DOI: https://doi.org/10.21323/2414-438X-2026-11-1-45-56
Received 19.12. 2025
Accepted in revised 07.03.2026
Accepted for publication 12.03.2026

Available online at https://www.meatjournal.ru/jour
Review article
Open Access

THE RUMEN MICROBIOME: A BRIEF REVIEW

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Keywords: *rumen, rumen microbiology, microbiome, anaerobic bacteria, fungi*

Abstract

The rumen microbiome is a complex dynamic community of microorganisms that participate in digestion and provide an utmost impact on the cattle efficiency. Despite significant advancements in microbiome research, understanding the formation and management of the rumen microbiome still remains a significant scientific challenge. This topic holds both economic and environmental importance. The purpose of this literature review is to analyze and arrange structurally the current knowledge about the composition and functions of the rumen microbiome for further application of this knowledge in livestock farming. The article emphasizes that diet is an important factor that defines the composition and variability of the microbiome. This work demonstrates that the main functions of the rumen are provided by mutually coordinated groups of bacteria, methanogenic archaea, bacteriophages, protozoa, and fungi. The review covers various microbial groups in the rumen and their functions, as well as the factors that influence changes in the microbial community. Traditional methods of studying the rumen microbiome, based on culture-based techniques, have been significantly improved by the introduction of modern sequencing technologies. The review also explores the history of microbiome research and the "Hungate 1000 collection" project. This work demonstrates how metagenomics, metatranscriptomics, and metaproteomics have not only discovered numerous previously unknown microorganisms, but also provided insights into their functional roles. The systematization of knowledge presented in this review provides a comprehensive understanding of the rumen microbiome as a dynamic object for innovation targeted at improving the productivity, sustainability, and environmental safety of modern livestock farming.

For citation: **Novikova, A.A., Maksimenko, A.A., Sidorenko, A.V., Podvolotskaya, A.B., Tekutyeva, L.A.** (2026). The rumen microbiome: A brief review. *Theory and Practice of Meat Processing*, 11(1), 45–56. https://doi.org/10.21323/2414-438X-2026-11-1-45-56

Funding:

This work was supported by the Ministry of Education and Science of the Russian Federation (Agreement on the provision of grants from the federal budget in the form of subsidies in accordance with paragraph 4 of Article 78.1 of the Budget Code of the Russian Federation No. 075-15-2022-1143 dated July 7, 2022). The Institute of Biotechnology, Bioengineering, and Food Systems, Far Eastern Federal University, advanced engineering school.

Introduction

The rumen microbiome is a complex and dynamic ecosystem that in major scope participates in the digestion and metabolism of ruminants. Its study is of utmost importance for improving livestock efficiency and reducing environmental impacts.

Recent researches have significantly expanded our understanding of the versatile diversity and functions of microorganisms in a rumen. For example, the rumen microbial community of cattle has been analyzed across various geographic locations [1] and periods (such as the late perinatal period [2]). The importance of protozoa in shaping rumen ecosystems and their influence on metabolism has been noted [3], and changes in composition of fungal cultures community have been examined with various diets [4] and among various cattle [5]. Also, a meta-analysis has identified the main microbiota of the rumen epithelium [6].

The rumen cavity is divided with numerous internal and external structures, thus forming five separate rumen bags: the cephalic, dorsal, caudal-dorsal cecum, ventral, and caudal-ventral cecum. These names refer to their locations. In combination with environmental factors, these distinct bags create distinct ecological niches within a rumen [6–8]. The ventral compartments contain the largest amount of liquid chyme components, while the dorsal compartments feature more gaseous space and solid feed particles. There is evidence of differences in the structure of specific microbial communities in different fractions of the rumen contents, which is associated with differences in the composition of these contents [9,10].

The rumen microbiome is a complex system of microorganisms that interact with each other in the same environment. These include bacteria, protozoa, archaea, fungi, and viruses [11]. The ruminants' rumen microbiota metabolizes a variety of feed components, including cellulose and starch, as substrates for growth. This process produces volatile fatty acids, which are the primary energy source (approximately 70 %) for the host animal and which are absorbed through the rumen epithelium. Furthermore, microbial growth in the rumen leads to the formation of microbial biomass, which serves as an important source of metabolizable protein for the host animal due to its high concentration, optimal composition of amino acids, and good digestibility [12].

In recent decades researches of the rumen microbiome has focused primarily on bacteria, archaea, protozoa, and, in lesser extent, on fungi. Rumen viruses, which remained poorly studied for pretty long time, are now attracting increased scientific attention due to their potential applications in biotechnology and their possible impact on the ruminants' health and productivity [12].

Recent achievements in molecular techniques, including high-throughput sequencing and multi-omics approaches, have revolutionized our understanding of the rumen microbiome. These methods have revealed a greater diversity of microorganisms and allowed for a better understanding of their functional capabilities [13]. For example, studies have shown that the rumen microbiome can significantly influence feed efficiency, methane emissions, and milk composition in dairy cattle [14].

