goats fed SoyPlus+AmCl compared to goats consuming DDGS+AmCl or SBM+AmCl. Perhaps more monosaccharides escaped ruminal fermentation and enzymatic digestion, thus passing to the hindgut when SoyPlus+AmCl was fed. Since microbiome analyses simply report on which bacterial and archeal populations are present rather than those that are being coded for, there is a possibility that some abundances are influenced by microbes from the rumen.

Methanogens use end products of microbial fermentation, specifically H_2 and CO_2, to maintain the partial pressure of H_2 that may otherwise inhibit microbial enzymes (Morgavi et al., 2010). *Methanobrevibacter* increased in goats fed SBM+AmCl compared to those fed DDGS+AmCl or SoyPlus+AmCl. This was unexpected, and since fermentational end products

and nutrient digestibility were not evaluated in the current study it is unclear why this change occurred.

Alpha diversity refers to diversity within a sample while beta diversity allows for comparison of bacterial community structure between samples. Operational taxonomic unit counts did not differ between treatments. Furthermore, based on the Shannon index, another alpha diversity measure that further evaluates how rich and evenly dispersed microbes are within each treatment, the current study found no differences between treatment groups. This indicates bacteria were relatively evenly balanced within sample populations.

Carcass characteristics

Castro-Pérez et al. (2014) found the *Longissimus thoracis* area to decrease linearly in goats consuming DDGS at a rate up to 45% of the diet in place of SBM. Likewise, goats fed diets containing SBM, regardless of chloride source, had greater LEA compared to those consuming DDGS in the current study. Other studies provide antithetical data whereby protein source had no impact on LEA in lambs (Huls et al., 2006; Schauer et al., 2008; Crane et al., 2017) and goats (Gurung et al., 2009). Despite greater ADG, DMI and G:F in goats fed diets containing SoyChlor compared to AmCl, regardless of protein source, carcass characteristics were not different. Hot carcass weight, carcass yield, and BWT were unaffected by treatment, as was expected based on previous studies in sheep (Huls et al., 2006; Shauer et al., 2008; Crane et al., 2017) and goats (Gurung et al., 2009). Huls et al., (2006) reported increased backfat depth in goats fed 22.9% DDGS in place of SBM (DM basis), however others have reported no difference (Shauer et al., 2008; Gurung et al., 2009; Crane et al., 2017).

Conclusion

In summary, protein source did not alter final BW, ADG, DMI, or G:F despite the fact RUP differing between diets. While goats fed SBM had greater LEA, other carcass characteristics were uninfluenced by protein source. Despite differences noted in Euryarchaeota and some genera, microbial community diversity was unchanged by diet. By including SoyChlor in place of AmCl, ADG, DMI, and G:F increased while carcass characteristics were unchanged. SoyChlor improved growth performance and produced a greater value of gain; however, diets containing AmCl was less expensive. The diet containing DDGS was the most economical in this study when considering feed cost and feed cost/kg of gain. Altering protein and chloride source may be a viable option for increased gain, but more research is needed to further understand how nutrient digestibility and fermentation parameters alter the hindgut microbiome of feedlot goats.

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Tables and Figures

Table 4.1. Dietary composition (dry matter basis)[1]

	Treatment[2]				
Item	**SBM+ AmCl**	**DDGS+ AmCl**	**SoyPlus[3]+ AmCl**	**SBM+ SoyChlor[3]**	**SoyPlus[3]+ SoyChlor[3]**
Ingredient, %					
Soybean meal	18.72	-	-	17.24	-
DDGS[4]	-	34.35	-	-	-
SoyPlus[3]	-	-	22.02	-	20.01
Ammonium chloride	0.75	0.75	0.75	-	-
SoyChlor[3]	-	-	-	4.83	4.83
Corn	52.75	48.31	53.33	49.39	51.38
Soybean hulls	25.93	15.04	18.81	24.07	19.23
Vitamin/mineral pack[5]	0.51	0.05	0.62	0.66	0.64
Nutrient composition					
Dry matter, %	88.60	88.60	88.60	89.40	89.00
Crude protein, %	18.30	18.90	19.40	17.20	17.70
Ruminal undegradable protein, % CP[6]	55.00	74.00	71.00	67.00	75.00
Acid detergent fiber, %	14.50	9.80	11.60	13.70	12.00
Neutral detergent fiber, %	21.70	19.10	22.90	22.30	22.60
Crude fat, %	2.70	4.90	4.90	2.60	3.80
Total dietary nutrients[7], %	80.00	82.00	81.00	78.00	80.00
Net energy for gain, Mcal/kg	1.30	1.38	1.35	1.25	1.31
Calcium, %	1.00	1.18	0.87	0.75	0.93
Phosphorus, %	0.50	0.51	0.54	0.50	0.56
Sulfur, %	0.22	0.38	0.27	0.22	0.23

