LUMEN ET VIRTUS

RUMEN MICROBIOLOGY FROM A METAGENOMIC PERSPECTIVE: A REVIEW OF NEW FINDINGS AND TRENDS

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ABSTRACT

Rumen microbiology studies the microorganisms present in the rumen, the compartment of the ruminant stomach responsible for the fermentation of food. These microorganisms, which include bacteria, protozoa, fungi, and archaea, play a crucial role in the digestion of cellulose and other complex carbohydrates, allowing ruminants to take advantage of nutrients from fibrous plants. Traditionally, the study of the rumen microbiota was based on in vitro culture techniques, limited to organisms that could be grown in the laboratory. However, advances in metagenomics (an approach that allows for the direct analysis of microbial DNA from environmental samples, without the need for cultivation) have revolutionized the field. With metagenomics, scientists can identify and characterize all microbial diversity present in the rumen, including previously unknown or non-cultivable organisms. This method has revealed a much more complex and diverse microbiota than previously thought, offering new insights into microbial functions, interactions between species, and their influence on ruminant health and productivity. This new knowledge is key to improving feed efficiency, reducing the production of methane (a potent greenhouse gas), and developing more precise nutritional and therapeutic strategies for ruminant

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management. Metagenomics, therefore, is driving a new era in rumen microbiology, with the potential to transform animal production sustainably and efficiently.

Keywords: Microbial Diversity. Feed Efficiency. Ruminants.

INTRODUCTION

Ruminants belong to the class of herbivorous ungulate mammals, and to the order Artiofactyla, and are capable of using forage as their main source of food (HUNGATE, 1966). They are animals with a differential, mainly due to their complex gastrointestinal tract, composed of fermentative chambers responsible for digestion, nutrient absorption and the general health of the animal. The gastrointestinal tract of these animals contains a diverse and abundant microbial ecosystem (BORDIM et al., 2016).

The expansion of knowledge about the rumen microbiome has allowed detailed evaluations of the microbial community, its characteristics and functions. These advances have provided significant progress in ruminant feeding, allowing for greater efficiency in the use of nutrients and, consequently, better use by the animal (MORGAVI et al., 2013).

Studies investigating microbial communities, as well as rumen traits, are key to understanding and manipulating ruminant performance and health (HUWS et al., 2018). Several studies have shown that ruminants, together with their microbiome, have evolved over time, becoming adaptable to a wide variety of diets, thanks to the functional plasticity of the communities present in the rumen (MORGAVI et al., 2013).

In addition to evolutionary characteristics, other factors also influence the dynamics of rumen microorganisms, such as age (FONTY et al., 2007), the use of antimicrobial medication (KLEEN et al., 2003), animal health (RUSTOMO et al., 2006), geographic location (SUNDSET et al., 2007), stress level (LYNOMO et al., 2010), among others. In this way, metagenomics becomes an indispensable tool to understand the complex microbial community present in the rumen.

METHODOLOGY

This article is based on a literature review, in which information was collected from a broad search in the existing literature. The survey involved scientific productions related to rumen microbiology, focusing on the knowledge derived from studies in metagenomics, including basic activities of identification, analysis and interpretation of data.

The search platforms used for data collection included: Elsevier, SciELO, Capes Journal Portal, Sci-Hub, Google Scholar and digital books, in addition to other sources of technical dissemination. The inclusion criteria focused on articles published in the last ten years, theses and dissertations, as well as other scientific productions available on the internet, totaling 90 works analyzed. This review is descriptive and provides detailed information on the application of metagenomics in the study of rumen microbiology.

DISCUSSION

CHARACTERIZATION OF THE RUMEN ENVIRONMENT

The rumen ecosystem is made up of a dynamic and diverse microbial community, composed of bacteria, archaea, protozoa and fungi. These microorganisms interact intimately with each other and have a mutualistic symbiotic relationship, which favors the host (MIZRAHI, 2013). The metabolic activity of these microorganisms converts complex fibrous substrates into volatile fatty acids and microbial protein, which are used by the animal for maintenance, growth, and lactation (RIBEIRO et al., 2017).

The rumen is a cavity that presents favorable conditions for the survival and growth of microorganisms. An anaerobic fermentation chamber, with a relatively constant temperature (38-40°C) and pH between 5.5 and 6.9 (HUNGATE, 1966; CLARKE, 1977; DEHORITY, 2003). According to Orskov and Tyle, (1990), the pH of the rumen together with the substrates available for fermentation are factors that determine the permanence of rumen microorganisms. In the rumen, the redox potential is usually between -250 and -450 mV, proving the absence of oxygen and the high reducing potential (VAN SOEST, 1994).

The end products of rumen fermentation include short-chain fatty acids mainly acetate, propionate and butyrate, gases such as CO2 and CH4. Volatile fatty acids are the main route of energy supply for the animal. This fermentation process results in many intermediate products, such as lactate, succinate, furnace, H2 and NH3 (HUNGATE, 1966).

According to Stewart et al. (1997), for a species to be considered part of the rumen microbiota, it must be anaerobic, produce by-products in the rumen and develop actively, with a metabolism compatible with the normal conditions of this environment.

Thus, the rumen microbiota can be classified into four types of subpopulations, the first population is integrated with liquids, and is composed of planktonic microorganisms suspended in the rumen fluid, comprising those that separate from food particles and those that consume soluble food fragments in the rumen fluid (MCALLISTER et al., 1994). The second is populations incorporated into the solid fraction, which includes microorganisms moderately or strongly adhered to food particles, this group is considered essential in the digestion process (MCALLISTER et al., 1994) in addition to corresponding to approximately 75% of the microorganisms present in the rumen. The third group is the one adhered to the rumen epithelium, which corresponds to 1% of the total rumen population (CZERKAWSKI, 1986).

