APPLICATION OF MOLECULAR TECHNIQUES TO BETTER UNDERSTAND THE ROLES OF RUMEN MICROBIOTA IN CATTLE FEED EFFICIENCY

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ABSTRACT

Feed efficiency, simply expressed as less feed inputs versus animal production outputs, can be measured in several ways, such as feed conversion ratio (FCR) and residual feed intake (RFI). FCR is a common measurement in beef cattle operations, and is the ratio of feed intake to live-weight gain. RFI is defined as the difference between actual and predicted feed intake after taking into account variability in maintenance and growth requirements. Rumen microbiota, which inludes bacteria, archaea, protozoa, and fungi, play an essential role in the digestion of lignocellulosic plant biomass, and can provide more than 70% of the host ruminants energy requirements *via* the production of volatile fatty acids (VFAs). Methane, a potent greenhouse gas (GHG), is produced in large quantities by the rumen microbiota, and is a known contributor to the global increase in GHG emissions. Studies have shown a negative relationship between methane emission and feed efficiency. Therefore, there is a need to study the feed efficiency from a rumen microbiome perspective and explore the probability of improving feed efficiency and hence reduce methane production in cattle by manipulating the rumen microbiome. The development of high-throughput sequencing technologies incuding metagenomics and metatranscriptomic analyses in the past decade has led to a sharp increase in understanding the rumen microbiota and associated function. As such, this mini-review will focus on the new findings during the last decade in cattle feed efficiency and the rumen microbiome.

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KEYWORDS

- RFI
- Rumen microbiota
- Pyrosequencing
- Next Generation Sequencing
- Metagenomics

INTRODUCTION

The term feed efficiency implies a ratio of outputs to inputs. Therefore, feed efficiency of beef cattle is the relative ability of the animal to turn feed nutrients into animal products (8). Feed efficiency in cattle is important since it is directly associated with economic profit and enteric greenhouse gas emission from agriculture sectors. For farm owners, 66% of costs in calf management are spent in feed, rising to 77% in yearling finishing systems (1). Fox *et al.* (11) estimated that while a 10% improvement

in daily gain would increase profitability by 18%, and improving growth efficiency by 10% could increase profits by 43% in feedlot cattle. Another study demonstrated that improvement in feed efficiency has 7–8 times greater economic impact than similar improvements in daily gain (27). Other than the economic effect, improving feed efficiency can reduce GHG emission, as cattle with higher feed efficiency are reported to produce 20% to 30% less methane than inefficient ones under the same conditions (33). Given its importance in production systems, measuring the feed efficiency trait in cattle is important. Several different measurements of feed efficiency have been developed and used by industry, such as feed conversion ratio (FCR) and residual feed intake (RFI). FCR is a common measurement in beef cattle operations, which is the ratio of feed intake to live-weight gain (8). While FCR is useful for evaluating management, feed quality, and environment on efficiency in growing and finishing cattle, it has limited value with genetic improvement. FCR has a strong correlation with growth traits, meaning that the selection by lower FCR will increase cow mature size, rather than reduce feed inputs (8). Nowadays, researchers use RFI more generally than FCR for feed efficiency measuring in selecting beef cattle. The concept of RFI is defined as the difference between actual and predicted feed intake after taking into account variability in maintenance requirement and growth; therefore, when cattle consume less feed than expected for their body size and rate of gain, they are considered to have a negative RFI, which means a higher feed efficiency status as compared to positive RFI (17). Compared to FCR, selection by RFI would produce efficient offspring in all segments, because the progeny would be similar to their low RFI parents in yearling weight and average daily gain after almost two generations, but the progeny have been reported to have a lower feed intake (4). The moderate heritability of RFI indicated that selection from the low RFI herd will result in progeny that consume less feed than the high RFI herd (3).

Feed efficiency in cattle is influenced by multiple factors, such as variation in breeding, feed formulation and the rumen microbiota. This review will focus on the role of rumen microbiota in cattle feed efficiency, and will explore the feasibility of improving feed efficiency in beef cattle by modifying the rumen microbiota.

RUMEN FUNCTION

The cattle rumen is the largest of the four compartments of the stomach; with the other three being reticulum, omasum and abomasum. The rumen is a fermentation chamber where fibers are broken down into smaller digestible components by symbiotic microbiota (28). Rumen epithelium can efficiently absorb lactic acid, electrolytes, water and volatile fatty acids (VFAs). VFAs produced by rumen fermentation can meet more than 70% of the ruminant's energy requirement, and are absorbed across the ruminal epithelium for metabolism in the liver (28).

