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Effects of Dietary Change on Viral-Bacterial Interactions in the Rumen of Cattle

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Summary

This ongoing study investigates the impact of diet and bacteriophage activity on the structuring of rumen microbial community composition and diversity. Fistulated cattle were acclimated to a given diet for 21 days before samples were collected and subsequently enriched for viral particles with tangential flow filtration. Taxonomic identification, abundance, and functional attributes were assigned to both bacterial and viral communities. Principle coordinate analysis of the bacterial communities revealed significant clustering based on diet. While diet drives the structuring of rumen bacterial communities, bacteriophages may maintain high, constant bacterial diversity.

Introduction

Cattle, like other animals (including humans), are a complex supra-organism composed of not only their own gene collection, but also those of their associated microbes living on and within the host. The majority of these microbes are found in the gastrointestinal tract, playing an important role in shaping the immune system, gut function and development, nutrition acquisition, and host metabolism. This is especially true in ruminants such as bovine where microbial fermentation in the rumen aids in feed degradation, digestion, and later absorbance. Disruption of the "normal" microbiota may have dramatic consequences for health of the animal. Factors believed to influence rumen bacterial composition include other microbial life such as viruses, stress, colonization history, diet, and host genotype interactions.

Bacteriophages are a subset of viruses that infect and potentially lyse microbial cells, exerting significant influence on bacterial community structure. It is proposed bacteriophages help to maintain bacterial diversity by keeping bacterial species in check, allowing for diverse biological activity in the rumen. Through culture independent methods, this study aims to investigate the impact of dietary change on viral and bacterial composition and diversity while beginning to elicit the impact of bacteriophage-bacteria interactions on rumen function and animal performance traits such as feed efficiency.

Procedure

Five ruminally fistulated bovine cattle rotated through a series of four diets: 55% corn silage, 27% corn distillers solubles (27% CDS), 40% modified distillers grains plus solubles (40%MDGS), and a corn-based diet (Table 1). After 21 days of acclimation to a given diet, a total rumen evacuation was performed and contents were mixed, providing a homogenous sample. Bacterial DNA was extracted using MagMAX™ Pathogen RNA/DNA Kit (Life Technologies, Corp., Carlsbad, Calif.). Bacterial metagenomes (total rumen bacterial DNA) and V1-V3 variable regions of amplified 16s rRNA genes were sequenced with 454-pyrosequencing technology. Information regarding bacterial community composition and abundance are gleaned from 16s rRNA sequences through bioinformatic software packages mothur and QIIME. Dietary and host effects on community structure were analyzed using multivariate analysis (Wilk's Lambda) within JMP statistical software. Bacterial metagenome sequences were compared to curated databases to assign functional attributes to the microbial community.

Viruses were enriched from rumen contents via tangential flow-filtration on a 0.2 micron filter and subsequently concentrated on a 100-kilodalton filter. Concentrated viral particles were pelleted with ultracentrifugation at 100,000 X g and DNA was extracted (MagMAX Pathogen RNA/DNA Kit, Life Technologies) then amplified using multiple displacement amplification with phi29 DNA polymerase (New England BioLabs). Viral metagenomes were prepped and sequenced with 454-pyrosequencing. Sequences were searched against databases to assign taxonomic and functional characteristics to the viral community.

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Table 1. Dietary composition (%) on DM basis.

Ingredient, % DM	Control	27% CDS	40% MDGS	55% Corn Silage
High-Moisture Corn	51.25	36.3	28.5	—
Dry-Rolled Corn	36.25	24.2	19	—
CDS[1]	—	27	—	—
MDGS[1]	—	—	40	40
Corn Silage	—	—	—	55
Brome Hay	7.5	7.5	7.5	—
Supplement[2]	5	5	5	5

[1]CDS = Condensed distillers solubles; MDGS = Modified distillers grains plus solubles.
[2]Provided to contain 336 mg/head/day Rumensin® and 90 mg/head/day Tylan®.

