

RUMEN MICROBIOLOGY

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Keywords: Rumen, fermentation, cellulose, biofilm, volatile fatty acids, methane, diversity, bacteria, protozoa, fungi

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Summary

Ruminants are herbivores that utilize a symbiotic relationship with the microorganisms in their forestomach (rumen) to exploit fibrous feeds as a source of energy and nutrients. Ruminal content (fluid and feed material) contains 10^{10} to 10^{11} microorganisms per milliliter, including prokaryotic (bacterial and archaeal) and eukaryotic species. Microbial fermentation of ingested plant materials is a crucial step in the digestion of feed by the host animal. Rumen microorganisms usually adhere to feed particles and form biofilms to degrade plant material. Most microorganisms have different roles in feed digestion and act synergistically to ferment plant carbohydrates and proteins, but

antagonistic relationships can develop if different microbes occupy a similar niche. Microbial populations change with feed type as well as with other environmental influences such as the inclusion of antibiotics in the diet. Plant carbohydrates and proteins are fermented to volatile fatty acids by microbes and both these and the microbial protein produced provide nutrients that are directly utilized by the ruminant host. Rumen microorganisms can also detoxify many feeds but occasionally they also produce end products (e.g., nitrate, cyanide derivatives, lactic acid) that may be detrimental to the host. Manipulation of rumen fermentation through proper diet formulation or through the use of additives can often alter microbial populations in a manner that alleviates these problems. Application of the tools of modern molecular biotechnology is enhancing our understanding of this extremely complex microbial ecosystem in a manner that should provide opportunities for further optimization of rumen fermentation.

1. Introduction

Plant cell walls represent the most abundant source of organic carbon on earth – over 10^{14} kilograms are synthesized annually. Ruminants are unique in their ability to transform the complex polysaccharides in plant cell walls into microbial protein. The fact that wild ruminants occupy ecological niches from the tropics to the high arctic attests to the evolutionary fitness of the ruminant digestive system. Ruminants themselves, however, do not produce the enzymes required for the degradation of complex plant cell wall polysaccharides. Rather, they have developed a symbiotic relationship with anaerobic (i.e., oxygen intolerant) bacteria, fungi and protozoa that establish residence in the ruminant digestive tract within a few weeks of birth. These microorganisms ferment plant carbohydrates ingested by the ruminant host – this anaerobic process yields volatile fatty acids (VFAs), vitamins and microbial protein as end products. The ability of the microbial populations to produce vitamins and protein from recalcitrant plant material is the reason why ruminants can adapt to nutrient-sparse environments such as deserts and the high arctic.

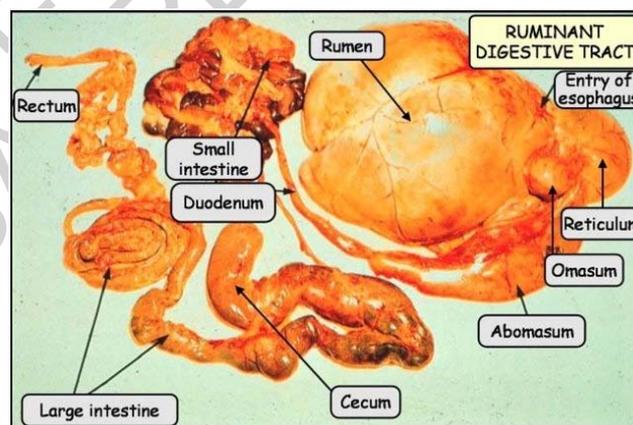


Figure 1. The ruminant digestive tract Adapted from: Russell, J. B. (2002). *Rumen Microbiology and Its Role in Ruminant Nutrition*. J.B. Russell, Ithaca, NY.

Many of the important domesticated animals that are utilized by humanity for food or draft animals (e.g., cattle, goats, sheep, camels) are ruminants. Ruminants possess four

stomachs: the reticulum, the rumen, the omasum and the abomasum (Figure 1). Whereas some other herbivores are hindgut fermenters (e.g., rabbits, horses), fermentation in ruminants occurs in the forestomach which comprises the reticulum and rumen. Fermentation in the forestomach offers several nutritional advantages over hindgut fermentation because it allows for the end products of the fermentation to be digested and absorbed within the lower digestive tract of the host. Fermentation products and undigested feed, flow from the rumen through the omasum and into the abomasum. The abomasum functions as a ‘true’ (i.e., gastric) stomach, playing an important role in protein digestion similar to the stomach in monogastric or ‘simple-stomached’ animals. Thus, the high quality microbial protein derived in the reticulo-rumen from low quality recalcitrant plant sources can be digested efficiently in the abomasum, thereby meeting a large proportion of the protein requirements of the host. Moreover, rumination (i.e., regurgitation of feed from the rumen) enables repeated mastication of feed, which enhances the ability of the microbial population to ferment the feed. Some ruminal microbes also possess the capacity to degrade toxic substances in feed (e.g., mycotoxins, glucosinilates) to harmless intermediates that do not impact the health of the host.

2. Rumen Ecosystem

The volume of the rumen varies with species, ranging from 3 to 15 L in sheep and from 35 to 120 L in cattle and accounting for approximately 70% of the total digestive tract volume. As a consequence, the digestive tract generally accounts for a larger proportion of total body weight in ruminants than it does in most other mammals. Efficient microbial fermentation in the rumen requires strictly anaerobic (i.e., oxygen-free) conditions. Trace amounts of oxygen that enter this system either with ingested feed or through diffusion across the rumen wall are quickly consumed by facultative anaerobic bacteria residing in the rumen. Temperature in the rumen is closely regulated at approximately 37 °C and the pH is usually between 5.8 and 6.8, but can decline below 5.0 when high-starch diets are fed.