RUMEN MICROBIOTA

The rumen microbiota, comprised of bacteria, archaea, protozoa and fungi, play an essential role in the digestion of recalcitrant lignocellulosic plant matter. Consequentially, examination of this microbiota has been of interest for many years. Wilson and Briggs applied a counting based method in 1955 by obtaining material from diluted rumen content and found that there were about 10^8–10^{10} microorganisms per gram in rumen contents (36). Wilson and Briggs results were similar to a more recent study, in which the mean population densities of bacteria, Archaea, protozoa, and fungi were reported as 10^{10}–10^{11}, 10^7–10^9, 10^4–10^6, and 10^3–10^6 (cells/ml rumen conten), respectively (33). This population, however, can be affected by many other factors, such as the time of the day, host, and diet. The microbes of the rumen may be separated into three distinct populational niches: solid adhered, free in the fluid, and attached to the epithelium wall (16). Bacteria are the most diverse microbes in rumen content, and are largely involved in digesting lignocellulosic feed and producing VFAs for host maintenance and growth, most notably acetate, propionate and butyrate (33). *Fibrobacter succinogenes, Ruminococcus flavefaciens*, and *Ruminococcus albus* are some of the main species involved in cellulose and hemicellulose digestion (32). Recently, Henderson *et al.* (13) studied rumen microbiota composition in ruminants from 35 countries using a deep sequencing approach, and found that the dominant microbes in rumen change with diets, host species, and geography. Despite this, their results also showed the existence of a core rumen microbiota, with the 30 most abundant bacterial groups present in over 90% of the samples, regardless of the factors mentioned above. The most abundant bacterial groups in all samples included *Prevotella*, *Butyrivibrio*, and *Ruminococcus*, as well as unclassified *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidales*, and *Clostridiales*.

Methanogenic archaea are also established members of the rumen microbial community, with the phylum *Euryarchaeota* dominating (33). Henderson *et al.* (13) found that rumen archaea are much less diverse than rumen bacteria because the two largest groups, *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* clades, were found in almost all samples, accounting for 74% of all archaea. Together with a *Methanosphaera* sp. and two *Methanomassiliicoccaceae*–affiliated groups, the five dominant methanogen groups comprised 89.2% of the archaeal communities.

Protozoan species of the rumen microbiota are in lower abundance than the prokaryotes, but may account for as much as 50% of the microbial biomass, due to their larger size (34). Protozoa exist in close association with other microbial groups by scavenging oxygen, transferring nitrogen from bacteria to the host, and regulating microbial population through predation (33). The majority of known rumen protozoa have been assigned to one of 12 genera (13). The rumen protozoal community structure has strong host individuality (35).

Anaerobic fungi in the rumen are also significant players in plant fiber digestion, degrading the lignocellulosic biomass by invasive rhizoidal growth and production of polysaccharide–degrading enzymes (18).

Rumen microbes are different in functions, but they also interact with each other by digestion. Fungi can assist bacteria and other microbes by the initial colonization of fiber. Bacteria, fungi and protozoa break down the indigestible lignocellulosic and release hydrogen, which archaea can utilize. Other fermentation end-products, carbon dioxide, formate and methyl-containing compounds are important substrates for methanogenesis by archaea (9).

RUMEN MICROBIOTA AND FEED EFFICIENCY

Because of the importance of rumen microbiota, there are emerging studies focused on understanding its role in feed efficiency in cattle. The first attempt to link rumen microbiota and cattle efficiency was reported by Guan and colleagues, who showed that bacterial profiles detected by fingerprint of L-RFI animals were grouped together, which was distinct from H-RFI animals (12). Guan *et al.*'s study indicated that specific bacterial groups may only inhabit efficient steers and host genetics may play an important role in rumen microbial structure. Hernandez *et al.* and Zhou *et al.* confirmed the difference in rumen bacteria and methanogens between H- and L- RFI beef steers under both low and high energy diets (14,15,37,38). Hernandez *et al.* found the abundance *Eubacterium* spp. was significantly ($P<0.05$) different between RFI groups that were only on the high-energy diet and observed correlations between the abundance *Robinsoniella* sp. and RFI ($P<0.05$) for H-RFI animals (15). Zhou *et al.* found *Methanobrevibacter gottschalkii* was linked to the low-energy diet, whereas *Methanobrevibacter smithii* and *Methanobrevibacter* sp. AbM4 were associated with the high-energy diet (38). For RFI groups in Zhou *et al.*'s study, *Methanosphaera stadtmanae* was detected more frequently in L-RFI animals, and *Methanobrevibacter ruminantium* more likely to appear in H-RFI animals with *Methanobrevibacter smithii* was observed only for H-RFI animals (38). A later study by Carberry *et al.* (6) showed that *Prevotella* abundance was higher ($P<0.0001$) in inefficient animals and other bacterial populations had relationship with different diets.