[1]Proximate analysis performed using wet chemistry with minerals by a commercial laboratory (Dairy One Forage Lab, Ithaca, NY). Diets were balanced to be ≥ crude protein and net energy for gain requirements (dry matter basis) for 25 kg Boer doelings and male castrates gaining 100 to 150 g·head^{-1}·day^{-1} (NRC, 2007)

[2]Complete pelleted diets: SBM+AmCl = soybean meal + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride; SBM+SoyChlor = soybean meal + SoyChlor; SoyPlus+SoyChlor = SoyPlus + SoyChlor

[3]SoyPlus and SoyChlor are trademarks of Landus Corporation (Dairy Nutrition Plus, Ames, IA)

[4]DDGS = Dried distillers grains with solubles

[5]Pack contains: Cu Sulfate, Zn Oxide, Monocalcium Phosphate, Se Selenite, Vit. A 30,000, Vit. D 30,000, and Vit. E 30,000.

[6]Crude protein

[7]Calculated

Table 4.2. Effect of protein and chloride source on feedlot goat growth performance and economic parameters

Item	Treatment[1]					Pooled SEM	*P*-value				
	SBM + AmCl	DDGS + AmCl	SoyPlus[2] + AmCl	SBM + SoyChlor[2]	SoyPlus[2] + SoyChlor[2]		TRT[3]	SBM vs. DDGS[4]	SBM vs. SoyPlus[2,4]	DDGS vs. SoyPlus[2,4]	AmCl vs. SoyChlor[2,4]
Body weight, kg											
d 0	24.71	23.35	22.23	24.05	23.29	1.07	0.57	0.44	0.15	0.66	0.81
d 42	30.09	28.99	27.04	31.43	29.79	1.39	0.28	0.31	0.11	0.74	0.15
Average daily gain, kg	0.13	0.13	0.11	0.18	0.16	0.02	0.10	0.38	0.31	0.96	0.01
Average daily feed intake, kg	2.81	2.78	2.39	3.23	2.80	0.21	0.14	0.37	0.06	0.49	0.08
Dry matter intake, kg	2.49	2.46	2.12	2.89	2.49	0.19	0.12	0.33	0.06	0.51	0.06
Gain:feed, kg gain:kg DMI	0.051	0.055	0.053	0.061	0.062	0.004	0.28	0.92	0.72	0.69	0.04
Feed cost, $/goat[5]	6.53^{bc}	5.68^{c}	6.01^{bc}	8.61^{a}	7.71^{ab}	0.42	0.0003	0.002	0.12	0.03	<0.0001
Feed cost, $/kg gain[6]	3.87	2.83	3.97	3.51	3.62	0.37	0.20	0.07	0.78	0.10	0.59
Value of gain[7]	7.29	6.92	7.31	6.46	6.64	0.08	0.08	0.89	0.69	0.85	0.01

[1]Complete pelleted diets: SBM+AmCl = soybean meal + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride; SBM+SoyChlor = soybean meal + SoyChlor; SoyPlus+SoyChlor = SoyPlus + SoyChlor

[2]SoyPlus and SoyChlor are trademarks of Landus Corporation (Dairy Nutrition Plus, Ames, IA)