RUMEN BACTERIAL COMMUNITY

The bacterial community in the rumen is predominantly composed of strictly anaerobic microorganisms. However, there is also the presence of some facultative anaerobic species, which play an important role in maintaining the anaerobic conditions of the environment. Rumen bacteria not only exhibit great diversity, but also have significantly elevated metabolic activity. They have an estimated population density of $^{10\text{-}11}$ cells/mL of rumen content, followed by archaea (108-9 mL-1), ciliated protozoa (106 mL-1), and fungi with 106 mL^{-1} (WANG et al., 2017).

The first studies that allowed the identification and understanding of the metabolism of bacteria in the rumen arose from research that used bacterial cultivation techniques under anaerobic conditions (HUNGATE, 1966). According to phylogenetic analyses, it has been shown that the central microbiome is composed mainly of bacteria from the phyla Firmicutes and Bacteroidetes, which are widely present in ruminants, regardless of geographic region (HENDERSON et al., 2015).

Through the sequencing of the 16S gene of the bacterial rRNA, it was found that the phyla Bacteroidetes and Firmicutes, regardless of species, age or diet, can represent up to 80% of the bacterial population in the rumen (MENEZES et al., 2011; Henderson et al., 2013; Mohammed et al., 2014). On the other hand, the phyla *Proteobacteria, Fibrobacter, Verrucomicrobia, Tenericutes* and *Spirochaetes* are present in lower concentrations (MENEZES et al., 2011).

Bacterial species in the rumen can present themselves in different forms, such as cocci, spirilli, bacilli or vibrios. They can also be classified as mycoplasmas (which do not have a cell wall), gram-positive, or gram-negative. Gram-positive cells have a thick cell wall, formed by a single layer of peptidoglycan, while gram-negative cells have a more complex structure, with a double outer membrane connected by a layer of glycopeptides (PURVES et al., 2002; KOZLOSKI, 2011). In most cases, species that degrade structural carbohydrates are usually gram-positive.

In the rumen environment, it is possible to identify a wide variety of highly specialized bacteria, capable of using specific, varied or intermediate substrates (HUNGATE, 1966). Thus, rumen bacteria can be classified according to their metabolic activity, as fibrolytic, amylolytic (*Selenomonas ruminantium, Streptococcus bovis),* proteolytic (*Prevotella spp*.), lipolytic (*Anaerovibrio lipolytica*), lactate-producing (*Streptococcus bovis and Selenomonas ruminantium*) and lactate-consuming (*Megasphaera elsdenii*) (HUNGATE, 1966; MCCANN et al., 2014).

Despite the wide variety of cellulose-degrading bacteria, some species, such as *Fibrobacter succinogenes, Ruminococcus flavefaciens and R. albus*, demonstrate a superior ability to digest cellulose compared to other known cellulolytic species (ARCURI et al., 2011). This is possibly due to the fact that these species have several genes that encode specific enzymes for fiber degradation (MATTHEWS et al., 2019).

Most cellulolytic bacteria prefer a pH range close to 6. pH sensitivity is a general feature of bacterial growth. When the rumen pH falls below this value, the activity of these microorganisms is limited; even a slight reduction can inhibit the digestion of cellulose (RUSSEL and WILSON, 1996). Some rumen bacteria, however, have developed resistance and the ability to keep their intracellular pH low, as well as preserve a pH gradient across the cell membrane, preventing the intracellular accumulation of anions.

In addition, cellulolytic activity can be inhibited by the presence of starch in the diet, which favors the growth of amylolytic (gram-negative) bacteria and negatively affects fiber digestion due to the competition between these groups (STEENBAKKERS et al., 2008).

Pectin, in turn, is fermented by both bacteria and protozoa (DEHORITY, 1969), and the main bacteria involved in this process are *Butyrivibrio fibrisolvens, Prevotella ruminicola, bacteroides ruminicola and Lachnospira multiparus*. These bacteria produce and release pectinolytic enzymes into the rumen environment; pectin lyases are the primary enzymes that hydrolyze pectin into oligogalacturonides (DUSKOVA; MAROUNEK, 2001).

Species belonging to the genus Bacteroides play an important role in the degradation of non-structural carbohydrates, such as starch, in the rumen environment, through the action of the enzyme amylase (KAMRA, 2005).

In the 1960s, the proteolytic characteristics of species such as B*. amylophilus, B. ruminicola, Butyrivibrio sp. and S. ruminantium* had already been evidenced (ARCURI et al., 2011). However, most rumen bacteria depend on carbohydrates as their main source of energy, and some species cannot grow using amino acids as the only substrate. Many produce small amounts of proteolytic enzymes, which contribute to the extensive proteolytic activity in the rumen (NOCEK, 1988).

Rumen proteolytic bacteria are responsible for the degradation of proteins present in the diet, allowing the synthesis of short-chain volatile fatty acids (VFAs), which are used as a source of energy and ammonia by the animal (ARCURI et al., 2011).

The group of organisms capable of hydrolyzing lipids in the rumen is limited, due to the low oxide-reduction potential of the rumen environment, characteristic of anaerobic environments (DEHORITY, 2003). The bacterial species *Anaerobivrio lipolytica* is able to hydrolyze lipids, while using ribose, fructose, glycerol, and lactate as energy sources. The

other substrates are converted into acetate, propionate, and CO_2, while glycerol is transformed into propionate and succinate. All these processes result in the production of H_2 (STEWART et al., 1997).

Adhered to the rumen wall is a group of bacteria composed of facultative anaerobic bacteria, such as *actobacillus sp., Streptococcus sp.,* which digest withered epithelial cells and offer an important ureolytic activity. These microorganisms play an important role in maintaining low oxygen levels in the rumen, even though they represent only 1% of the total rumen microbiota (ARCURI et al., 2011).

Lactate is an intermediate product of rumen fermentation, in starchy diets, the population of this type of bacteria capable of using lactic acid is increased (COUNOTTE et al., 1981). *Megasphaera elsdenii is the main species responsible for the metabolization of lactic acid*, playing an important role in the prevention of acidosis during the adaptation period of diets rich in concentrate (COUNOTTE et al., 1981).

