The Role of Rumen Microbiome on Feed Efficiency of Grazing Cattle

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Introduction

Feed efficiency of cattle directly affects the profitability, production efficiency, and is a determining factor for the sustainability of the beef industry, since feed cost could account for 50-70% of gross expenses (Cottle and Kahn, 2014). During cattle growth, approximately 75% of total dietary energy is used for maintenance (Arthur et al., 2001). Therefore, selection, breeding, and management of feed-efficient animals is a priority for the beef industry. Residual feed intake (**RFI**) is a measure of feed efficiency that is independent of growth and body weight. It has been identified as a selection tool for feed-efficient cattle based on this trait (Arthur et al., 2001; Basarab et al., 2003). It has been speculated that variation in RFI could be associated with many biological processes that are influenced by genetic and environmental factors; however, the molecular mechanisms underlying RFI are largely unknown.

In ruminant animals, the rumen plays a vital role in feed digestion and fermentation that produces short-chain fatty acids (**SCFAs**), which contribute up to 80% of the cattle's total energy requirements (Wolin, 1979). Diet can directly influence rumen function by altering the microbial population and fermentation activities (Bevans et al., 2005). A study by Durunna et al. (2011) revealed a re-ranking of RFI of individual cattle as they underwent a dietary change from a growing diet to a finishing diet in a feedlot production system. Therefore, we hypothesized that differences in the rumen microbiota could contribute to the observed variation of cattle feed efficiency. Our previous studies have revealed that particular microbes may be associated with cattle performance parameters including average daily gain, dry matter intake, feed conversion ratio, and RFI (Guan et al., 2008; Hernandez-Sanabria et al., 2010). The impact of these microbial populations on rumen function (fermentation measurements), RFI (Hernandez-Sanabria et al., 2012) and CH_4 emissions (Zhou et al., 2009; Zhou et al., 2010) has also been documented. Furthermore, particular microbial phylotypes in cattle arising from differing sires can influence rumen microbial metabolic processes and ultimately RFI (Hernandez-Sanabria et al., 2013). These suggest that rumen function (presence or absence of particular microbes) can be regulated by the interaction between 'gene (genotypes of the host)' and 'environment (diet, management)', which subsequently impact RFI ranking. Currently, there is no existing DNA marker for rumen function and the particular host mechanisms responsible for variation in the microbial populations, and their interactions with diet and impact on host feed efficiency are unknown.

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Current Understanding of Rumen Microbiota

Ruminants are foregut fermenters characterized by their pre-gastric anaerobic fermentation in the rumen, where harbors variety of microbes including bacteria, archaea, protozoa, and fungi. The complex association of different microbes acts synergistically for the conversion of cellulosic feeds into volatile fatty acids (**VFAs**) and proteins that fulfill the nutrient requirement of animals (Frey et al., 2010). Rumen microbiology research has evolved in the last decade to understand their diversity, metabolic functions, and different interactions especially with the intervention of molecular biology techniques. To date, hundreds to thousands of microbial phylotypes have been identified from various rumen systems using the culture-independent molecular-based approaches (Brulc et al., 2009; Henderson et al., 2015). Such diverse microbial composition suggests that the rumen microbiome (collective genomes of rumen microbiota) contains 100 times more genes than the host animal (McSweeney and Mackie, 2012), providing genetic and metabolic capabilities to digest fibers and provide host animals with nutrients. The molecular microbial ecology studies have allowed the identification of uncultured and low abundant microbes, discovery of potential interactions among different microbial groups, and the quantitative exploration of this complex ecosystem which is co-evolved with their host. Many factors have been identified to affect rumen microbial diversity, density, and functions including diet, breed, age of the animal, physiological conditions and growth stages of the animals, season, geographic location, feed additives, feeding strategies, intensities, intake level, and animal health as well as medical treatment (antibiotic usage) (Weimer et al., 2000; Romero-Perez et al., 2011; Hernandez-Sanabria et al., 2012; McCann et al., 2014).
To date, numerous studies of analyzing rumen microbial communities using next generation sequencing estimated that the rumen microbiota contains up to approximately 7,000 bacterial species of which ~ 30% of them remain unidentified (McSweeney and Mackie, 2012). Among them,19 existing bacterial phyla have been identified with phyla of Firmicutes, Bacteroidetes, and Proteobacteria and genera of *Prevotella*, *Bacteroides,* and *Clostridia* dominating in most of the cattle rumens (Brulc et al., 2009; Cai et al., 2013). In addition, *Methanobrevibacter* (>60%), *Methanomicrobium* (~15%), and *Methanomassiliicoccales* (a group of uncultured rumen archaea previously referred to as rumen cluster C (RCC, ~16%) are the predominant genera in rumen archaeal community (St-Pierre and Wright, 2012; Borrel et al., 2014). Similar to other microbial groups, knowledge of protozoa has been significantly increased with the application of molecular techniques (Skillman et al., 2006). The most prevalent protozoans in the rumen can be classified under genus level, including *Epidinium*, *Entodinium*, *Diplodinium*, and *Holotrich* ciliates (Williams and Coleman 1992). Currently, more than 18 species of anaerobic rumen fungi have been described with the implementation of molecular biological techniques such as specific qPCR technique and high throughput sequencing technology (Denman et al., 2008). Rumen fungi have been classified into six genera; namely, the monocentric *Neocallimastix*, *Caecomyces, Piromyces,* and the polycentric *Anaeromyces*, *Orpinomyces,* and *Cyllamyces* (Ishaq, 2015). Last, it is noticeable that viruses, especially phages, are dense and diverse in the rumen. These were first identified in the 1960s but very few studies were done until the 1990s. Bacteriophages are abundant (10^7 to 10^9 particles per ml) in the rumen