The rumen microbiome is very diverse and dynamic; it is influenced by various factors such as diet, age, and host animal's genetics. Understanding these interactions is crucial for developing rumen ecosystem management strategies to enhance cattle productivity and reduce impacts of environmental factors. For example, recent studies have been aimed to identification and isolating of potential probiotic strains from the rumen that are able to improve nutrient digestion and suppress pathogenic bacteria growth [15].

Researches of the rumen microbiome have enormous importance for the sustainable development of livestock production. They allow developing the new feeding strategies, improving animal health, and reducing greenhouse gas emissions. In the future, studying the rumen microbiome is expected to help develop more effective probiotics, methods for methanogenesis reducing, and to optimize using of feed resources.

Materials and methods

The literature review was composed on the basis of search of data published before 2025 in well-known scientific databases, such as PubMed, Web of Science, and ScienceDirect. A variety of keywords were used for the search, including "rumen", "rumen microbiome", "rumen bacteria", "ruminal phages", "rumen archaea", "rumen protozoa", "rumen methanogenic archaea", "rumen fungi", and "Hungate 1000". Then the literature from the scientific databases was thoroughly classified and reviewed for its relevance with the objectives of this study.

Evolution of rumen microbiome research technologies

Understanding the rumen microbiome is important for developing methods for producing food from ruminants. Rumen research technology has evolved over time, driven by technological and scientific progress. In the last century compiling of the Hungate 1000 collection[1] began worldwide, providing a better understanding of the microbiome picture. The creation of a shared catalogue now makes it possible to trace the genome of a cultured isolate without fearing of cross-species contamination or the integration of fragments from other species. Genome sequencing technologies have provided a broader view of protein functions, along with the potential to predict their structure. Sequencing has also enabled analysis and clearer evolutionary correlations, provided more data for taxonomy and classification, and enabled comparison of rumen functions, taxa, and metabolism across various countries [16].

As in many studies, rumen researches started small. In the 19th and 20th centuries, the majority of researches were biochemical and morphological ones. Researches were conducted all over the world and covered various areas of interest: from the study of acidity to the study of microorganisms. In 1939 Monroe and Perkins measured pH values of rumen components in cows with rumen fistula [17]. While studying the microbiome, in 1942 and 1943, Hungate conducted studies of the rumen when he discovered that some protozoa, such as *Diplodinium maggii, D. multivesciculatum, D. denticulatum* in the rumen digest cellulose [18,19]. Various ruminants were under research. In 1947, Gall and colleagues compared rumen bacterial communities in sheep and calves, and examined the differences in the bacterial population under different modes of feeding [20]. In 1947, Hungate [21] was already engaged in the cultivation of cellulose-digesting rumen microorganisms. In 1960, he published an article discussing the microbial ecology of the rumen [22], where he described the correlation between bacteria and protozoa.

A lot of previous researches were conducted through culturing and maintaining cultures, which process was able not only influence on certain microorganisms, but also alter them. However, culturing is necessary before sequencing, as sometimes it is not possible to isolate and culture a microorganism outside a body. It is known that not all rumen microorganisms are culturable.

Since the last century, with each study, data on the microbiome has expanded, and now we have a certain picture of a microbiome concept in which bacteria, fungi, protozoa, viruses, bacteriophages, and amoebae coexist. In 1995, the first bacterial genomes (human pathogens) of *Haemophilus influenzae* Rd [23] and *Mycoplasma genitalium* [24] were sequenced, and only eight years later, in 2003, the first sequenced and analyzed genome of a rumen microorganism — *Wolinella* (*Vibrio*) *succinogenes* DSMZ 1740 — was published [25].

When Illumina technology appeared (developed by the same name company, founded in 1998), the laboratories began using it for their researches purposes. The company Pacific Biosciences of California, Inc. also became known for its PacBio product, which also enabled genome sequencing. It was Illumina, along with PacBio, that formed the basis for the creation of the "Hungate 1000 Collection" catalogue. The availability of sequencing allowed defining

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[1] "Hungate 1000 collection". Retrieved from www.hungate1000.org.nz Accessed Novemder 20, 2025.

the correlation of bacterial and archaeal communities with important rumen functions [6,26].

PacBio and Illumina can be used together in researches, such as the one conducted by Brede et al. [26], where they used the technologies to confirm complete recovery of the bacterial community after subacute rumen acidosis.

Since the early 2000s, the genome has been actively analyzed, and in 2018, the largest dataset — almost 500 genomes from the “Hungate 1000 Collection” — was published. Since then, the number of completed genome sequences has only grown. The project “Hungate 1000 collection” included genomes sequenced with the help of Illumina and PacBio technologies. The resulting collection included several genomes of prevalent species and isolates representing a variety of the cultured organisms. Although most samples were obtained from cattle, the collection also included genomes of bacteria isolated from non-ruminants, as these isolates are known to also inhabit the rumen [27]. As of 2018, the collection contained 27,755 putative genes engaged in carbohydrate breakdown, and scientists also expressed 90 candidate proteins. Of these data, 57 % were enzymatic, that showed positive activity toward cellulosic substrates. Also 15 uncultured microbial genomes were also included into the collection. In total, numerous findings and datasets have been collected that are valuable for analyzing correlations in the rumen microbiome [28]. Similar isolates were also detected in both rumen fluid samples and those collected from the human intestine. There were several of those isolates at the time of the study [29]. This suggested that these isolates possess similar functions for both organisms.