[3]TRT = main effect of treatment

[4]Pre-planned contrast statements: Soybean meal (SBM) vs. Dried distillers grains with solubles (DDGS); SBM vs. SoyPlus; DDGS vs. SoyPlus; AmCl vs SoyChlor

^{abc} Means within the same row with a different superscript are different ($P \leq 0.05$)

[5]Based on local ingredient prices on September 1, 2018

[6]Feed cost/kg gain = feed cost / total gain per goat

[7]Value of gain, income ($)/gain = [(Ending weight*$4.88) – (beginning weight*4.40)] / (ending weight – beginning weight)

Table 4.3. Most abundant bacterial and archaeal phyla in fecal samples of feedlot goats fed varying protein sources[1]

	Treatment[2]				*P*-value			
Phylum	SBM+ AmCl	DDGS+ AmCl	SoyPlus[3]+ AmCl	Pooled SEM	TRT[4]	SBM vs DDGS[5]	SBM vs SoyPlus[3,5]	DDGS vs SoyPlus[3,5]
Bacteroidetes	39.59	33.48	36.22	1.99	0.11	0.04	0.23	0.33
Firmicutes	37.81	34.21	32.16	2.60	0.3	0.34	0.13	0.58
Spirochaetes	17.82	23.36	19.49	3.41	0.51	0.26	0.73	0.43
Proteobacteria	2.48	4.62	5.53	1.64	0.4	0.36	0.19	0.70
Actinobacteria	0.09	0.16	4.84	1.90	0.14	0.98	0.08	0.09
Fibrobacteres	0.13	2.21	0.61	0.76	0.15	0.06	0.65	0.15
Euryarchaeota	1.42	0.19	0.35	0.18	0.0004	0.003	0.0001	0.34
Verrucomicrobia	0.31	0.27	0.37	0.21	0.94	0.16	0.22	0.09
Elusimicrobia	0.07	0.76	0.10	0.29	0.19	0.11	0.94	0.12
Tenericutes	0.18	0.15	0.19	0.04	0.77	0.65	0.79	0.47
Cyanobacteria	0.08	0.15	0.09	0.06	0.67	0.39	0.86	0.49
Firmicutes:Bacteroidetes	1.01	1.06	0.93	0.10	0.63	0.71	0.55	0.34

[1]Phyla making up > 0.1% relative abundance

[2]Complete pelleted diets: SBM+AmCl = soybean meal (SBM) + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles (DDGS) + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride

[3]SoyPlus is a trademark of Landus Corporation (Dairy Nutrition Plus, Ames, IA)

[4]TRT = main effect of treatment

[5]Pre-planned contrast statements: Soybean meal (SBM) vs. dried distillers grains with solubles (DDGS); SBM vs. SoyPlus; DDGS vs. SoyPlus

Table 4.4. Most abundant bacterial and archaeal genera in fecal samples of feedlot goats fed varying protein sources[1]

	Treatment[2]				*P*-value			
Genera	SBM+ AmCl	DDGS+ AmCl	SoyPlus[3]+ AmCl	Pooled SEM	TRT[4]	SBM vs DDGS	SBM vs SoyPlus[35]	DDGS vs SoyPlus[35]
Treponema	16.53	22.46	18.67	3.37	0.46	0.22	0.65	0.43
Bacteroides	9.65	12.22	10.56	1.67	0.55	0.29	0.70	0.49
Clostridium	9.67	6.39	5.46	0.86	0.003	0.01	0.001	0.45
Prevotella	6.24	4.23	10.69	1.90	0.06	0.46	0.10	0.02
Ruminococcus	7.43	5.76	6.87	1.10	0.56	0.29	0.71	0.48
Eubacterium	3.88	4.35	3.53	0.73	0.73	0.65	0.73	0.43
Paludibacter	4.35	3.25	2.16	0.36	0.0005	0.04	0.0001	0.04
Coprobacter	4.59	2.55	2.38	0.77	0.09	0.07	0.04	0.87
Lachnoclostridium	4.55	2.55	1.58	0.88	0.06	0.12	0.02	0.44
Tannerella	4.33	1.64	1.99	0.63	0.008	0.005	0.01	0.69
Rikenella	1.43	2.64	2.60	1.35	0.77	0.53	0.54	0.98
Turicibacter	2.06	1.95	2.05	0.32	0.96	0.81	0.98	0.82
Faecalibacterium	0.66	3.03	1.87	0.68	0.06	0.02	0.21	0.24
Parabacteroides	1.70	1.49	1.68	0.28	0.85	0.61	0.96	0.64
Bifidobacterium	0.03	0.07	4.53	1.82	0.14	0.99	0.08	0.09
Succinivibrio	1.18	2.53	0.51	0.81	0.21	0.25	0.55	0.09
Blautia	1.21	0.84	1.18	0.23	0.47	0.27	0.93	0.31
Coprococcus	1.18	1.37	0.70	0.39	0.47	0.74	0.39	0.24
Campylobacter	0.08	0.59	2.48	0.92	0.16	0.70	0.07	0.16
Spirochaeta	1.29	0.90	0.82	0.27	0.42	0.31	0.22	0.84
Methanobrevibacter	1.42	0.57	0.34	0.18	0.0003	0.002	0.0001	0.38

