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Summary

To understand the relationship between microbial community and methane, the microbial community of the rumen was examined by esophageal-tubing cattle on a common diet and on 10 treatment diets. Microbial community analysis via 16S taq sequencing displayed structuring of microbial communities (Bacteria and Archaea) by diet. This study demonstrates that diet influences microbial community composition within the rumen, and the potential capacity to develop dietary intervention strategies for methane mitigation and animal performance.

Introduction

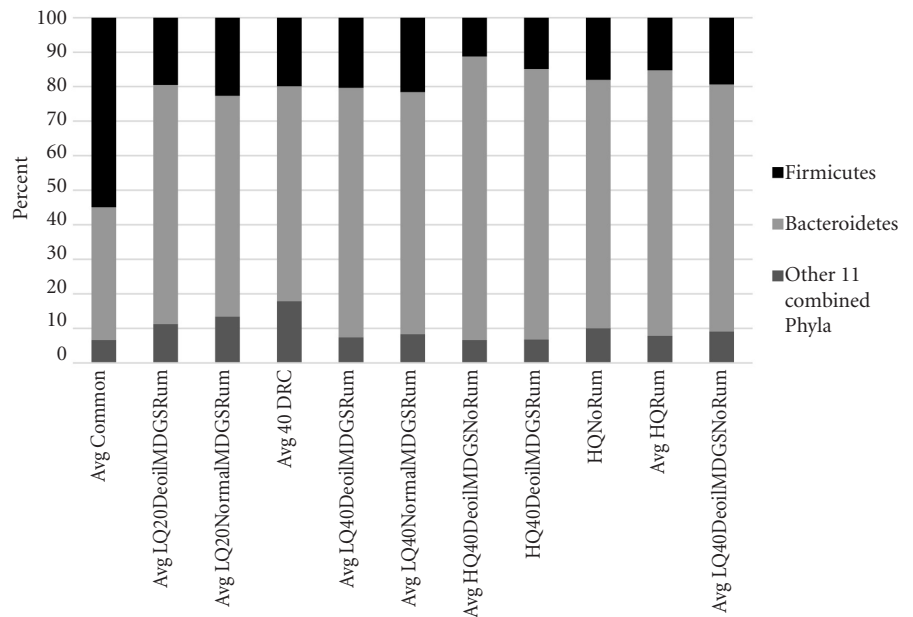
Methane is a potent greenhouse gas that traps heat 21 times more than carbon dioxide. The livestock industry is a contributor to the anthropogenic methane produced. Rumen microbes are responsible for the breakdown of plant material and conversion of those products into usable energy for the animal through fermentation. As a result of this process, byproducts are formed such as volatile fatty acids and methane; methane carbon is not a usable energy by cattle and leads to reduced animal performance and efficiency. At the heart of methane production are microbes, and these microbes are known to change based on substrate availability in the diet. As diet can change microbial communities, dietary intervention can be used

to reduce greenhouse gas emissions from cattle by controlling microbial populations. Dietary intervention strategies for mitigation of methane are being explored (2014 Nebraska Beef Cattle Report, pp. 29-31). Understanding the relationship between diet, methane, and microbial community will help identify microbial species associated with methane to develop new intervention strategies. The purpose of this study was to identify the role diet plays on the rumen microbiota, and how this will affect methane emissions in growing cattle.

Procedure

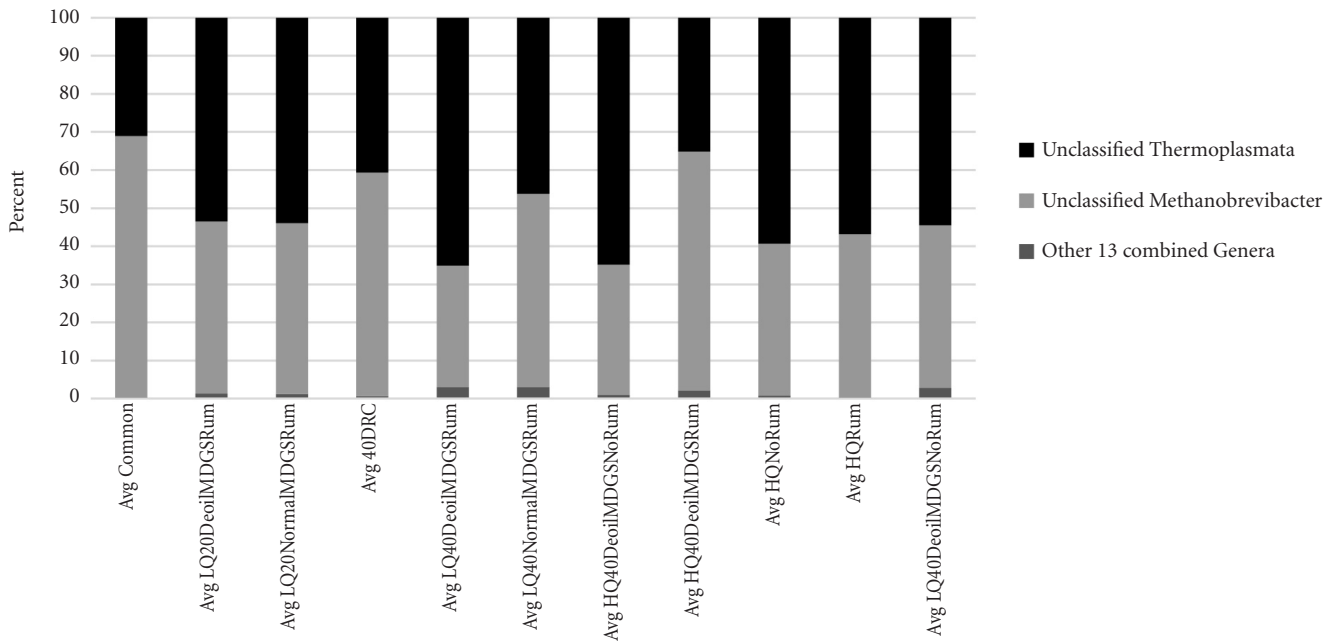
An 84-day growing study was performed starting in January 2013 to identify interactions between diet, methane, and microbial community. Rumen samples were collected by esophageal tubing 120 steers on a common diet containing alfalfa and Sweet Bran[®] at a 50/50 ratio. The cattle were then switched to one of 10 treatment diets containing high and low quality forage, with and without Rumensin[®], with 20 or 40% MDGS supplementation (2014 Nebraska Beef

