
An Overview of Rumen Microbial Ecosystem

Keshav Gaur¹, Monika Karnani^{2*}, Sheela Choudhary³, Manju², Geeta Choudhary¹

¹M.V.Sc. Scholar, animal Nutrition Division, RAJUVAS-PGIVER, Jaipur (Rajasthan)

²Assistant Professor, animal Nutrition Division, RAJUVAS -PGIVER, Jaipur (Rajasthan)

³Professor and HOD, animal Nutrition Division, RAJUVAS -PGIVER, Jaipur (Rajasthan)

* Correspondence author - dr.monikakarnani@gmail.com

abstract

The rumen microbial ecosystem is diverse and complex and the complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses. These microorganisms work symbiotically to break down feedstuffs consumed by ruminant animals. The microbiome controls the production efficiency of the animal, with certain pathways (such as those associated with methane production) resulting in energy loss in the animal. The microbiome also affects end-product quality (milk and meat) but also contributes to environmental pollution. Understanding the rumen microbiome and its connection to the ruminant itself; is important for producing quality products, increasing profitability and reducing environmental impacts.

Introduction

The rumen can be viewed as an anaerobic and methanogenic fermentation vat that contains microorganisms that have the ability to utilize, and increase the productivity of, cellulolytic feeds (i.e. straw, hay, silage and grass). The rumen dynamics are almost solely responsible for providing nutrients to the host animal. The rumen and its microbiota play a particularly important role in the degradation of feedstuffs. As a result of fermenting feedstuffs, carbon dioxide (CO₂) and hydrogen (H₂), which are the main electron acceptors and donors of the ecosystem, are produced in the rumen. The rumen microbiome, i.e., the community of microorganisms that inhabits the rumen, is characterized by its high population density, extensive diversity (encompassing bacteria, archaea, protozoa and fungi) and complexity of interactions. The continuous fermentation carried out by these microorganisms leads to broken down of ingested fibrous feed into their subcomponents.

There are 3 intersecting micro-environments found in the rumen that contain these microbes; the **liquid phase** making-up 25% of the microbial mass, the **solid phase** making-up 70% of the microbial mass, and the **rumen epithelial cells and protozoa**, containing 5% of the

microbial mass. a nutritionally balanced diet is important as it provides an atmosphere that maximizes the growth and activity of these microbes.

A rumen microorganism is anaerobic or facultatively anaerobic, and produces end products that are either directly utilized by the host or by other microorganisms as energy. The ruminal pH is kept relatively constant (Generally 6 to 7), but may vary depending on diet. Such variations can result in a change in the microbial populations, and the levels of volatile fatty acids (VFAs) produced. These fatty acids such as propionate and butyrate, can be absorbed across the gut wall to serve as an energy source for the ruminant.

Buffering of the rumen to maintain a relatively constant pH is facilitated by the large quantity of saliva produced by the ruminant, which is high in sodium and potassium bicarbonate and urea. The saliva is swallowed into the rumen and then absorbed through the rumen walls. Further buffering is provided by ammonia produced during fermentation, which can then be used for microbial growth in the rumen. Ruminants, through the action of their microbiota, can utilize indigestible components of feed i.e. lignocellulose. The interplay between the host and microbes in the rumen is synergistic, i.e. the host provides heat, moisture and food, while the microbiomes produce protein and by-products of digestion, such as VFAs, for use by the host. The complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses.

1. Rumen bacteria –

The most abundant microbes in the rumen liquor with concentration $10^{10} - 10^{11}$ cells/ml and are of over 200 species. The bacterial composition of rumen liquor depends upon a number of factors including precedence of certain diets, energy requirements, and resistance to some toxic metabolic end-products. Rumen bacteria are mainly Gram negative (when fed on high forage diet), but on high grain diets more Gram positive bacteria are seen in rumen liquor. The use of molecular techniques for the analysis of rumen microbiology for example, the conserved 16S rRNA gene to determine bacterial composition, predict their functionalities or enumerate targeted microbes within a complex ecosystem without the need for culturing. This is important because only 20% of the rumen microbiota can be cultured using standard techniques. Sequencing (using 16S rRNA) also reveals that *Prevotella*, *Butyrivibrio* and *Ruminococcus* are the most dominant bacteria in the rumen, and that community structure is affected by changes in the diet of the host.