Archaea in rumen are responsible for methane production by phylum Euryarchaeota, which is usually the only phylum found in rumen (33). Studies have showed that methanogens were greatly affected by different diets (high energy vs low energy), as well as feed efficiency (L-RFI vs H-RFI) (36). Carberry et al. (7) also conducted a study that focused on the rumen methanogen microbiota of cattle divergent for phenotypic RFI across two contrasting diets (high forage vs high grain). Results showed that *Methanobrevibacter* spp. was the dominant methanogens in rumen, with *Methanobrevibacter smithii* being the most abundant species. The abundance of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* were detected from the low forage diet group; but irrespective of diet, *Methanobrevibacter smithii* was different between H-RFI and L-RFI animals, which was significantly overrepresented in H-RFI animals.

Other than bacteria and rchaea, researchers have also tried to examine the relationship between cattle rumen feed efficiency and fungi or protozoa. In Carberry *et al.*'s study (6), there was no evidence that the total abundance of fungi could be influenced by feed efficiency or diet, but a positive association between the abundance of fungi and CH_4 emission was observed in the study. Carberry *et al.* (7) also observed a negative relationship between protozoa and propionate concentration and positive relationships between protozoa and butyrate, isobutyrate, and acetate propionate (A:P) ratios. Since decreased A:P ratio is associated with decreased methane emissions, and propionate provides most energy requirement for weight gain as a major contributor to gluconeogenesis (5), improving feed efficiency in cattle may be implemented by removing protozoa (defaunation), thereby increasing propionate concentrations and reducing A:P ratio. However, Newbold *et al.* (26) studied the role of protozoa in rumen and the results suggested that the main drawbacks of defaunation is decreasing feed digestibility, since defaunation could limit the feed intake and feed utilization efficiency.

With the understanding of relation between rumen microbiota and feed efficiency, improving feed efficiency may be implemented by regulating the rumen microbiota. Since cattle with higher feed efficiencies are reported to produce 20% to 30% less CH_4 (33), it was thought that reducing methanogen populations in rumen would lead to the improvement of feed efficiency. However, Zhou *et al.* (38) found that total methanogen population did not correlate with differences in feed efficiency, diet, or metabolic measurements. Li *et al.* (20) tried to reduce the methane production during the fermentation in an *in vitro* continuous culture system (Rusitec) with *Eremophila glabra*, a native Australian shrub. After 33 days fermentation, the results showed that the total gas production, methane and volatile fatty acid concentrations were significantly reduced with the addition of *E. glabra*. The overall methane reduction was 32% and 45% with 150g/kg DM and 250g/kg DM respectively, compared to the control group. Though the total bacterial numbers did not change, the total methanogen population decreased by up to 42.1% (with 400g/kg DM) when compared to the control group. This suggests that reducing methane emission by changing the fibrous substrate is feasible. However, studies to date have shown a trend of short term effectiveness of dietary intervention, and there are practical impediments to on-farm use of ingredients that may be hard to obtain in some regions.

TECHNOLOGY USED FOR STUDYING RUMEN MICROBIOTA

The evolution of next generation sequencing (NGS) technology over the last 10 years has led to a sharp increase in studying the gastrointestinal microbiota in production animals, without the need for time-consuming cultivation studies. The first of the "next generation" sequencing technologies to emerge was 454, commercialized by Roche and based on pyrosequencing mechanism (22). Pyrosequencing can detect the pyrophosphate release upon nucleotide incorporation in real time, the pyrosequencing relies on the