[1]Genera making up > 1% relative abundance

[2]Complete pelleted diets: SBM+AmCl = soybean meal (SBM) + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles (DDGS) + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride

[3]SoyPlus is a trademark of Landus Corporation (Dairy Nutrition Plus, Ames, IA)

[4]TRT = main effect of treatment

[5]Pre-planned contrast statements: Soybean meal (SBM) vs. dried distillers grains with solubles (DDGS); SBM vs. SoyPlus; DDGS vs. SoyPlus

Table 4.5. Alpha diversity in fecal samples of feedlot goats fed varying protein sources

	Treatment[1]				*P*-value[2]		
Item	**SBM+ AmCl**	**DDGS+ AmCl**	**SoyPlus[3]+ AmCl**	**SEM**	**SBM vs DDGS**	**SBM vs SoyPlus[3]**	**DDGS vs SoyPlus[3]**
Observed OTU[4] index	463.80	459.36	438.07	19.24	0.83	0.92	0.98
Shannon Index[5]	6.17	6.06	5.77	0.18	0.41	0.27	1.00

[1]Complete pelleted diets: SBM+AmCl = soybean meal (SBM) + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles (DDGS) + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride

[2]Comparisons were made using Kruskal-Wallis pairwise comparisons: soybean meal (SBM) vs. dried distillers grains with solubles (DDGS); SBM vs. SoyPlus; DDGS vs. SoyPlus

[3]SoyPlus is a trademark of Landus Corporation (Dairy Nutrition Plus, Ames, IA)

[4]Operational taxonomic units

[5]Alpha diversity index accounting for both richness and evenness

Table 4.6. Feedlot goat carcass characteristics in goats fed varying sources of protein and chloride

	Treatment[1]						*P*-value			
Item	SBM + AmCl	DDGS + AmCl	SoyPlus[2] + AmCl	SBM + SoyChlor[2]	SoyPlus[2] + SoyChlor[2]	Pooled SEM	TRT[3]	SBM vs. DDGS[4]	SBM vs. SoyPlus[24]	DDGS vs. SoyPlus[24]	AmCl vs. SoyChlor[24]
Hot carcass weight, kg	15.60	14.50	13.10	16.40	14.70	1.09	0.26	0.25	0.06	0.67	0.23
Carcass yield[5], %	50.70	49.40	48.30	50.70	49.60	1.11	0.52	0.34	0.12	0.74	0.50
Loin eye area[6], cm[2]	10.80[a]	9.40[ab]	9.50[ab]	11.40[a]	8.80[b]	0.66	0.05	0.04	0.005	0.78	0.75
Loin eye depth[6], cm	2.60	2.40	2.40	2.60	2.30	0.11	0.14	0.12	0.02	0.72	0.78
Backfat depth[7], mm	0.90	1.20	1.00	1.10	1.20	0.17	0.71	0.38	0.51	0.73	0.46
Body wall thickness, cm	1.50	1.60	1.50	1.70	1.50	0.13	0.76	0.93	0.52	0.53	0.44