It has been also discovered that diet complexity favors increased microbial diversity. Due to the high forage diets of ruminants, particularly grass-based diets, cellulose digesters are more in number. These **cellulolytic bacteria** degrade the main components of plant fiber i.e. cellulose and hemicellulose. The ability to degrade cellulose is strongly dependent on the type of forage, crop maturity and the accessibility of the cellulolytic bacterial communities. The plant fiber matrix is complex in nature and are composed of β -1, 4 linked glucose residues for cellulose and β -1, 4 linked xyloses for hemicellulose, and thereby require the coordination of a number of hydrolytic enzymes in order to break it down. Although there are many cellulose degrading bacteria, *Fibrobacter succinogenes* and *Ruminococcus albus* are the most desirable cellulose degraders. Their ability to digest cellulose is much higher than that of other cellulolytic bacteria, which is due to the fact that they possess a number of genes encoding enzymes which are involved in fibre degradation. Fermentation end products of cellulolytic bacteria include acetate, butyrate, propionate and CO₂. Formic acid and lactic acid are also formed but are quickly used by other bacteria.

Starch is also an important constituent of the ruminant diet, in particular for highly productive dairy cows. High grain diets result in an increase in the amount of starch in the rumen. *Streptococcus bovis* (an **amylolytic** bacterium) is normally present in low numbers in cows fed high forage diets and in high abundance in cows that consume high grain diets. *S. bovis* requires low pH for their optimum growth and its high abundance following consumption of high grain diets; is attributed to a sudden increase in glucose levels in the rumen and the loss of protozoa due to the more acidic environment created by high grain diets. More specifically, lactic acid is produced from starch and, as lactic acid is not metabolized by the animal, therefore get absorbed through the rumen wall; causing an increase in lactic acid in the blood and reduced blood pH.

Sudden change in animal diet, results in an accumulation of VFAs in the rumen liquor, causing a detrimental effect on the microbiota and the host animal. These severe and sudden changes lead to a decrease in ruminal pH and an increase in *S. bovis* and *Lactobacillus* species. Some anaerobic bacteria acquire energy from the pectin degradation, with the most important **pectinolytic** species, *Lachnospira multiparus*, *Prevotellaruminicola* and *Butyrivibrio fibrisolvens*, being capable of reducing pectin to oligo-galacturonides, yielding large quantities of acetate. Citrus by-products like citrus pulp are widely used in ruminant feeding systems and contain a high percentage of pectin substances. These by-products can be used as an alternative to highly

fermentable grains, preventing the excessive growth of *S. bovis*, and associated ruminal acidosis, and some researchers suggest that they may even improve the feed utilization efficiency for milk production.

Microorganisms associated with milk fat yield are **Firmicutes** and **Bacteroidetes**. A decreased abundance of Bacteroidetes in comparison to Firmicutes results in increased milk fat %.

2. Methanogenic archaea –

Archaea, due to their broad spectrum of unusual and distinctive metabolisms, can survive in a variety of different environments. Rumen archaea are strictly anaerobic and are the only known rumen microorganisms to produce methane (CH₄). Such archaea are referred to as methanogens. Archaea are found in the rumen in the range of 10⁶ to 10⁸ cells/ml, accounting for less than 4% of the microbial community. Archaea are present at the bottom of trophic chain due to their need to use the end products of fermentation as their substrates.

The domain archaea is broken into two different kingdoms-

- A) Euryarchaeota, consisting of methanogens and extreme halophiles, and
- B) Crenarchaeota, consisting of hyperthermophiles and non-thermophiles.

Methanogens of kingdom Euryarchaeota; requires very low redox potential and are strictest anaerobes known. According to meta-analysis of global data, 90% of rumen methanogens belong to the following genera; *Methanobrevibacter* (63.2% of methanogen population), *Methanomicrobium* (7.7% of methanogen population), *Methanosphaera* (9.8%) "Rumen Cluster C", now referred to as *Thermoplasma* (7.4%) and *Methanobacterium* (1.2%).