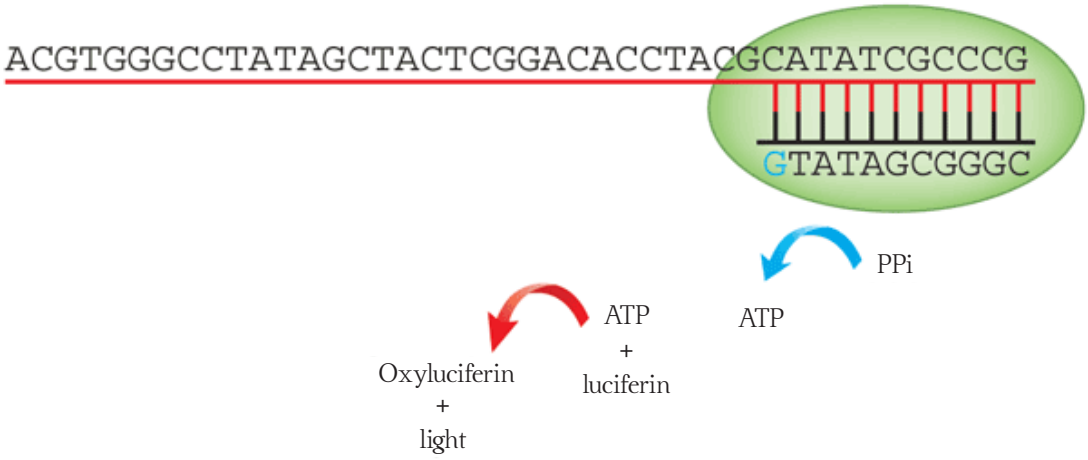

Figure 1. The template strand is represented in red, the annealed primer is shown in black and the DNA polymerase is shown as the green oval. Incorporation of the complementary base (the blue "G") generates inorganic pyrophosphate (PPi), which is converted to ATP by the sulfurylase (blue arrow). Luciferase (red arrow) uses the ATP to convert luciferin to oxyluciferin, producing light (32).

cooperation of four different enzymatic reactions (Fig. 1) (2,32). Pyrosequencing was initially used for single-nucleotide polymorphism (SNP) based genotyping, rather than standard DNA sequencing, because of the short read-lengths (31). 454 sequencing is a parallelized version of pyrosequencing, which was the first NGS available as a commercial product. Such technologies have dramatically increased the throughput of microbiota studies, as hundreds of samples may be multiplexed on a single sequencing run. For example, the latest version of 454 sequencing named "GS FLX Titanium XL+" can provide up to 1,000 base pairs (bps) read length with 700 Mbps throughput in a single 23-hour run, compared to Sanger Sequencing which can only provide 1000-1200 bps each time with the limitation of electrophoresis (24,29). McCann *et al.* (23) reviewed the recent bovine rumen metagenomic publications and found that the Roche 454 FLX platform is commonly used today because of the longer read lengths. However, the increased throughput and lowered cost of Illumina platforms (MiSeq, HiSeq), has led to increases in their use. Illumina platforms also allow for paired end sequencing, as an alternative to the expensive long-read system employed by 454. Fouts *et al.* (10) pointed out that next generation sequencing technologies provide promises to help us better understand how rumen microbial community structure and function affects ruminant feed efficiency, biofuel production, and environmental impact.

Metagenome is the DNA sequence information of a community as a whole (21), which is commonly used today to study rumen microbiota. The complex nature of the rumen environment is difficult to replicate in the laboratory. In determining an accurate rumen microbiome, a whole microbial community database would be the most promising option (25), therefore, a metagenome system is essential in the study. Ross *et al.* (30) developed a reference metagenome to compare rumen metagenomic profiles for individual cattle. When the reads from the study were aligned to a rumen metagenome

reference, rumen metagenome profiles were repeatable (P <0.00001) within sample regardless of the location of sampling rumen fluid. Consequently, determining the accurate microbiome by metagenomics analysis strategy can help researchers study the role of particular groups of microbiome in the whole community and the connection with feed efficiency.

Additionally, metagenomic studies also allow for elucidation of the functional potential of the rumen microbes. While function can be predicted from amplicon studies using bioinformatic tools like PICRUSt, metagenomic investigations will yield definitive data on the fibrolytic enzymes encoded by the ruminal metagenome (19). However, while amplicon sequencing has decreased sharply in cost in recent years, metagenomic studies remain, for many people, prohibitively expensive due to the depth of sequencing required.

CONCLUSION

In summary, with the evolution of new molecular biology techniques, researchers can determine more about rumen microbiota composition, function, and its relationship with feed efficiency, as well as attempting to improve feed efficiency by manipulation of rumen microbiome. However, it is still hard to permanently change the rumen microbiome.

While this review has focused solely on literature of the rumen microbiota and its relationship with feed efficiency, future studies should examine microbiota throughout the GIT, as intestinal microbiota also play important roles in feed efficiency. The interaction between the host and resident microbes also warrants further study in terms of feed efficiency. Also, studies of beef cattle rumen microbiota mainly focused on feedlot cattle, the differences between feedlot cattle and free grazing cattle rumen microbiota are still unknown.

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