[1]Complete pelleted diets: SBM+AmCl = soybean meal + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride; SBM+SoyChlor = soybean meal + SoyChlor; SoyPlus+SoyChlor = SoyPlus + SoyChlor

[2]SoyPlus and SoyChlor are trademarks of Landus Corporation (Dairy Nutrition Plus, Ames, IA)

[3]TRT = main effect of treatment

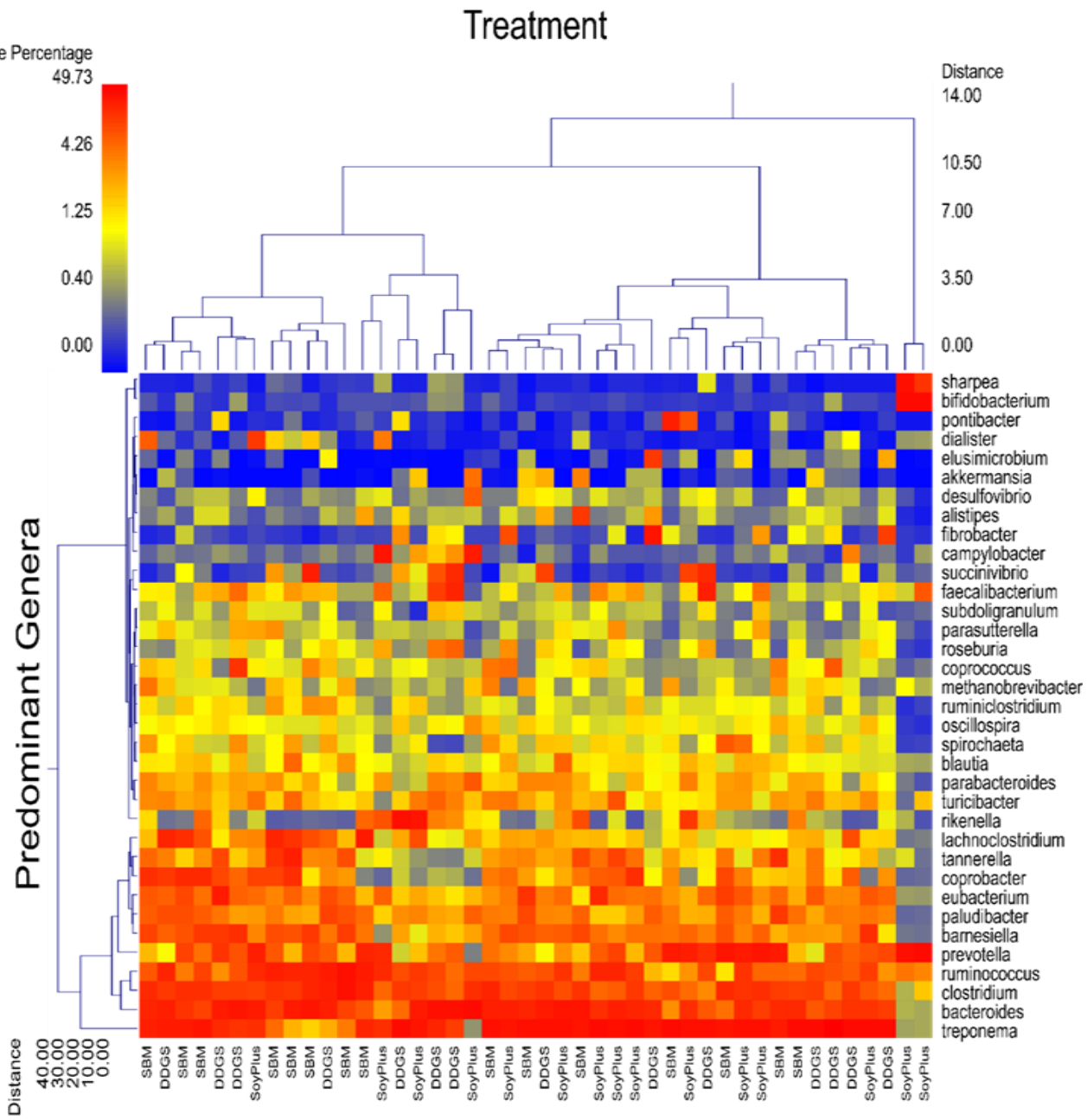
[4]Pre-planned contrast statements: Soybean meal (SBM) vs. dried distillers grains with solubles (DDGS); SBM vs. SoyPlus; DDGS vs. SoyPlus; AmCl vs SoyChlor[5]Carcass yield, %, calculated by dividing hot carcass weight by live weight recorded before transport to packing plant