METHANE-PRODUCING BACTERIA (*ARCHAEA)*

Methane-producing bacteria in the rumen are an important group that regulate rumen fermentation through efficient H2 removal. They belong to the *domain Archaea* and the phylum *Euryarchaeota* (MORGAVI et al., 2010).

The removal of H2 through the reduction of CO2 to CH4 is important for the growth of other rumen microorganisms and for the efficient fermentation of substrates (YOKOYAMA; JOHNSON 1988). This group can act freely or associated with protozoa.

The production of methane as a final product of rumen fermentation is considered a loss of energy by ruminants, representing about 2-12% of the ingested food energy (MOHAMMED et al., 2004), in addition to contributing negatively to the greenhouse effect (GARNSWORTHY et al., 2012). Therefore, ruminal methanogens have attracted a lot of research attention in the last decade, with the aim of understanding their diversity, community structure, relationship with other rumen microorganisms and with feed efficiency, CH_4 emission in responses to dietary interventions.

The process of methanogenesis is necessary to maintain low concentrations of H+ in the rumen environment, using CO2 as an electron acceptor (BODAS et al., 2012).

Although H+ is one of the main end products of the fermentation of bacteria, protozoa, and fungi, it does not accumulate in the rumen because it is quickly used by some microorganisms that are part of the ecosystem. In the rumen, there is an interrelationship between H+ producing and using species that is called "H+ interspecies transfer. The production of methane in the rumen environment is a clear example of this

process, where there is an association between species that produce and use H+. Methane is generated by methanogens that use carbon dioxide and hydrogen (VAN ZIJDERVELD et al., 2011).

When H+ is not used by methanogens, NADH can be reoxidized by a dehydrogenase to produce ethanol or lactate. This process occurs rapidly in animals fed high amounts of fermentable sugars (MOSS et al 2000).

In addition to CO2 to produce CH4, these microorganisms can also degrade substrates containing methyl (CH3-) or acetyl ($CH3OO^{-),}$ such as methanol and acetate that act as electron receptors (LIU; WHITMAN 2008). In vivo studies have also shown that inhibition of methanogens decreases the acetate:propionate ratio, reflecting changes in fermentation to volatile fatty acids (VFA) (PATRA et al., 2012).

According to public databases, 90% of the identified ruminal methanogens belong to known genera, with *Methanobrevibacter* being represented by 63.2% of the sequences analyzed, followed by Methanomicrobium (7.7%), *Methanosphaera* (9.8%) and *Methanobacterium* (1.2%). The order *Thermoplasmatales*, previously called Rumen *Cluster C (RCC*), is represented by 7.4% of the total sequences analyzed (MATTHEWS et al., 2019).

RUMEN PROTOZOA

Protozoa are unicellular organisms, eukaryotes, with a cuticle and complex internal organization with a digestive tract containing the mouth, stomach, rectum, and anus. vary in size from 20 to 200 µm, being about 10 to 100 times larger than bacteria (DEHORITY, 2003) use bacteria as their source of amino acids and N (HUNGATE, 1966).

They have a macronucleus, micronucleus and contractile vacuoles. Contractile vacuoles can act in the excretion of gases and liquid waste products (HUNGATE, 1966). They reproduce by binary fission (WARNER, 1966). They were the first microorganisms discovered in the rumen around 1843 (HUNGATE, 1966). There are about 104 to 106 protozoan cells per gram of rumen content (DEHORITY, 2003).

Most protozoa present in the rumen are ciliated, however, flagellates are also present. Ciliates are divided into two groups (subclasses): *Holotricha and Entodiniomorpha*.

Entodinium is the most abundant genus in the rumen (IBRAHIM et al. 1970; DEARTH et al. 1974). For evolutionary reasons, ciliates have become highly specialized for the rumen ecosystem (DEHORITY, 2003), use carbohydrates (FINDLEY et al., 2011) and consequently release H2, used by methanogenic microorganisms (SKILLMAN et al., 2006; TYMENSEN et al., 2012).

They use phagocytosis on bacteria as a way to control the number of bacteria in the rumen (SERRANO et al., 2011). They have the ability to ingest and use starch, insoluble protein and also ferment carbohydrates and as a final result acetate, formate, butyrate, propionate, H2 and CO2 can be generated (ABRAR et al., 2016).

They are efficient in using high amounts of starch and at the same time are able to use their bodies to store it (WILLIAMS; COLEMAN, 1985). The use of this mechanism to engulf allows for the delay of fermentation by bacteria and the formation of acids that lower the pH (MACKIE et al., 1978).

Protozoa play a fundamental role in animals fed low-protein diets or subjected to short periods of restriction (YOKOYAMA; JOHNSON, 1988). They are capable of converting bacterial proteins into protozoan proteins, when they cannot efficiently utilize NH3 as a nitrogen source.

FUNGOS ANAERÓBIOS RUMINAIS

In the rumen environment, anaerobic fungi are recognized as being the main players in the degradation of plant biomass in the rumen (AKIN *et al*., 1988 ; LEE et al.; 2000). They belong to the phylum *Neocallimastigomycota, which* currently comprises six genera: *Neocallimastix, Piromyces, Caecomyces, Orpinomyces, Anaeromyces* different in morphological characteristics: morphology of the thallus and hyphae (rhizoidal vs. bulbous) and flagellation of the zoospore (monoflagellated vs. polyflagellate) and shape of the zoospore (ovoid, piriform, spherical, ellipsoid) (HO; BARR, 1995; OZKOSE *et al*., 2001).

The presence of fungi in the rumen was questioned, due to the belief that fungi were obligatory aerobes. However, Orpin (1977) evidenced that the cell wall of these organisms contained chitin, confirming their correct classification in the fungi kingdom. Since then, research has been developed to understand the mechanisms that allowed these fungi to survive in anaerobic environments.

Regarding the adaptations present in this type of fungus, the absence of mitochondria, cytochromes and other biochemical characteristics that are part of the oxidative phosphorylation pathway is included (YARLETT *et al*., 1986 ; YOUSSEF *et al.,* 2013). However, these anaerobic fungi have specialized organelles, called hydrogenosomes, that couple glucose metabolism to produce cellular energy in the absence of oxygen.