ecosystem, and their population structure and symbiotic relationship are poorly understood (McSweeney and Mackie, 2012). Several studies have pointed out the influence of rumen viruses on other microbial population structure and density through cell lysis and possible lateral gene transfer (Hegarty and Klieve, 1999). Recent metagenomic analysis of bovine rumen virome identified 28,000 different viral genotypes belonging to several families (*Siphoviridae, Myoviridae, Podoviridae, Unclassified, Herpesviridae, Phycodnaviridae, Mimiviridae, Poxviridae, Baculoviridae, Iridoviridae, Polydnaviridae, Adenoviridae,* and *Bicaudaviridae*) (Berg Miller et al., 2012). They may play beneficial roles by balancing the bacterial populations, involving in lateral gene transfer, and adding novel enzymes to the rumen ecosystem and host animals, along with introducing detrimental effects such as reducing feed efficiency and transferring toxin genes (Gilbert and Klieve, 2015).

Advanced Methodologies to Study Rumen Microbiome

Rumen microbiome usually refers to the total genetic information of the rumen microbiota. There are two key questions when studying the rumen microbiome: Who are they and what are they doing in the rumen? By assesses the genomic information of the microbiota using metagenomics, it can help to identify the composition of the entire microbial community, to understand the symbiosis relationships between microbes and hosts, and to reveal the competition and communications within the microbiome (Handelsman, 2004). Brulc et al. (2009) firstly studied the metagenome of the rumen content collected from three beef steers, and have revealed fundamental variations in the glycoside hydrolases (GH) content of the steers fed on forages and legumes compared to that in the hindgut of the termite fed on wood. Hess et al. (2011) applied metagenomic analysis on the rumen microbiome of cows and have identified 27,755 putative carbohydrate-active genes, and expressed 90 candidate proteins among which 57% were active against cellulosic compounds of the feed. Besides, they have also assembled 15 unculturable microbial genomes, complementing the rumen microbial reference database. Microbial plasmids also encode essential functional genes. As reported by Kav et al. (2012), besides of the genes allowing the microbes to confer their host with advantages within the ecological riche, rumen microbial plasmidomes in cows also enriched in functions such as proximity to plasmid backbone functions and biosynthetic pathway function. Metagenomics help to discover the functional potentials within the rumen microbiome, but its actual activity has not been revealed. Thus, metatranscriptomics which study the active transcripts of microbial genes was then employed. Findley et al. (2011) isolated total RNA from cow rumen fluid and examined the transcripts of protozoan GHs, and identified four novel genes among which two (type 1-7.1 and type 2-8.6) were characterized in downstream biochemical assays. Metatranscriptomic analyses performed in cow rumen (Dai et al., 2012) have proved that the GHs produced by *Ruminococcus*, *Fibrobacter*, and *Prevotella* were the predominant degraders against plant cell wall polysaccharides (PCWP), with GH48 cellobiohydrolases and cellulosome-like structures contributed significant roles in efficient PCWP degradation. Getting the complete insight of rumen microbiome, it is also important to identify the microbial metabolites which can be utilized by the host or can influence rumen environment and host health. *Butyrivibrio proteoclasticus* B316^{T}, a