Extensive interactions occur among the members of the rumen microbial community and numerous examples of both synergistic and antagonistic relationships have been documented. These include both synergistic and inhibitory relationships between bacterial species, predation of ruminal bacteria by ciliate protozoa, and opportunistic access to plant tissues by bacterial species following initial invasion by fungal hyphae. The rumen is a dynamic system, in which resident microbes must adapt continuously to changes in diet composition, form, quantity and frequency of consumption. Nutrient composition, physical structure and presence of additives in the diet all affect species distribution within the ruminal ecosystem, and thus the overall digestive activity of the microbial community. Rapid changes in the diet can lead to instability in this community, giving rise to digestive upsets such as bloat or acidosis which can cause illness or even death of the host. No clearer example of this exists than when ruminants are misguidedly switched from a forage-rich to a grain-rich diet over a short duration (i.e., 1 to 3 days), a practice that often results in fatalities. Given time to adapt, however (i.e., 14 to 21 d), microbial communities can make this transition with no adverse affects on the host and in fact, ruminants are often more productive on high-grain diets than on high-forage diets. The naturally tendency of the microbial community to return to a

steady state condition is another factor that undoubtedly contributes to the environmental fitness of ruminants. Consequently, many of the same microbial species are identified from ruminants from vastly different geographical areas.

3. Microbial Species

In the 1950s, Robert E. Hungate, the father of rumen microbiology, developed the roll tube anaerobic technique for culturing and isolating ruminal microorganisms. This opened the door to what had previously amounted to a digestive “black box”, and an appreciation for the incredible complexity of the rumen microbial community began to emerge. Subsequent advances in molecular biology indicated that the majority of microbes in the rumen were not culturable using the Hungate technique, thus molecular methods have been increasingly utilized to study the rumen ecosystem. Many microbes are now identified only by their 16S or 18S ribosomal DNA sequences and new molecular procedures such as metagenomic analysis are currently being used to characterize the nature of this unique ecosystem.

3.1. Bacteria

Ruminal content contains 10^{10} to 10^{11} bacteria per mL, and these bacteria are responsible for the majority of fermentative activity in the rumen. Comparisons of 16S ribosomal DNA sequences have documented a high degree of diversity among rumen bacteria and new species continue to be identified and described. Rumen bacteria play a critical role in the biotransformation of complex polysaccharides into simple sugars, which are subsequently fermented to produce VFA. To accomplish this degradative task, most structural carbohydrate-degrading bacteria adhere tightly to their substrates during the digestion process. Three bacterial species, namely, *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*, are considered the most active and predominant of the cellulolytic bacteria, although other minor cellulose-degrading species have been reported. These three species all produce an array of glycoside hydrolases such as cellulases, xylanases, and carbohydrate esterases. However, the mechanisms by which these organisms degrade cellulose apparently differ between *F. succinogenes* and the ruminococci.

Cellulose degradation in Gram-positive ruminococci is similar to that of non-ruminal anaerobes such as *Clostridium thermocellum*. Both exoglucanases (e.g., cellobiohydrolases) and endoglucanases are produced, and act synergistically during cellulose degradation. Also in similarity with *C. thermocellum*, the ruminococci produces multi-protein complexes known as cellulosomes that mediate the attachment of cellulases and bacterial cells to cellulose (see Section 4.1). In contrast, Gram-negative *F. succinogenes* apparently utilizes a different strategy for cellulolysis, as neither cellobiohydrolases or the cellulosome complex have been identified through biochemical or genomic means.

Hemicellulose and pectin within plant cells can be utilized by ruminococci and some strains of *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*. It is interesting to note that *F. succinogenes* can also degrade hemicellulose, but is unable to ferment the xylose that is liberated. Sequencing of the *F. succinogenes* genome has confirmed the presence

of all but one of the key enzymes involved in pentose metabolism, which explains why this bacterium is unable to utilize five-carbon (C₅) sugars. Bacterial species predominance in the rumen is altered in response to changes in the animal's diet. For example, the starch utilizers *Ruminobacter amylophilus* and *Succinomonas amylolytica*, present in the rumen at low numbers when forages are fed, proliferate dramatically when grain is introduced into the diet. In addition to the bacteria that serve as primary digesters of structural carbohydrates, some (e.g., *Treponema bryantii*, *Selenomonas ruminantium*) derive energy from fermentation of end products such as succinate and lactate. Although these bacteria do not participate in the direct hydrolysis of plant cell walls, they play an important role in preventing inhibition of fermentation through end product accumulation.

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Biographical Sketches

Meng (Samuel) Qi is a postdoctoral research fellow with expertise in microbiology, fermentation engineering and ruminal genomics. His Ph.D. research at the University of Guelph, in Ontario, Canada, included characterization of mechanisms of cellulose degradation by *Fibrobacter succinogenes* and comparative genomic analysis of *F. succinogenes* and *F. intestinalis*. Samuel joined Dr. McAllister's research team in 2008.

Katherine Jakober holds a B.Sc. in Cellular and Microbial Biology, and has provided technical and editorial support to the rumen microbiology and nutrition research group at the Lethbridge Research Centre for 25 years.

Tim McAllister is an authority on rumen microbiology and conducts research in a variety of areas related to Canadian beef production. In rumen microbiology, he is best known for his Ph.D. work that characterized the role of microbial attachment and biofilms in starch digestion. This work has been used in the development of management strategies to avoid digestive disturbance such as bloat and acidosis in ruminants. He is presently a Principal Research Scientist with Agriculture and Agri-Food Canada and contributes extensively to the training and education of graduate students through the adjunct professorship appointments he holds at seven Canadian and three international universities. He spends his spare time cycle touring and mountain biking with his family in the Canadian Rockies.