[6]*Longissimus dorsi*

[7]Subcutaneous fat depth measured over the 12th rib

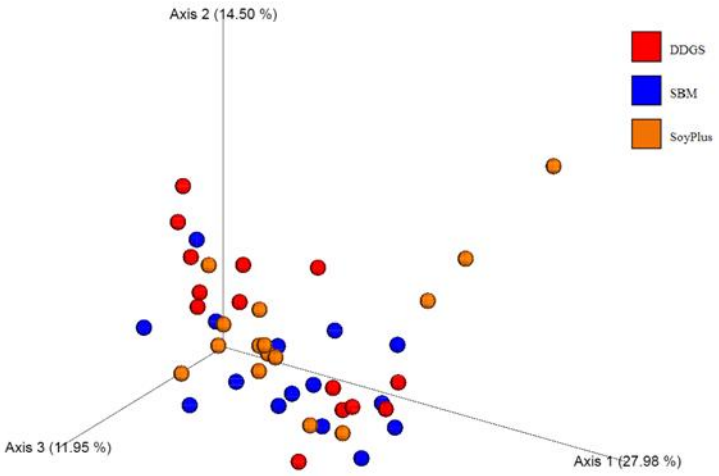
[abc] Means within the same row with a different superscript are different ($P \leq 0.05$)

Figure 4.1. Most abundant bacterial and archaeal genera in fecal samples of feedlot goats fed varying protein sources[1]



[1]A dual hierarchal dendrogram is used to visually evaluate genera populations, with each sample clustered on the X-axis labeled based on treatment. Samples that mathematically cluster closer together are more similar in consortium of genera with the lines located at the top of the heatmap (the shorter the line between samples indicates more similar genera consortium). The predominant genera are located along the right Y-axis and the heatmap represents the relative percentages of each genus. Samples are represented by protein source varied in complete pelleted diet: soybean meal (SBM); dried distillers grains with solubles (DDGS); Soyplus (Dairy Nutrition Plus, Ames, IA).

Figure 4.2. Beta diversity of fecal samples in feedlot goats fed varying protein sources[1]



[1]A principal plot of weighted UniFrac data was used to visualize the microbial community structure of samples and pairwise analysis of similarities (ANOSIM) was utilized to determine differences between communities. In the principal coordinate plot, the red dots indicate DDGS+AmCl samples, the blue dots indicate SBM+AmCl samples, and the orange dots indicate SoyPlus+AmCl samples. Based on the ANOSIM, there was no relationship between SBM+AmCl and DDGS+AmCl samples (r = 0.09), SBM+AmCl and SoyPlus+AmCl (r = 0.05), or DDGS+AmCl and SoyPlus+AmCl (r = -0.003). Dots represent protein source varied in complete pelleted diet: Soybean meal (SBM); Dried distillers grains with solubles (DDGS); Soyplus (Dairy Nutrition Plus, Ames, IA).

