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Factors influencing ruminal bacterial community diversity and composition and microbial fibrolytic enzyme abundance in lactating dairy cows with a focus on the role of active dry yeast

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ABSTRACT

The objective of the current study was to employ a DNA-based sequencing technology to study the effect of active dry yeast (ADY) supplementation, diet type, and sample location within the rumen on rumen bacterial community diversity and composition, and to use an RNA-based method to study the effect of ADY supplementation on rumen microbial metabolism during high-grain feeding (HG). Our previous report demonstrated that the supplementation of lactating dairy cows with ADY attenuated the effect of subacute ruminal acidosis. Therefore, we used samples from that study, where 16 multiparous, rumen-cannulated lactating Holstein cows were randomly assigned to 1 of 2 dietary treatments: ADY (*Saccharomyces cerevisiae* strain Y1242, 80 billion cfu/animal per day) or control (carrier only). Cows received a high-forage diet (77:23, forage:concentrate), then were abruptly switched to HG (49:51, forage:concentrate). Rumen bacterial community diversity and structure were highly influenced by diet and sampling location (fluid, solids, epimural). The transition to HG reduced bacterial diversity, but epimural bacteria maintained a greater diversity than fluid and solids. Analysis of molecular variance indicated a significant separation due to diet × sampling location, but not due to treatment. Across all samples, the analysis yielded 6,254 nonsingleton operational taxonomic units (OTU), which were classified into several phyla: mainly *Firmicutes*, *Bacteroidetes*, *Fibrobacteres*, *Tenericutes*, and *Proteobacteria*. High forage and solids were dominated by OTU from *Fibrobacter*, whereas HG and fluid were dominated by OTU from *Prevotella*. Epimural samples, however, were dominated in part by *Campylobacter*. Active dry yeast had no effect on bacterial community diversity or structure. The phylum SR1 was more abundant in all ADY samples regardless of diet or sampling location. Furthermore, on HG, OTU2 and OTU3 (both classified into *Fibrobacter succinogenes*) were more abundant with ADY in fluid and solids than control samples. This increase with ADY was paralleled by a reduction in prominent *Prevotella* OTU. Metatranscriptomic profiling of rumen microbiome conducted on random samples from the HG phase showed that ADY increased the abundance of the cellulase endo-β-1,4-glucanase and had a tendency to increase the hemicellulase α-glucuronidase. In conclusion, the shift from high forage to HG and sampling location had a more significant influence on ruminal bacterial community abundance and structure compared with ADY. However, evidence suggested that ADY can increase the abundance of some dominant anaerobic OTU belonging to *F. succinogenes* and phylum SR1. Further, microbial mRNA-based evidence suggested that ADY can increase the abundance of a specific microbial fibrolytic enzymes.

Key words: dairy cow, active dry yeast, subacute ruminal acidosis, rumen microbiome, metatranscriptome

INTRODUCTION

Ruminants are unique in their ability to convert indigestible plant materials through microbial fermentation into a range of VFA (C2 to C6) that constitute the main energy source for the host animal. The ruminal microbial population is complex, diverse, and dominated in number by bacteria (10^{10} to 10^{11} viable cells per gram). It also includes bacteriophages (10^{7} to 10^{9}), ciliate protozoa (10^{4} to 10^{6}), anaerobic fungi (10^{2} to 10^{4}; Mackie et al., 2001), and archaea (5% of total bacteria + archaea; Frey et al., 2010). The rumen microbiome contains approximately 100 times more genes than the host animal, which provides ruminants with genetic and metabolic capabilities to hydrolyze and ferment inaccessible nutrients (McSweeney and Mackie, 2012). Therefore, the performance of ruminants relies on the

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quantity and quality of fermentation end products, which are in turn a function of the quantity and quality of ingested feed and type and activities of rumen microbes. Due to the important role of the rumen microbiome, it is referred to as the "second" genome of the ruminant (Bath et al., 2013).

The ability to study the entire microbiome from complex communities such as the rumen has been impaired by classical methods. Culture-based techniques accounted for only 10 to 20% of the bacterial species present in the rumen (McSweeney and Mackie, 2012). However, the recent advances in nucleic-acid-based techniques, namely high-throughput DNA sequencing, provided the means to study rumen and gut ecology.