Results

Numerous factors influence the structuring of the rumen bacterial community, but diet is the main driving factor. A change in dietary substrate allows different microbes to flourish, causing a shift in the bacterial community composition and diversity (Figure 1). Steers on the 55% corn silage diet consistently had the highest bacterial community diversity, while those on the corn-based diet the lowest. Principle coordinate analysis taking into account taxonomic differences and phylogenetic relationships demonstrates that bacterial communities of steers on the same diet cluster together (Figure 2), indicating these communities are more similar to each other than to samples from other diets. Multivariant analysis confirmed the principle coordinate analysis; there is a significant difference between bacterial communities based on diet (Wilk's Lambda, $P < 0.05$), but not by individual animal (Wilk's Lambda $P > 0.05$).

Viruses in the rumen outside of a bacterial host were enriched by tangential flow filtration. Enriched viral communities were nearly completely free of bacterial and eukaryotic contamination. Bacterial and viral metagenomic analysis is ongoing. This data will give us an indication of potential bacterial metabolic functions and contains signals of bacteriophages that have embedded themselves into the bacterial genome of a host (prophages). Preliminary work suggests prophages are highly abundant, but their influence on community structure is incomplete at this point. Continued work in this area will aid in understanding the role of bacteriophages on bacterial communities and identify bacteriophages that can be used to control microbes in hopes of improving the health, performance, and feed efficiency of cattle.

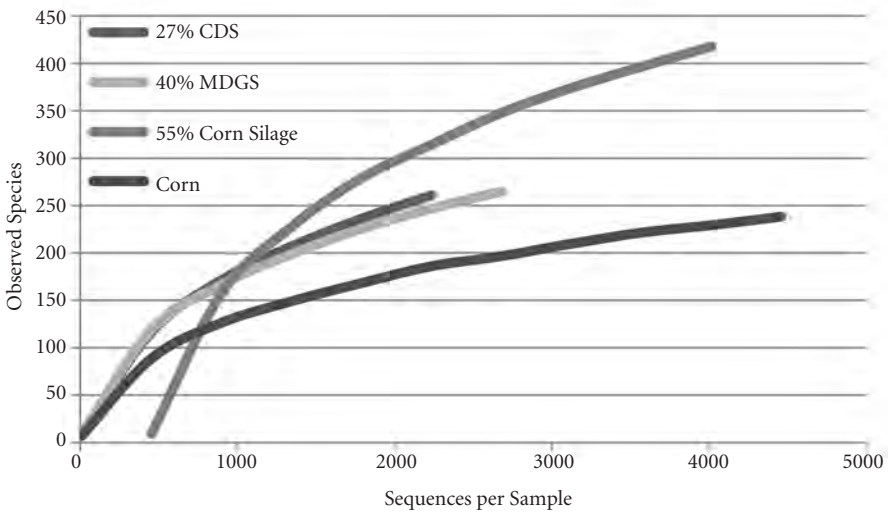

Figure 1. Average species richness of bacterial communities from four experimental diets.

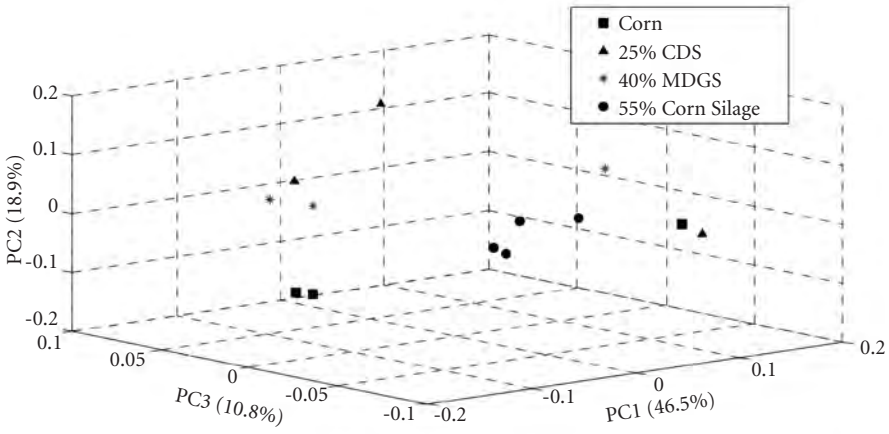

Figure 2. Principle Coordinate Analysis of the bacterial communities from each diet.

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