There is evidence to support the function of fungi as primary colonizers and digesters of fiber in the rumen. Zoospores develop into mycelia, which then envelop and break down the plant structure. As a result of their activity and growth, they penetrate and disrupt plant

tissue, which also increases the exposed area of the substrate, facilitating access to other fiber-using microorganisms in the rumen, such as bacteria and protozoa (YOUSSEF et al., 2013).

Anaerobic fungi are able to break down lignocellulosic material by expressing a variety of fiber-metabolizing enzymes in addition to the invasive growth of their vegetative mycelium. Free complexes and also multiple enzymes, such as cellulosomas, have been identified in ruminal fungal species for which genome sequence is available (YOUSSEF et al., 2013; GILMORE et al., 2015).

Rumen fungi have a close association with methanogens, a mutually beneficial relationship in which the metabolic activity of the latter maintains H2 at low levels, which favors the activity of hydrogenase in hydrogenosomes (BAUCHOP; MOUNTFORT, 1981)

NON-RUMEN ENVIRONMENT VIRUS

Despite being abundant in the rumen environment, viruses, especially bacteriophages, have little information about their interaction with other microorganisms present in the rumen. Pioneering studies on bacteriophages in the rumen have recognized different morphological types in both cattle and sheep (KLIEVE; BAUCHOP, 1988) belonging to three families of viruses, *Podoviridae, Myorividae, Syphorividae* (KLIEVE et al., 1996).

Bacteriophages play an important role in limiting the number of bacteria in the ecosystem, allowing the balance of rumen communities, through the natural selection of phage-resistant bacteria (MILLER et al., 2012).

They are present in an approximate amount of 109 particles per mL (TARAKANOV, 2006). They are facilitators of horizontal gene transfer (HGT) (RODRIGUEZ et al., 2009; ROHWER; THUBER, 2009). HGT is defined as a common and widespread phenomenon in microbial communities, contributing to the evolution of microorganisms in these places (KOONIN; WOLF, 2008; AMINOV, 2011).

Techniques for enteric methane mitigation include bacteriophage therapy (PATRA, 2012), so that bacteriophages are able to reach and lyse unwanted bacterial species (KLIEVE et al., 1999; BACH et al., 2002).

METAGENOMIC APPROACH IN THE RUMEN ENVIRONMENT

Since the development of the Hungate Technique, which allowed the cultivation of strictly anaerobic rumen bacteria outside the rumen (HUNGATE, 1950), rumen microbiology has experienced a significant advance. Although culture-based techniques have been

successful in isolating several microorganisms, due to the enormous diversity of species present in the rumen, these methods are not the most suitable for monitoring changes in the structure of microbial communities. The number of species of microorganisms isolated and characterized from the rumen is still considered low.

According to a study conducted by Han et al. (2015), who evaluated the diversity of rumen bacteria in goats before and after weaning, analyzing the genomic DNA of the microorganisms extracted from the rumen, it was observed that 72.14% of the identified bacteria could not be cultured. These results highlight the large number of bacterial species present in the rumen that have not yet been fully characterized.

However, with the expansion of molecular techniques, the use of high-performance sequencing, and the emergence of the so-called Next-Generation Sequencing techniques, as well as the development of bioinformatics tools, it has been possible to significantly expand knowledge about microbial diversity in different ecosystems (MATTHEWS et al., 2019).

Among the methods used to study microbial diversity, metagenomics stands out as an effective alternative, allowing more comprehensive access to the diversity of microorganisms present in the analyzed environment, enabling genomic studies of the microbiota without the need to cultivate the microorganisms (TESSLER et al., 2017).

Metagenomics refers to the technique of sequencing the whole genomic material of a sample. Crucially, by sequencing the genomes of all organisms present, rather than focusing on a single marker gene, it is possible to gain insight into gene function, genome structure and organization, as well as identify new genes and biocatalysts, and explore evolutionary relationships within the community (ROUMPEKA et al., 2017).

There are two main methods for studying the microbiome using high-throughput sequencing: marker gene sequencing and whole-genome metagenomics (WGS). In marker gene studies, generic primers are designed to amplify, by PCR, specific genes, such as 16S rRNA for bacteria and archaea, and 18S rRNA for fungi (ROUMPEKA et al., 2017).

The use of these techniques allows changes to be detected in individual species, provided that the appropriate primer is used. Quantification of species is also possible. However, only a small fraction of the rumen microbiota is represented by these enumerated species. Consequently, changes in species that are not quantified may not be detected (MIDDELBOS et al., 2007).

The main aspect that differentiates genomic sequencing from metagenomic sequencing is the need to isolate microorganisms under laboratory conditions before performing DNA sequencing in genomic sequencing. In contrast, metagenomics allows the

analysis of DNA sequences collected directly from the environmental sample, providing fundamental biological information for understanding the microbial community as a whole (MATTHEWS et al., 2019).

Whole Genome Shotgun Sequencing (WGS) is a technique that involves the complete fragmentation of DNA molecules collected directly from the environment into small pieces, which are sequenced completely randomly. In this way, it is possible not only to assess microbial diversity and the abundance of microorganisms in the respective environments, but also to assemble complete genomes, predict genes, identify enzymes and metabolic pathways, and quantify functional genomic elements (KUNIN et al., 2008; HUWS et al., 2018).

After the metagenomic sequencing of the sample, it is necessary to analyze the generated content, using bioinformatics tools, which help in the generation of large volumes of data. As reported by Dudhagara et al. (2015), there are several options of offline tools available that allow classifying metagenomic readings using a known dataset as a reference, such as Metagenomics Rapid Annotation software package, Software Integrated Microbial Genomes and Metagenomes, METAVIR, Metagenomics Reports, MyTaxa.