polysaccharide-degrading and butyrate-producing bacteria prevalent in the rumen, was reported to produce intracellular debranching enzymes, implicating a plausible model that this species is capable of conducting extracellular digestion of hemicellulose to oligosaccharides, followed by transporting the oligosaccharides to the cytoplasm for further digestion by intracellular enzymes (Dunne et al., 2015). Having these 'omics'-based approaches, it is possible to study the composition, activities, and functions of the rumen microbiome systematically.

Rumen Microbiome and Feed Efficiency

Beyond the previous predicted functions of microbes under specified experimental conditions, recent studies have added focus on the co-evolution (Ley et al., 2008; Hernandez-Sanabria et al., 2012) of gut microbes with the host and the possible interaction of animal's genotype with rumen microbes. With the developing knowledge about the rumen microbiota, it is feasible to explore the impacts of rumen microbiome to host performance by associating microbial measurements with host phenotypes. One of such application is to define the roles of rumen microbiota in affecting host feed efficiency. Hernandez-Sanabria et al. (2012) analyzed the rumen microbiome in beef cattle with varied RFI under growing and finishing diets, and found that the abundance of *Succinivibrio* sp. was associated with host dry matter intake and average daily gain in L-RFI (efficient) animals, *Robinsoniella* sp. abundance was associated with H-RFI (inefficient) animals, whereas the abundance of *Eubacterium* sp. differed between RFI groups when animals were fed with feedlot finishing diet. With a deeper coverage of sequences, Myer et al. (2015) reported that although Bacteroidetes and Firmicutes were the dominant phyla regardless of host feed efficiency differences, proportion of *Succiniclasticum*, *Lactobacillus*, *Ruminococcus*, and *Prevotella* differed among animal groups with varied feed intake and body weight gain. Jami et al. (2014) identified a tentative correlation between the relative abundance of bacteria order RF39 and host RFI (R=0.51) in dairy cows. Besides the findings on bacteria, both Zhou et al. (2009; 2010) and Carberry et al. (2014) reported that although the total methanogen population was similar, changes of particular archaeal genotype abundance may have attributed to the variation in host methane production and thereafter impacting host RFI.

Different Microbes are Associated With Beef Cattle Feed Efficiency

Recently, we applied a metatranscriptomic based approach to study the relationship between active rumen microbiome and feedlot cattle RFI. Both the bacterial community structure and the archaeal community structure were different ($P < 0.001$, using weighted UniFrac test) between H- and L-RFI groups. The relative abundance of three bacterial families including *Lachnospiraceae*, *Veillonellaceae*, p-2534-18B5, and one archaeal taxon *Methanomassiliicoccales* were different ($P < 0.05$) or tended to be different ($P < 0.10$) between H- and L-RFI steers (**Figure 1**). *Lachnospiraceae* has been reported to be associated with feed efficiency and fermentation traits in beef cattle in a previous study using DNA-based methods (Hernandez-Sanabria et al., 2010). Results of the current study showed that the H-RFI group possessed a larger relative abundance of *Lachnospiraceae* than the L-RFI group, further supporting its association with host RFI. In the rumen, *Veillonellaceae* can ferment lactate into acetate and