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Diet Abbreviation	Diet
Avg Common	50/50 Alfalfa Hay and Sweet Bran
Avg LQ20DeoilMDGSRum	Low Quality Forage 20% Deoiled MDGS plus Rumensin
Avg LQ20NormalMDGSRum	Low Quality Forage 20% Normal MDGS plus Rumensin
Avg 40 DRC	Low Quality Forage 40% DRC
Avg LQ40DeoilMDGSRum	Low Quality Forage 40% Deoiled MDGS plus Rumensin
Avg LQ40NormalMDGSRum	Low Quality Forage 40% Normal MDGS plus Rumensin
Avg HQ40DeoilMDGSRum	High Quality Forage 40% Deoiled MDGS no Rumensin
Avg HQ40DeoilMDGSRum	High Quality Forage 40% Deoiled MDGS plus Rumensin
Avg HQNoRum	High Quality Forage 20% MDGS no Rumensin
Avg HQRum	High Quality Forage 20% MDGS plus Rumensin
Avg LQ40DeoilMDGSRum	Low Quality Forage 40% Deoiled MDGS no Rumensin

Figure 1. Bacterial taxonomic distribution at the phylum level on common diet and 10 treatment diets.



Diet Abbreviation	Diet
Avg Common	50/50 Alfalfa Hay and Sweet Bran
Avg LQ20DeoilMDGSRum	Low Quality Forage 20% Deoiled MDGS plus Rumensin
Avg LQ20NormalMDGSRum	Low Quality Forage 20% Normal MDGS plus Rumensin
Avg 40 DRC	Low Quality Forage 40% DRC
Avg LQ40DeoilMDGSRum	Low Quality Forage 40% Deoiled MDGS plus Rumensin
Avg LQ40NormalMDGSRum	Low Quality Forage 40% Normal MDGS plus Rumensin
Avg HQ40DeoilMDGSNoRum	High Quality Forage 40% Deoiled MDGS no Rumensin
Avg HQ40DeoilMDGSRum	High Quality Forage 40% Deoiled MDGS plus Rumensin
Avg HQNoRum	High Quality Forage 20% MDGS no Rumensin
Avg HQRum	High Quality Forage 20% MDGS plus Rumensin
Avg LQ40DeoilMDGSNoRum	Low Quality Forage 40% Deoiled MDGS no Rumensin

Figure 2. Archaeal taxonomic distribution at the genus level on common diet and 10 treatment diets.

Cattle Report, pp. 29-31). The animals were tubed every 21 days to evaluate volatile fatty acids and microbial community structure. The samples collected were placed in liquid nitrogen to freeze the contents instantly and inhibit continued microbial growth. DNA was extracted from all rumen samples and purified utilizing the MoBio PowerMag[®] Soil DNA Isolation Kit (Carlsbad, Calif.). The V3 region of the 16S rRNA genes from the rumen bacterial and V6 region of the 16S rRNA genes from archaea communities were amplified using the polymerase chain reaction (PCR) technique. The resulting amplicons were sequenced using the Ion Torrent Personal Genome Machine[®] (PGM[™]).

The resulting sequence reads were analyzed using published bioinformatics pipelines UPARSE (*drive5.com/uparse/*, Edgar, 2013) and QIIME (*qiime.org/*). Statistical analysis was performed using the phantom package within MATLAB[®].

Results

Taxonomic distribution at the phylum level shows that Bacteroidetes and Firmicutes dominate the bacterial populations in the rumen (Figure 1). The genus level of distribution for archaea is presented in Figure 2 and shows that the archaea population in the rumen is predominated by methanogens. Unclassified Thermo-

plasmata and Methanobrevibacter are the major Archaeal genera present in the rumen. The bacterial community composition in Figure 3 shows that microbial community composition changes significantly ($P < 0.05$) based on forage quality (high and low). The archaeal microbial communities are displayed in Figure 4, where changes in methane producing archaea are seen in low and high quality forages when MDGS is supplemented at 20%. Archaeal community differs from the common diet but were not different between high and low quality forage at 40% supplementation.