Integrated research using metagenomics, bioinformatics, nutrition, and statistical inferences has provided a unique opportunity for ruminant nutritionists and rumen microbiologists to work synergistically. This joint work aims to improve efficiency in the use of nutrients, minimize waste production and reduce methane and ammonia emissions, thus increasing the productivity of herds and reducing negative environmental impacts.

In a study conducted by Delgado et al. (2019), which evaluated the associations between rumen microbiota and traits related to feed efficiency in Holstein cattle through metagenomic sequencing, the results revealed that more efficient cows had a higher relative abundance of *Bacteroidetes and Prevotella*, and a lower abundance of *Firmicutes.* In addition, it was observed that *methanobacteria* and the genus *Methanobrevibacter* were less abundant in animals with higher feed efficiency. The study also identified differences in the composition of the microbiota of these animals, both at the taxonomic and genus levels.

The influence of the host on the rumen microbiome was highlighted in a study by Weimer et al. (2010). They observed that even after the exchange of almost all of the rumen content between cows, the bacterial composition was restored to the original conditions, including rumen pH values and volatile fatty acid concentrations. In a similar study conducted by Weimer et al. (2017) with low and high feed efficiency Holstein cows, the ability of hosts to regenerate the original rumen microbiota was again evidenced.

Wallace et al. (2015), when investigating the microbial differences that result in bovine phenotypes with high and low methane emission, observed that the abundance of the 16S rRNA gene, indicative of the predominantly *Methanobrevibacter archaea*, was 2.5 times higher in large methane emitters. On the other hand, bacteria of the *Succinivibrionaceae family*, belonging to the phylum *Proteobacteria,* were four times less abundant. Lower methane emissions were associated with a higher abundance of *Succinivibrionaceae,* as well as changes in acetate and hydrogen production, which resulted in lower methanogenesis.

In another study, Singh et al. (2014) evaluated the rumen microbiota of buffaloes as a genetic resource for the extraction of microbial enzymes applicable to the production of biofuels. They identified contigs that encode enzymes involved in the degradation of plant biomass, such as glycoside hydrolases, carbohydrate binding modules, glycosyltransferases, carbohydrate esterases, and polysaccharide lyases. These findings demonstrate that the rumen microbiome of buffaloes is rich in functional genes related to the degradation of polysaccharides, which suggests a great potential for the discovery of new molecules applicable to the biofuel industry.

Although fungi are the most studied microorganisms in relation to the enzymes responsible for the degradation of plant biomass, such as cellulases*, xylanases, mannanases, glucosidases and glucanases* (WHITE et al., 2014), almost all commercially available enzymes for the deconstruction of lignocellulosic material are derived from fungi. This is due to the higher production of enzymes by fungi compared to bacteria, facilitating their extraction and purification. Genera such as *Trichoderma, Penicillium, Aspergillus, Fusarium, and Humicola* are frequently mentioned in the literature, with Trichoderma being considered the most efficient in the production of cellulases and xylanases (LYND et al., 2002). Penicillium and Trichoderma are widely applied in the commercial production of lignocellulases (GUSAKOV, 2011).

Therefore, an in-depth understanding of the rumen ecosystem can only be achieved if all aspects pertinent to this microbiome are taken into account. The integration of genomic, metagenomic and nutritional resources will allow a more detailed interpretation of the interactions between rumen microorganisms. Studies in this area can help in the adoption of more efficient feeding strategies, resulting in higher animal productivity and lower methane emissions, thus contributing to sustainability in production systems.

FINAL CONSIDERATIONS

In view of the research mentioned above, it is concluded that metagenomics, by allowing the study of microbial communities without the need for isolation and cultivation in culture medium, provided a deeper understanding of the vast population of microorganisms present in the rumen and their role in the organism of ruminants.

The detailed study of these microorganisms enabled significant advances in feed conversion, reduction of methane emissions and the development of more effective therapeutic and nutritional strategies for the herd. This is especially relevant considering that many of these microorganisms were previously unknown or could not be grown in the laboratory.

REFERENCES

1. Abrar, A., et al. (2016). Diversity and fluctuation in ciliate protozoan population in the rumen of cattle. *Animal Science Journal*, 87(9), 1188-1192. https://doi.org/10.1111/asj.12591

2. Akin, D. E., & Benner, R. (1988). Degradation of polysaccharides and lignin by ruminal bacteria and fungi. *Applied and Environmental Microbiology*, 54(5), 1117-1125. https://doi.org/10.1128/aem.54.5.1117-1125.1988

3. Aminov, R. I. (2011). Horizontal gene exchange in environmental microbiota. *Frontiers in Microbiology*, 2(2), 158. https://doi.org/10.3389/fmicb.2011.00158

4. Arcuri, P. B., Lopes, F. C. F., & Carneiro, J. C. (2011). Microbiologia do rúmen. In T. T. Berchielli, A. V. Pires, & S. G. Oliveira (Eds.), *Nutrição de ruminantes* (Vol. 2, No. 4, pp. 115-147). Jaboticabal: Funep.

5. Bach, S. J., et al. (2002). Transmission and control of Escherichia coli O157: H7—a review. *Canadian Journal of Animal Science*, 82(4), 475-490. https://doi.org/10.4141/A02-021

6. Bauchop, T., & Mountfort, D. O. (1981). Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogens. *Applied and Environmental Microbiology*, 42(6), 1103–1110. https://doi.org/10.1128/aem.42.6.1103-1110.1981

7. Bodas, R., et al. (2012). Manipulation of rumen fermentation and methane production with plant secondary metabolites. *Animal Feed Science and Technology*, 176(1-4), 78-93. https://doi.org/10.1016/j.anifeedsci.2012.07.010

8. Bordim, S., et al. (2016). Microscópicos e eficientes: Importância dos microrganismos no ambiente ruminal. *Revista Brasileira de Zoociências*, 17(2).

9. Clarke, R. T. J. (1977). Protozoa in the rumen ecosystem. In R. T. J. Clarke & T. Bauchop (Eds.), *Microbial ecology of the gut* (pp. 251–275). Academic Press.