Most methanogens remove hydrogen gas by reducing CO₂ with H₂ gas to form CH₄. In contrast, *Methanosphaera stadtmanae* only produces methane through the reduction of methanol with H₂, having one of the strictest energy metabolisms of all methanogenic archaea. Producing methane; keeps low ruminal hydrogen concentrations, allowing methanogens to promote the growth of other species, and thereby enabling a more efficient fermentation.

Eructation of methane; which is also a green-house gas (GHG), leads to atmospheric pollution. Therefore, following efforts to mitigate emissions of rumen methane includes -

- I. Vaccines (targeting rumen methanogens through the generation of antibodies to selected methanogen antigens that enter via saliva, binding to targets on the methanogens)

- II. Small-molecule inhibitors (targets enzymes essential for the growth of methanogens)
- III. Additives and Breeding approaches.

In a study carried out by *Goopy et al.* (2014), it was found that sheep that emitted low methane levels had a smaller rumen in comparison to high methane-emitting sheep. There was no difference in dry matter intake or digestibility between the two groups. The study also found that low methane-emitting animals had a shorter mean retention time for both solid and liquid phase. Hence, this may be the basis for breeding animals with a smaller rumen size to reduce methane emissions. However, most effective and straightforward method of lowering rumen methane emissions is dietary manipulation because, as selective breeding is slow and selection of specific traits may affect favorable variants.

Methane production by archaea represents an energy loss of about 2–12% of gross energy intake (GE_i), means this energy is no longer available for animal growth, lactation, maintenance or pregnancy. Manipulating the ruminant's diet; to reduce the number of methanogens would therefore be helpful to reduce the negative impact on the environment, and will also improve the efficiency of livestock production.

Methanogenesis is the only mechanism of ATP synthesis for methanogenic archaea. The methyl coenzyme M reductase (McrA, encoded by *mcrA* gene) catalyzes the final step in methanogenesis. Thus, use of anti-methanogens, such as bromochloromethane, can be used to inhibit methane production. Because, Bromochloromethane reacts with reduced vitamin B₁₂ and inhibits the cobamide-dependent methyl transferase step, which is responsible for McrA synthesis. Therefore, targeting this step may break the pathway and inhibit production.

A recent study, investigated the use of an invasive species of macroalgae *asparagopsis taxiformis* as a means of reducing methane emissions in vitro by its secondary metabolite-bromoform, which is similar to bromochloromethane in its ability to decrease methanogenic activity.

3. Ciliate protozoa –

Ciliate protozoa are found in the range of 10⁴ – 10⁶ cells/ml in rumen liquor and are amenable for 30 to 40% of overall fiber digestion. They are also relatively active in lipid hydrolysis and can produce hydrogen via their hydrosomes. The *Entodinium* genus is the most dominant protozoan in high grain diet and it rapidly degrades starch, engulfs it and converts it to an

iodophilic storage polymer. Degradation occurs through a combination of debranching, amylase and glucosidase enzymes.

Defaunation is a mechanism used to prevent the growth and/or remove, protozoal population from the rumen. This can be done by several different means such as through the isolation of calves after birth, chemical defaunation through the use of CuSO₄, calcium peroxide, alcohol ethoxylate, coconut oil, linseed oil or soya oil hydrolysate. Another method of defaunation, involves an invasive method to remove the rumen contents from the host, followed by carefully washing of rumen mucosa and then the rumen contents are treated by either heating or freezing to eliminate protozoa. The rumen contents are then returned back to the rumen. This method also removes protozoa-associated methanogens (methanogens sequestered within rumen protozoa) which account for approximately 37% of methanogenesis in ruminants, and as defaunation would also eliminate them, leading to decrease in methane production.

Some studies revealed that methane emissions increased with protozoal abundance and stated that protozoa showed the strongest link with methane emissions (in comparison to other domains) after qPCR using DNA extracted from rumen contents. The presence of protozoa can probably have both beneficial and negative effects on the rumen microbiome, as protozoa engulf bacteria and other smaller microbes and particles in the rumen, but also larger molecules including proteins and carbohydrates. They actively ingest the bacteria as a source of protein and also act as a stabilizing factor for end products of fermentation.