The common diet was utilized as a baseline for comparison to the treatment diets. Therefore, when animals

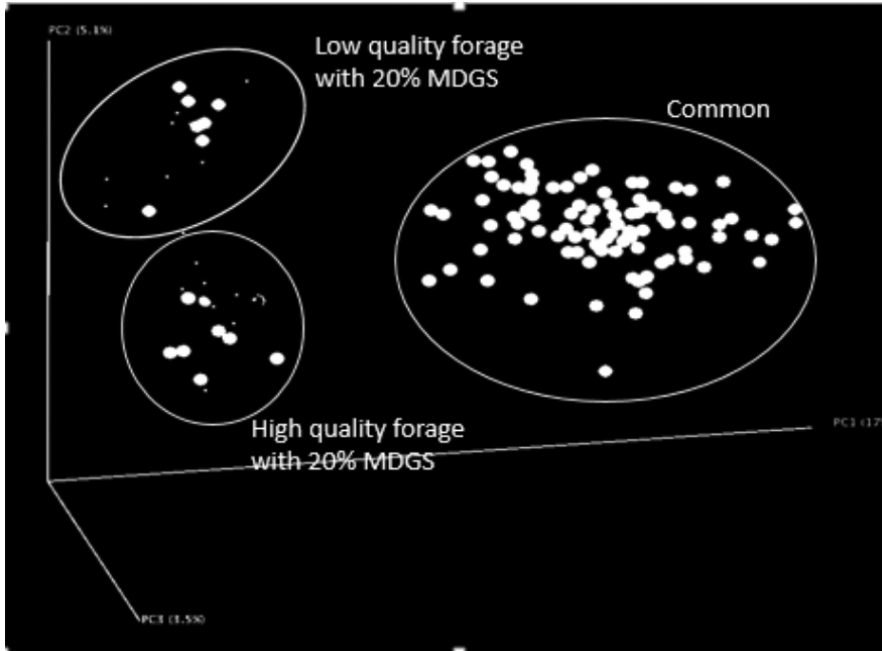


Figure 3. Bacterial community composition — high and low quality forage with 20% MDGS supplementation.

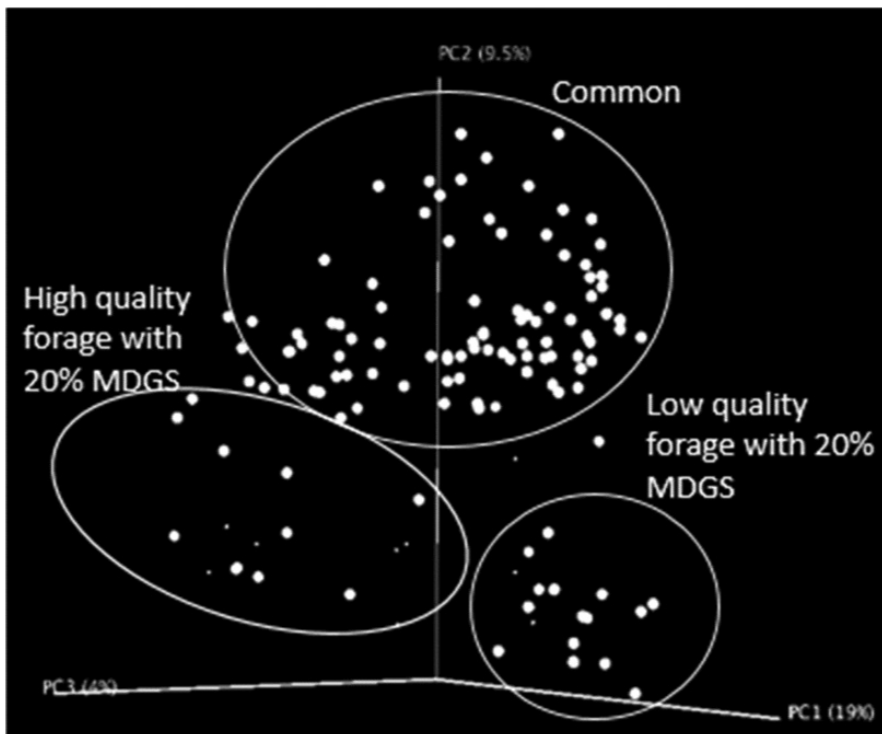


Figure 4. Archaeal community composition — high and low quality forage with 20% MDGS supplementation.

are shifted from the common diet to treatment diets, microbial communities change showing that diet influences rumen microbial community composition.

Methane is produced by a group of microbes known as methanogens which are found in the domain Archaea. Little is known about this group of organisms. However, to develop management based mitigation strategies, continued research in this area is crucial. Identifying the functions and the roles methanogens play towards digestion and hydrogen recycling within the rumen, may lead to methods that decrease methane emissions and improve cattle performance.

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