10. Counotte, G. H. M., et al. (1981). Role of Megasphaera elsdenii in the fermentation of DL-[2-13C] lactate in the rumen of dairy cattle. *Applied and Environmental Microbiology*, 42(4), 649-655. https://doi.org/10.1128/aem.42.4.649-655.1981

11. Czerkawski, J. W. (1986). *An introduction to rumen studies*. Pergamon Press.

12. Dearth, R. N., Dehority, B. A., & Potter, E. L. (1974). Rumen microbial numbers in lambs as affected by level of feed intake and dietary diethyletiboeatrol. *Journal of Animal Science*, 38, 991-996. https://doi.org/10.2527/jas1974.385991x

13. Dehority, B. A. (1969). Pectin-fermenting bacteria isolated from the bovine rumen. *Journal of Bacteriology*, 99(1), 189-196. https://doi.org/10.1128/jb.99.1.189-196.1969

14. Dehority, B. A. (2003). *Rumen microbiology*. Nottingham University Press.

15. Delgado, B., et al. (2019). Whole rumen metagenome sequencing allows classifying and predicting feed efficiency and intake levels in cattle. *Scientific Reports*, 9(1), 11. https://doi.org/10.1038/s41598-018-36673-w

16. Dudhagara, P., et al. (2015). Web resources for metagenomics studies. *Genomics, Proteomics & Bioinformatics*, 13(5), 296-303. https://doi.org/10.1016/j.gpb.2015.10.003

17. Duskova, D., & Marounek, M. (2001). Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rumen bacterium Lachnospira multiparus. *Letters in Applied Microbiology*, 33(1), 159-163. https://doi.org/10.1046/j.1472-765x.2001.00970.x

18. Findley, S. D., et al. (2011). Activity-based metagenomic screening and biochemical characterization of bovine ruminal protozoan glycoside hydrolases. *Applied and Environmental Microbiology*, 77(22), 8106-8113. https://doi.org/10.1128/AEM.05925-11

19. Fonty, G., et al. (2007). Establishment and development of ruminal hydrogenotrophs in methanogen-free lambs. *Applied and Environmental Microbiology*, 73(20), 6391-6403. https://doi.org/10.1128/AEM.00181-07

20. Garnsworthy, P. C., et al. (2012). Variation among individual dairy cows in methane measurements made on farm during milking. *Journal of Dairy Science*, 95(6), 3181-3189. https://doi.org/10.3168/jds.2011-4606

21. Gilmore, S. P., Henske, J. K., & O'Malley, M. A. (2015). Driving biomass breakdown through engineered cellulosomes. *Bioengineered*, 6(4), 204–208. https://doi.org/10.1080/21655979.2015.1060379

22. Gusakov, A. V. (2011). Alternatives to Trichoderma reesei in biofuel production. *Trends in Biotechnology*, 29(9), 419-425. https://doi.org/10.1016/j.tibtech.2011.04.004

23. Han, X., et al. (2015). Rumen bacterial diversity of 80 to 110-day-old goats using 16S rRNA sequencing. *PloS One*, 10(2), e0117811. https://doi.org/10.1371/journal.pone.0117811

24. Henderson, G., et al. (2013). Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. *PloS One*, 8(9), e74787. https://doi.org/10.1371/journal.pone.0074787

25. Henderson, G., et al. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, 5(1), 14567. https://doi.org/10.1038/srep14567

26. Ho, Y. W., & Barr, D. J. S. (1995). Classification of anaerobic gut fungi from herbivores with emphasis on rumen fungi from Malaysia. *Mycologia*, 87(5), 655-677. https://doi.org/10.1080/00275514.1995.12026582

27. Hungate, R. E. (1950). Mutualisms in protozoa. *Annual Review of Microbiology*, 4(1), 53-66. https://doi.org/10.1146/annurev.mi.04.100150.000413

28. Hungate, R. E. (1966). *The rumen and its microbes*. New York: Academic Press. Department of Bacteriology and Agricultural Experiment Station. University of California.

HUWS, S. A. et al. Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. Frontiers in microbiology, v. 9, p. 2161, 2018. DOI: https://doi.org/10.3389/fmicb.2018.02161

29. Ibrahim, E. A., Ingalls, J. R., & Stanger, N. E. (1970). Effect of dietary diethylstilboestrol on populations of ciliate protozoa in dairy cattle. *Journal of Animal Science, 50*(1), 101-106. https://doi.org/10.4141/cjas70-012

30. Kamra, D. N. (2005). Rumen microbial ecosystem. *Current Science, 89*(1), 124-134.

31. Kleen, J. L., et al. (2003). Subacute ruminal acidosis (SARA): a review. *Journal of Veterinary Medicine Series A, 50*(8), 406-414. https://doi.org/10.1046/j.1439-0442.2003.00569.x

32. Klieve, A. V., & Bauchop, T. (1988). Morphological diversity of ruminal bacteriophages from sheep and cattle. *Applied and Environmental Microbiology, 54*(6), 1637-1641. https://doi.org/10.1128/aem.54.6.1637-1641.1988

33. Klieve, A. V., et al. (1999). Genetic homogeneity and phage susceptibility of ruminal strains of *Streptococcus bovis* isolated in Australia. *Letters in Applied Microbiology, 29*(2), 108-112. https://doi.org/10.1046/j.1365-2672.1999.00596.x

34. Klieve, A. V., Swain, R. A., & Nolan, J. V. (1996). Bacteriophages in the rumen: types present, population size and implications for the efficiency of feed utilisation. In *Proceedings of the Australian Society of Animal Production* (pp. 92-94).