4. Amoeba –

Amoebae can represent an important reservoir for bacteria in the environment, but their role in the rumen is unclear. In the vegetative cycle (multiplication by binary fission), amoeba, similar to ciliate protozoa, survive by ingesting bacteria through phagocytosis. It is known that some bacteria can survive phagocytosis by protozoa and live as endosymbionts. For instance, *Campylobacter jejuni* has been shown to invade *Acanthamoeba polyphaga* and can replicate in vacuoles. *C. jejuni* and *C. fetus* can have large effects on cow fertility, immunity and overall health. Amoeba therefore may be important in rumen and general bovine health.

5. Fungi -

Rumen fungi ($10^3 - 10^6$ zoospores/ml) are anaerobic, falling into the class Neocallimastigomycetes, consisting of 6 genera (*Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces* and *Piromyces*) with 21 known species and, using

molecular techniques, 2 genera are recently discovered *Oontomyces* and *Buwchfawromyces*. anaerobic fungi may influence the rest of the microbial community since they produce H₂ during initial degradation of plant tissue and this H₂ can be used as fuel for the degradation mechanisms of other communities.

Fungi colonizes plant cell walls and accounts for approx. 8–12% of total ruminal microbial biomass, but this figure varies widely depending on the animal diet. While most plant fiber-associated fungi are retained in the solid phase, the liquid phase may contain smaller particulate matter that fungi may have attached to. Fungi are the best degraders and they produce high levels of celluloses hemicelluloses, as well as also possess the ability to break down xylan due to the production of xylanases enzyme. Fungi appear to initiate the feed breakdown process, indicating that anaerobic fungi may be pivotal for feed utilization efficiency and animal growth and production in pasture-fed ruminants.

By breaking down the carbohydrates, fungi produce metabolites which can be used for nutritional purpose by the host. They have many features which make them unique in comparison to fungi found outside the rumen, including polyflagellate zoospores (the reason why rumen fungi were originally mistaken for ciliate protozoa), cellulosomes, hydrogenosomes and a wide enzymatic spectrum.

6. Bacteriophage –

Bacteriophage are obligate pathogens of bacteria and they occur in dense populations of approximately $10^7 - 10^9$ particles/gram of digesta in the rumen. Like others; bacteriophage population are also influenced by external sources, meaning they may also be controlled through different strategies.

The bacteriophage and virus population found in a rumen sample is referred to as the **virome**. The high number of rumen bacteriophage suggests that they may have an important function in the balance of the ruminal ecosystem, but there is little known about the effect of the rumen virome on the system it inhabits. Some studies showed that this was important in the transfer of glycoside hydrolase between bacteria and rumen fungi. Also some research propose that this may also be important in the case of rumen ciliate protozoa, whereby horizontal gene transfer occurs from rumen bacteria in order to aid in protozoa adaptation to the carbohydrate-rich environment of the rumen, transferring genes encoding plant cell wall degradation. This information indicates that bacteriophage may represent a shared gene pool for the rumen system.

Metagenomics can also be used to determine the origin of these genes. In a study carried out by it was found that most viruses found in the rumen were associated with the most dominant phyla, namely Firmicutes, Bacteroidetes and Proteobacteria. Bacteriophage interaction can be identified by the presence of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins in the microbial population. These genes are fundamental to provide adaptive immunity in some bacteria and archaea, which enable the organism to respond and eliminate invading genetic material. Once infected by these invading genetic materials, the new DNA is integrated into the host CRISPR locus as new spacers, ultimately encoding a unique spacer sequence

CONCLUSION

The rumen microbiomes play an exigent role in shaping the host physiological parameters. The complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses. Although bacteria are the most prominent microorganisms in the rumen, but fungi are the best degraders. Identification of certain metabolic pathways of these microbiota and then further investigation of those pathways; may be helpful to determine the best diet for ruminants; in order to minimize the energy losses, to reduce the methane production and to increase the nitrogen utilization efficiency. Examination of the rumen microbiome can even identify the effects of diet on the microbiome and in turn, the effects on milk yield, milk fat %, protein percentages, urea percentage (used as an NPN indicator) and milk protein yield.

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