propionate (Dehority, 2003). The greater abundance of this phylotype in H-RFI animals suggested that lactate-reducing processes may be faster in H-RFI animals than in L-RFI animals. Future studies to measure the lactic acid in the rumen is needed to validate the role of this bacterial family in feed efficiency. *Methanomassiliicoccales* belongs to methylotrophic methanogens, which was the only methanogen known to use methylamines as the major energy and carbon sources (Poulsen et al., 2013). The higher relative abundance of *Methanomassiliicoccales* in L-RFI group indicated that more methylamines might be utilized during fermentation in L-RFI animals. Using 16S rRNA gene library sequencing on the same animals as the current study, Zhou et al. (2009) proposed that the varied methanogenesis substrate preferences may be one of the mechanisms leading to the variation in host CH_4 production between H- and L-RFI animals. The identified differential abundance of *Methanomassiliicocales* using transcriptomic analyses in current study further supported the linkage between methanogenic ecology and host feed efficiency, although the observed differences in arachael communities differed between the two studies. Additionally, *Methanomassiliicocales* is the only group encoding genes to synthesize pyrrolysine-containing proteins (Borrel et al., 2014), whose primary function is methylamine methyltransfer (Rother and Krzycki, 2010). The capability of utilizing a methyl-group from the methanogenesis substrates may lead to the variation of available energy and/or compounds to the host, and ultimately impacting host RFI. These results warrant further investigation to fully elucidate the relationship between available microbial metabolic substrates and host nutritional utilization pathways to better understand how microbial fermentation influences host feed efficiency.

Differential Microbial Functions Between H-RFI and L-RFI Animals

Functional analyses were also performed on the rumen microbiome of the 20 high and low efficiency steers. After quality control and removal of the ribosomal RNA (rRNA), the proportion of mRNA was 7.2 ± 0.5% (Mean ± SEM) of total reads among 20 samples. In total, 92,125,160 mRNA reads were subjected to the functional analysis. Between H-RFI and L-RFI groups, 1, 9 and 14 differential function features in the annotation sources of subsystems were detected at level 1, level 2, and level 3, respectively ($P < 0.05$). Among the listed functions that differed between H-RFI and L-RFI animals, it was noticeable that key metabolic pathways such as glycolysis and gluconeogenesis, purine and pyrimidine conversion, and pyruvate metabolism were more active in L-RFI steers, suggesting that the rumen microbiome in the L-RFI group were more active in digesting fibrous feed, and as such, supplied the host animals with more nutrients. In addition, the rumen microbiome in the L-RFI steers were more active in cell proliferation and survivability, and displayed higher tolerance to viral infection. These functional features may allow the rumen microbiome of L-RFI animals to better adapt to different environmental challenges, and as such improve rumen fermentation efficiency.

Implications for Grazing Systems

Although the current study was conducted on feedlot animals, the findings can be applied to animals in grazing systems. The grazing cattle production system is important

in North America for providing ecosystem goods and services such as forage, carbon storage, recreation as well as contributing to ecological diversity. Especially, for cow-calf production, grazing on summer pasture is a key period to produce beef at a lower cost, compared to feeding with grain. To date, the understanding of the rumen microbiota and its function in beef cattle has mainly focused on feedlot production systems, while the available information on grazing cattle is very limited due to the complexity and diversity of the production system (grazing rotation patterns, pasture diversity, intake monitoring and so on) and lack of access to allow the collection of phenotypic data and biological samples. Therefore, there is the need to perform more research in a collaborate manner to address the following fundamental questions: 1) what microorganisms are present in the rumen of cattle on pasture and what functional groups do they represent? 2) How does the nutritional and chemical composition of consumed forage from pasture affect the structure and function (microbial metabolites) of a microbial community? 3) Is the difference in rumen microbiota associated with cattle feed efficiency and predicted methane emission as is the case in feedlot production systems? This study aims to provide knowledge on the biological process (pasture digestion) of beef cattle production under grazing. With a more complete understanding of the microbial markers for better fiber digestibility and/or host feed efficiency, it is possible to design feed supplements for grazing animals to enhance their capability to utilize nutrients from pasture and/or improve feed efficiency thereby altering the rumen fermentation profiles. Furthermore, host genetics has been proposed to influence its symbiotic microbiota, and thus impact rumen fermentation processes. By defining the linkage between host genetics and microbial fermentation markers, it may be possible to provide novel tools from the microbial aspect to breed animals selectively, thus further enhancing animal performance and feed efficiency.