35. Koonin, E. V., & Wolf, Y. I. (2008). Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. *Nucleic Acids Research, 36*(21), 6688-6719. https://doi.org/10.1093/nar/gkn668

36. Kozloski, G. V. (2011). *Bioquímica dos ruminantes* (3rd ed.). Santa Maria: UFSM.

37. Kunin, V., et al. (2008). A bioinformatician's guide to metagenomics. *Microbiology and Molecular Biology Reviews, 72*(4), 557-578. https://doi.org/10.1128/mmbr.00009-08

38. Lee, W. L., Há, J. K., & Cheng, K. J. (2000). Relative contributions of bacteria, protozoa, and fungi to in vitro degradation of orchard grass cell walls and their interactions. *Applied and Environmental Microbiology, 66*(9), 3807–3813. https://doi.org/10.1128/AEM.66.9.3807-3813.2000

39. Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences, 1125*(1), 171-189. https://doi.org/10.1196/annals.1419.019

40. Lynd, L. R., et al. (2008). How biotech can transform biofuels. *Nature Biotechnology, 26*(2), 169-172. https://doi.org/10.1038/nbt0208-169

41. Mackie, R. I., et al. (1978). Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. *The Journal of Agricultural Science, 90*(2), 241-254. https://doi.org/10.1017/S0021859600055313

42. Matthews, C., et al. (2019). The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes, 10*(2), 115-132. https://doi.org/10.1080/19490976.2018.1505176

43. McAllister, T. A., et al. (1994). Microbial attachment and feed digestion in the rumen. *Journal of Animal Science, 72*(11), 3004-3018. https://doi.org/10.2527/1994.72113004x

44. McCann, J. C., Wickersham, T. A., & Loor, J. J. (2014). High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. *Bioinformatics and Biology, 3*(8), 109–125. https://doi.org/10.4137/BBI.S15389

45. Menezes, A. B., et al. (2011). Microbiome analysis of dairy cows fed pasture or total mixed ration diets. *FEMS Microbiology Ecology, 78*(2), 256-265. https://doi.org/10.1111/j.1574-6941.2011.01151.x

46. Middelbos, I. S., et al. (2007). A dose-response evaluation of spray-dried yeast cell wall supplementation of diets fed to adult dogs: effects on nutrient digestibility, immune indices, and fecal microbial populations. *Journal of Animal Science, 85*(11), 3022-3032. https://doi.org/10.2527/jas.2007-0079

47. Miller, M. E. B., et al. (2012). Phage–bacteria relationships and CRISPR elements revealed by a metagenomic survey of the rumen microbiome. *Environmental Microbiology, 14*(1), 207-227. https://doi.org/10.1111/j.1462-2920.2011.02593.x

48. Mizrahi, I. (2013). Rumen symbioses. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The Prokaryotes: Prokaryotic Biology and Symbiotic Associations* (pp. 533–544). https://doi.org/10.1007/978-3-642-30194-0_1

49. Mohammed, N., et al. (2004). Effects of ionophores, vitamin B6 and distiller's grains on in vitro tryptophan biosynthesis from indolepyruvic acid, and production of other related compounds by ruminal bacteria and protozoa. *Animal Feed Science and Technology, 116*(3-4), 301-311. https://doi.org/10.1016/j.anifeedsci.2004.07.017

50. Mohammed, R., et al. (2014). Bacterial communities in the rumen of Holstein heifers differ when fed orchardgrass as pasture vs. hay. *Frontiers in Microbiology, 5*, 689. https://doi.org/10.3389/fmicb.2014.00689

51. Morgavi, D. P., et al. (2010). Microbial ecosystem and methanogenesis in ruminants. *Animal, 4*(7), 1024-1036. https://doi.org/10.1017/S1751731110000546

52. Morgavi, D. P., et al. (2013). Rumen microbial (meta)genomics and its application to ruminant production. *Animal, 7*(s1), 184-201. https://doi.org/10.1017/S1751731112000419

53. Moss, A. R., Jouany, J. P., & Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. *Ann. Zootech, 49*, 231-253. https://doi.org/10.1051/animres:2000119

54. Nocek, J. E., & Russell, J. B. (1988). Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. *J. Dairy Sci., 71*, 2070-2107. https://doi.org/10.3168/jds.S0022-0302(88)79782-9

55. Orpin, C. G. (1977). The occurrence of chitin in the cell walls of the rumen organisms *Neocallimastix frontalis*, *Piromonas communis*, and *Sphaeromonas communis*. *J Gen Microbiol, 99*(1), 215-218.

56. Ørskov, E. R., & Tyle, M. (1990). *Energy nutrition in ruminants*. Cambridge: Elsevier Science Publishers.
57. OZKOSE, E. (2001). *Molecular Morphology and Ecology of Anaerobic Fungi*. Tese de Doutorado, Universidade de Gales, Aberystwyth.

58. PATRA, A. K. (2012). Enteric methane mitigation technologies for ruminant livestock: A synthesis of current research and future directions. *Environmental Monitoring and Assessment, 184*(4), 1929–1952. https://doi.org/10.1007/s10661-011-2090-y

59. PATRA, A. K., & YU, Z. (2012). Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. *Applied and Environmental Microbiology, 78*(12), 4271–4280. https://doi.org/10.1128/AEM.00309-12

60. PURVES, W. K., et al. (2002). *Vida: a ciência da biologia* (6ª ed.). Porto Alegre: Artmed.

61. RIBEIRO, G. O., et al. (2017). Repeated inoculation of cattle rumen with bison rumen contents alters the rumen microbiome and improves nitrogen digestibility in cattle. *Scientific Reports, 7*(1), 1276. https://doi.org/10.1038/s41598-017-01269-3

62. RODRIGUEZ, F. V., et al. (2009). Explaining microbial population genomics through phage predation. *Nature Precedings*, 1-1. https://doi.org/10.1038/npre.2009.3489.1

63. ROHWER, F., & THURBER, R. V. (2009). Viruses manipulate the marine environment. *Nature, 459*(7244), 207-212. https://doi.org/10.1038/nature08060

64. ROUMPEKA, D. D., ESCALETTES, F., & FOTHERINGHAM, I. (2017). A review of bioinformatics tools for bio-prospecting from metagenomic sequence data. *Frontiers in Genetics, 8*(2), 23-36. https://doi.org/10.3389/fgene.2017.00023