Appendix A - Supplemental tables

Table A.1. Effect of hay type and sampling location on bacterial classes that comprise ≥ 1% relative abundance[1]

	Brome		Alfalfa			
Class, %	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[2]**
Clostridia	36.82[a]	41.58[b]	31.05[a]	52.93[c]	2.39	L, F*L
Bacteroidia	52.00[a]	36.61[b]	51.03[a]	18.44[c]	2.93	F, L, F*L
Spirochaetes	2.38[a]	8.46[b]	2.32[a]	2.90[a]	1.09	F, L, F*L
Unassigned	1.27[a]	1.21[a]	8.61[b]	2.43[a]	0.85	F, L, F*L
Verruco-5	0.33[a]	5.39[b]	0.08[c]	6.36[b]	0.85	L
Bacilli	1.60[a]	0.78[a]	3.44[b]	4.13[b]	0.76	F
Erysipelotrichi	1.30[a]	1.83[a]	0.73[a]	5.79[b]	0.96	F, L, F*L
Mollicutes	1.19[a]	1.08[a]	1.47[a]	2.33[b]	0.17	F, L, F*L
Fibrobacteria	1.52[a]	1.27[a]	0.23[b]	0.30[b]	0.30	F
Methanobacteria	0.14	0.43	0.02	1.93	0.91	NS[3]

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed ad libitum to horses; sampling locations included the cecum and rectum

[2]F = main effect of hay type (alfalfa and brome); L = main effect of location (cecum and rectum); F*L = interaction between hay and location; $P \leq 0.05$

[3]NS = no significance detected; $P > 0.10$

[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Table A.2. Effect of hay type and sampling location on bacterial orders that comprise ≥ 1% relative abundance[1]

	Brome		Alfalfa			
Order, %	**Cecum**	**Rectum**	**Cecum**	**Rectum**	**SEM**	***P*-value[2]**
Clostridiales	36.83[a]	41.58[b]	31.05[a]	52.92[c]	2.48	L, F*L
Bacteroidales	52.00[a]	36.61[b]	51.03[a]	18.44[c]	3.31	F, L, F*L
Spirochaetales	2.38[a]	8.46[b]	2.32[a]	2.90[a]	1.11	F, L, F*L
Unassigned	1.27[a]	1.21[a]	8.61[b]	2.43[b]	1.02	F, L, F*L
WCHB1-41	0.22[a]	5.39[b]	0.08[c]	6.36[b]	0.85	L
Lactobacillales	1.60[a]	0.78[a]	3.43[ab]	4.12[b]	0.75	F
Erysipelotrichales	1.30[a]	1.83[a]	0.73[a]	5.79[b]	0.96	F, L, F*L
RF39	1.13[a]	0.88[a]	1.39[a]	2.04[b]	0.19	F, F*L
Fibrobacterales	1.52[a]	1.27[a]	0.23[b]	0.30[b]	0.32	F
Methanobacteriales	0.14	0.43	0.02	1.93	0.91	NS[3]

[1]Hay type [smooth bromegrass (brome) or alfalfa] was fed ad libitum to horses; sampling locations included the cecum and rectum

[2]F = main effect of hay type (alfalfa and brome); L = main effect of location (cecum and rectum); F*L = interaction between hay and location; $P \leq 0.05$

[3]NS = no significance detected; $P > 0.10$

[abc]Means within the same row with a different superscript are different ($P \leq 0.05$)

Table A.3. Formulated ingredient costs[1] and inclusion rates of dietary treatments[2]

	Cost,	Inclusion, %				Cost, $/ton			
Ingredient, %	**$/lb**	**0DDGS**	**10DDGS**	**20DDGS**	**30DDGS**	**0DDGS**	**10DDGS**	**20DDGS**	**30DDGS**
Corn dried distillers grains with solubles	0.09	0.00	10.30	20.50	31.05	0.00	18.54	36.90	55.89
Soybean Meal, 48%	0.20	15.45	10.26	5.12	0.00	61.80	41.04	20.48	0.00
Corn	0.06	52.75	51.17	49.61	48.31	63.30	61.40	59.53	57.97
Soybean Hulls	0.06	25.93	22.61	19.31	15.04	31.12	27.13	23.17	18.05
Molasses	0.02	2.50	2.50	2.50	2.50	1.00	1.00	1.00	1.00
Ammonium chloride	0.04	0.75	0.75	0.75	0.75	0.60	0.60	0.60	0.60
Limestone	0.02	1.60	1.60	1.59	1.79	0.64	0.64	0.64	0.72
Salt	0.04	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40
Copper sulfate	1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vitamin A 30,000	0.10	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08
Vitamin D 30,000	0.10	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
Zinc Oxide	0.81	0.01	0.01	0.00	0.00	0.16	0.16	0.00	0.00
Monocalcium Phosphate	0.32	0.45	0.25	0.05	0.00	2.88	1.60	0.32	0.00
Total	-	100.00	100.00	100.00	100.00	162.00	152.62	143.14	134.73