Conclusions

In conclusion, the exploration of the relationship between the activity of the rumen microbiome and host feed efficiency has revealed increased microbial metabolic functions in L-RFI steers, suggesting the important role of rumen microbiome in feed efficiency. Increased adaptability to negative environmental factors such as virus infection and higher cell survivability in L-RFI steers may enable their microbiome to adapt more quickly to adverse conditions, especially when animals are undergoing dietary challenges with poor quality diets. Given the dynamic nature of the rumen microbiome, future studies involving long-term monitoring of the microbial composition and functions are necessary to solidify its role in host RFI. Regardless, our findings provide new insights regarding the rumen microbiome in animals which differ in RFI ranking, providing the necessary knowledge to more fully understand rumen microbial fermentation, thereby enhancing nutrient utilization and improving animal feed efficiency through enhancing rumen fermentation.

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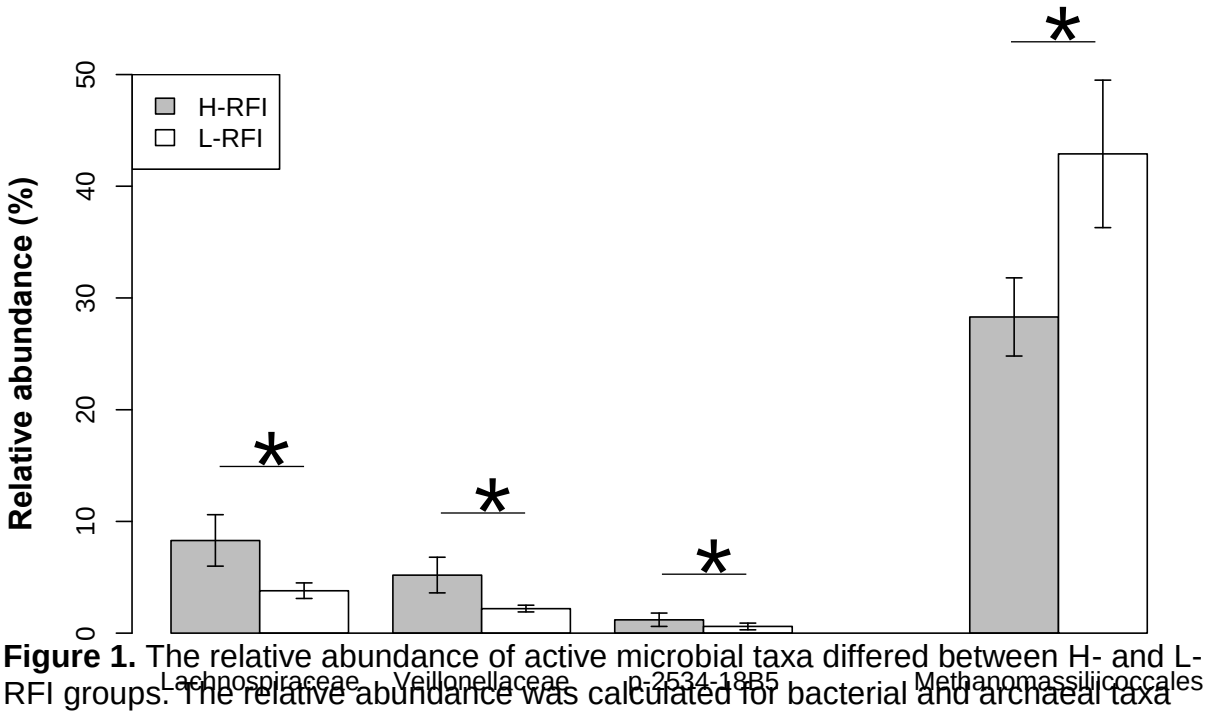

Figure 1. The relative abundance of active microbial taxa differed between H- and L-RFI groups. The relative abundance was calculated for bacterial and archaeal taxa separately. H- and L-RFI represent high and low residual feed intake, respectively. *P*-value was calculated using Metastats in Mothur and "*" represents *P*-value < 0.10.

SESSION NOTES