65. RUSSEL, J. B., & WILSON, D. B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of Dairy Science, 79*(8), 1503-1509. https://doi.org/10.3168/jds.S0022-0302(96)76510-4

66. RUSTOMO, B., et al. (2006). Acidogenic value of feeds I. The relationship between the acidogenic value of feeds and in vitro ruminal pH changes. *Canadian Journal of Animal Science, 86*(1), 109-117. https://doi.org/10.4141/A04-074

67. RUSTOMO, B., et al. (2006). Effects of rumen acid load from feed and forage particle size on ruminal pH and dry matter intake in the lactating dairy cow. *Journal of Dairy Science, 89*(12), 4758-4768. https://doi.org/10.3168/jds.S0022-0302(06)72525-5

68. SERRANO, R. D. C., & SIERRA, L. M. P. (2011). Técnicas de quantificação da síntese microbiana no rúmen: Uma revisão. *CES Medicina Veterinaria y Zootecnia, 6*(1), 46-53.

69. SINGH, K. M., et al. (2014). High potential source for biomass degradation enzyme discovery and environmental aspects revealed through metagenomics of Indian buffalo rumen. *BioMed Research International, 2014*, 267189. https://doi.org/10.1155/2014/267189

70. SKILLMAN, L. C., et al. (2006). Development and validation of a real-time PCR method to quantify rumen protozoa and examination of variability between Entodinium populations in sheep offered a hay-based diet. *Applied and Environmental Microbiology, 72*(1), 200-206. https://doi.org/10.1128/AEM.72.1.200-206.2006

71. STEENBAKKERS, P. J. M., et al. (2008). A serpin in the cellulosome of the anaerobic fungus Piromyces sp. strain E2. *Mycological Research, 112*(8), 999-1006. https://doi.org/10.1016/j.mycres.2008.01.021

72. STEWART, C. S., FLINT, H. J., & BRYANT, M. P. (1997). The rumen bacteria. In *The rumen microbial ecosystem* (pp. 10-72). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-009-1453-7_2

73. SUNDSET, M. A., et al. (2007). Novel rumen bacterial diversity in two geographically separated sub-species of reindeer. *Microbial Ecology, 54*, 424-438. https://doi.org/10.1007/s00248-007-9254-x

74. TARAKANOV, B. V. (2006). *The Phenomenon of Bacteriophagy in the Rumen of Ruminants*. Moscow: Nauchny mir.

75. TESSLER, M., et al. (2017). Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. *Scientific Reports, 7*(1), 6589. https://doi.org/10.1038/s41598-017-06665-3

76. TYMENSEN, L., BARKLEY, C., & MCALLISTER, T. A. (2012). Diversidade relativa e análise da estrutura da comunidade de protozoários ruminais de acordo com T-RFLP e métodos microscópicos. *Journal of Microbiological Methods, 88*(1), 1–6.

77. UYENO, Y., SEKIGUCHI, Y., & KAMAGATA, Y. (2010). rRNA-based analysis to monitor succession of faecal bacterial communities in Holstein calves. *Letters in Applied Microbiology, 51*(6), 570–577. https://doi.org/10.1111/j.1472-765X.2010.02937.x

78. VAN SOEST, P. J. (1994). *Nutritional ecology of the ruminant* (2. ed.). Ithaca: Cornell University Press.

79. VAN ZIJDERVELD, S. M., et al. (2011). Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. *Journal of Dairy Science, 94*(8), 4028-4038. https://doi.org/10.3168/jds.2011-4236

80. WALLACE, R. J., et al. (2015). Contribution of fungi, archaea, protozoa and ruminal bacteria to the suppression of methane caused by diets supplemented with oilseeds. *BMC Genomics, 16*(2), 839.

81. WANG, S., et al. (2017). Contribuição de fungos, arquéias, protozoários e bactérias ruminais para a supressão de metano causada por dietas suplementadas com sementes oleaginosas. *Frontiers in Microbiology, 8*, 1864. https://doi.org/10.3389/fmicb.2017.01864

82. WARNER, A. C. I. (1966). Diurnal changes in the concentrations of micro-organisms in the rumens of sheep fed to appetite in pens or at pasture. *Microbiology, 45*(2), 243-251. https://doi.org/10.1099/00221287-45-2-243

83. WEIMER, P. J., et al. (2010). Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. *Journal of Dairy Science, 93*(12), 5902-5912. https://doi.org/10.3168/jds.2010-3500

84. WEIMER, P. J., et al. (2017). Transient changes in milk production efficiency and bacterial community composition resulting from near-total exchange of ruminal contents between high-and low-efficiency Holstein cows. *Journal of Dairy Science, 100*(9), 7165-7182. https://doi.org/10.3168/jds.2017-12746

85. WHITE, B. A., et al. (2014). Biomass utilization by gut microbiomes. *Annual Review of Microbiology, 68*(1), 279-296. https://doi.org/10.1146/annurev-micro-092412-155618

86. WILLIAMS, A. G., & COLEMAN, G. S. (1985). Hemicellulose-degrading enzymes in rumen ciliate protozoa. *Current Microbiology, 12*, 85-90. https://doi.org/10.1007/BF01567397

87. YARLETT, N., et al. (1986). Hydrogenosomes in the rumen fungus Neocallimastix patriciarum. *Biochemical Journal, 236*(3), 729-739. https://doi.org/10.1042/bj2360729

88. YOKOYAMA, M. T., & JOHNSON, K. A. (1988). Microbiología del rumen e intestino. In D. C. CHURCH (Ed.), *El Ruminant Fisiologia Digestiva e Nutrición* (Cap. 7, pp. 137-157). Zaragoza: Editorial Acribia, S.A.

89. YOUSSEF, N. H., et al. (2013). The genome of the anaerobic fungus Orpinomyces sp. strain C1A reveals the unique evolutionary history of a remarkable plant biomass degrader. *Applied and Environmental Microbiology, 79*(15), 4620-4634. https://doi.org/10.1128/AEM.00821-13