Recent efforts to study the rumen microbiome have focused on analyzing the ruminal microbial communities (i.e., identification and quantification), whereas microbial activities are extrapolated based upon measuring changes in microbial communities (Jami et al., 2014). On the other hand, an mRNA-based metatranscriptomic approach can provide information on the functions of the community by identifying the pools of enzymes or pathways that are active within that microbial community (Helbling et al., 2012). The metatranscriptomic approach has been recently used to qualitatively characterize the association between the presence of specific transcripts and observed functions within microbial communities (Ettwig et al., 2010; McCarren et al., 2010). Furthermore, Helbling et al. (2012) demonstrated that the level of transcript abundance (as measured by quantitative PCR or next-generation sequencing) of a given enzyme within a microbial community has the potential to quantitatively predict the level of enzyme activity. The metatranscriptomic approach has been applied to study the marine microbiome (Gifford et al., 2011), the pig gut microbiome (Poroyko et al., 2010), and the rumen microbiome of wild herbivores (Qi et al., 2011). However, reports investigating microbial gene expression in the rumen microbiome of Holstein cows are scarce.

Rumen microbiologist and nutritionists have developed and used several supplementary products to manipulate the rumen microbiome and harness benefits, such as improved productivity. The effect of such products on productivity is variable and likely reflects the variation in available products, dosages, and terms of application. These products can include active dry bacteria, active dry yeast (**ADY**), and yeast culture products. The latter does not ensure the delivery of live microbial cells. In a previous report (AlZahal et al., 2014), we demonstrated that feeding ADY to lactating dairy cows before a rapid switch to a high-grain diet (**HG**) that commonly causes SARA attenuated the ruminal pH depression and the associated reduction in DMI and milk production.

The aim of the current study was to extend the previous investigation to study the influence of ADY on the rumen microbiome and its interaction with diet type and sampling location using a DNA-based, high-throughput sequencing method. The second aim was to use a metatranscriptomic approach to study the effect of ADY supplementation on rumen microbial metabolism during HG.

MATERIALS AND METHODS

Animals, Feeding and Treatments, and Rumen Sampling

This protocol has been detailed previously (AlZahal et al., 2014). Briefly, 16 multiparous, rumen-cannulated lactating Holstein cows were randomly assigned to 1 of 2 dietary treatments that included ADY (*Saccharomyces cerevisiae* strain Y1242; AB Vista, Marlborough, UK; 80 billion cfu/animal per day) or control (carrier only). During wk 1 to 6, all cows received a high-forage diet (**HF**; 77:23, forage:concentrate; CP = 14.3, NDF = 45.0, NFC = 31.5, % of DM). Cows were then abruptly switched during wk 7 to a HG diet (49:51, forage:concentrate; CP = 16.4, NDF = 28.2, NFC = 45.2, % of DM) and remained on the HG until the end of wk 10. Feed intake and milk yields were recorded daily. As mentioned in the previous report, cow no. 3641 (control) was removed due to a pre-existing SARA.

The DNA-based analysis assessed 3 bacterial communities (3 sampling locations); free-living in ruminal fluid (fluid), associated with feed particles (solids), and associated with ruminal epithelium (epimural); during HF and HG phases (15 cows by 3 rumen locations by 2 time points, one random sample was removed to fit a mock sample, totaling 89 samples). Rumen samples were collected from all cows during wk 5 (HF) and wk 10 (HG) at 1600 h midway to the ventral sac of the rumen. Samples were filtered through 4 layers of cheesecloth to separate rumen fluid from rumen solids. Rumen fluid and solids were diluted 1:1 with 100% ethanol and stored for bacterial genomic DNA isolation. For epimural sample collection, the rumen was partially evacuated. Then, the ventral sac was retracted to the fistula and a small area halfway into the ventral area was washed thoroughly with ice-cold PBS to remove any loosely adherent microbes, rumen fluid, or feed particles. Afterward, the washed area was swabbed using a sterile toothbrush. The toothbrush was shaken in a 50-mL tube containing 25 mL of PBS, and then