[1]Costs are based off commodity prices in Kansas in February 2018

[2]Treatment: Complete pelleted diets with 0DDGS = 0% dried disillers grains with solubles (DDGS) in place of soybean meal (SBM); 10DDGS = 33 % DDGS in place of SBM; 20DDGS = 66 % DDGS in place of SBM; 30DDGS = 100% DDGS in place of SBM

Table A.4. Formulated ingredient costs[1] and inclusion rates of dietary treatments[2]

		Inclusion, %					Cost, $/ton				
Ingredient, %	**Cost, $/lb**	**SBM+ AmCl[2]**	**DDGS+ AmCl[2]**	**SoyPlus[3]+ AmCl[2]**	**SBM+ SoyChlor[2,3]**	**SoyPlus[3]+ SoyChlor[2,3]**	**SBM+ AmCl[2]**	**DDGS+ AmCl[2]**	**SoyPlus[3]+ AmCl[2]**	**SBM+ SoyChlor[2,3]**	**SoyPlus[3]+ SoyChlor[2,3]**
SoyPlus[3]	0.16	0.00	0.00	22.02	0.00	20.01	0.00	0.00	68.26	0.00	62.03
Corn dried distillers grains with solubles	0.07	0.00	31.05	0.00	0.00	0.00	0.00	43.01	0.00	0.00	0.00
Soybean Meal, 48%	0.15	15.45	0.00	0.00	17.24	0.00	47.18	0.00	0.00	52.66	0.00
Corn	0.06	52.75	48.31	53.33	49.39	51.38	60.14	55.08	60.80	56.31	58.57
Soybean Hulls	0.07	25.93	15.04	18.81	24.07	19.23	35.91	20.83	26.05	33.33	26.63
Molasses	0.02	2.50	2.50	2.50	2.50	2.50	1.00	1.00	1.00	1.00	1.00
AmCl	0.06	0.75	0.75	0.75	0.00	0.00	0.90	0.90	0.90	0.00	0.00
Limestone	0.10	1.60	1.79	1.46	0.80	0.91	3.30	3.69	3.00	1.64	1.87
Salt	0.04	0.50	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40	0.40
Copper Sulfate	1.10	0.00	0.00	0.01	0.00	0.01	0.10	0.03	0.21	0.02	0.22
Vitamin A 30,000	0.10	0.04	0.04	0.01	0.01	0.01	0.07	0.07	0.03	0.03	0.03
Vitamin D 30,000	0.10	0.01	0.01	0.00	0.00	0.00	0.02	0.02	0.01	0.01	0.01
Vitamin E 20,000	0.40	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Zinc Oxide	0.81	0.01	0.00	0.01	0.01	0.01	0.16	0.00	0.16	0.14	0.16
Monocalcium Phosphate	0.16	0.45	0.00	0.59	0.01	0.61	1.47	0.00	1.92	0.03	1.97
Selenium Selenite	0.15	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	1.83	0.00
SoyChlor[3]	0.26	0.00	0.00	0.00	4.83	4.83	0.00	0.00	0.00	25.36	25.36
Total	-	100.00	100.00	100.00	100.00	100.00	150.67	125.04	162.76	172.76	178.25

[1]Costs are based off commodity prices in Kansas in August 2018

[2]Complete pelleted diets: SBM+AmCl = soybean meal + ammonium chloride; DDGS+AmCl = dried distillers grains with solubles + ammonium chloride; SoyPlus+AmCl = SoyPlus + ammonium chloride; SBM+SoyChlor = soybean meal + SoyChlor; SoyPlus+SoyChlor = SoyPlus + SoyChlor

[3]SoyPlus and SoyChlor are trademarks of Landus Corporation (Dairy Nutrition Plus, Ames, IA)