The comparative genomic analysis was conducted, and it revealed that most genes encoding growth hormone were acquired by rumen bacteria through horizontal gene transfer [30]. Meta-omics technologies have provided better understanding of metabolic activities, rumen community structure, and metabolic potential [31].

For functional assessment of the rumen microbiome, Wilkinson et al. in 2018 developed CowPI — a version of PICRUSt software [32]. CowPI predicts functional profiles that are compliant with assessments for both metagenomic and transcriptomic datasets. Today and further in the future the scientists will most likely use both the latest sequencing techniques and microbiological and biochemical methods developed in the last century.

Rumen microbiome

The rumen microbiome includes anaerobic bacteria, protozoa, archaea, fungi, and bacteriophages. Microbial activity in the first two sections of the digestive tract, the rumen and reticulum, satisfies most of the ruminants’ energy needs and plays a key role in the feed materials breakdown [33].

Bacteria are the most abundant microorganisms reaching concentrations of 10^{10}–10^{11} cells/mL of rumen fluid [34]. The major bacterial phyla are *Bacteroidetes* and *Firmicutes*, followed by *Proteobacteria* [35]. At the genus level, *Prevotella*, *Ruminococcus*, *Butyrivibrio*, and others predominate [2].

There are data on the rumen bacterial community that prove that specific community members are associated with milk yield efficiency in dairy cows during two lactation cycles [36]. During the experiment, it was observed that the most common genera detected in cows were *Succiniclasticum* (2.28 %), *Coprococcus* (2.29 %), *Ruminococcus* (2.35 %), *Butyrivibrio* (2.38 %), *Prevotella* (40.15 %), and the most common phyla were *Tenericutes* (2.17 %), *Proteobacteria* (5.67 %), with the highest rates for *Firmicutes* (39.32 %), *Bacteroidetes* (49.42 %).

Studies were also conducted in which the predominant identified phyla were *Fusobacteria* (1.1 %), *Synergistetes* (1.5 %), *Actinobacteria* (3.5 %), *Proteobacteria* (11.1 %), *Bacteroidetes* (30.9 %), and *Firmicutes* (51.9 %).

Cattle do not have genes that encode enzymes necessary for the breakdown of carbohydrates and rupture of glycosidic bonds in plant cell walls (cellulose, hemicellulose, lignin, pectin, etc.), so rumen microorganisms play a crucial role in these processes. For example, anaerobic bacteria such as *Ruminococcus*, *Fibrobacter, Megasphaera*, *Streptococcus*, and *Escherichia* participate actively in cellulose breakdown [37].

Henderson et al. [38] studied microbial communities in camelids and ruminants taking into consideration their geographic location and diet. They found that similar bacteria and archaea dominated in most samples, while protozoan communities were more diverse. In over 90 % of studies, the main bacterial groups were *Clostridiales, Ruminococcus, Bacteroidales, Lachnospiraceae, Ruminococcaceae, Butyrivibrio,* and *Prevotella*. These groups comprise a core of the rumen bacterial microbiome, but some of them still remain poorly studied.

Protozoa can comprise up to 50 % of the rumen microbial biomass, but their study is challenged by the lack of reference genomic sequences and the complexity of standard methods of analysis.

Functional variations in the rumen microbiome arise from differences between microbial communities which run specialized functions in different parts of the rumen. The microbial population in the gastrointestinal tract not only affects feed efficiency and feed digestion, but also the condition of immune system and overall behavior of cattle [39]. An increase in pathogenic bacteria in the gastrointestinal tract can impair animal behavior and reduce feed intake. Microbial therapy of the gastrointestinal tract can improve the health and overall well-being of cattle. Three types of therapy are used to modulate the microbial community in the gastrointestinal tract: intestinal microbiota transplantation, probiotics administration, and using of this type of therapy [40].

There are three main microbial communities in the rumen: 1) planktonic (free-floating in rumen fluid), 2) fiber-attached, and 3) epimural (associated with the rumen wall) [41,42]. The differences and modes of interaction between planktonic and fiber-attached communities have been extensively studied [42]. However, few studies have been conducted on rumen epimural communities. This may be due to their lower relative abundance and density compared to the rest of the rumen, as well as difficulties in sampling [42]. However, epimural communities, despite their lower abundance, perform specialized functions that are critical for maintaining a healthy rumen environment. These functions include host epithelial tissue recycling, oxygen uptake, and urea transport [6,43]. Epimural microorganisms localize along the rumen epithelium, allowing them to influence host gene expression through host-microbiome interactions [43–45].

Studies have shown that diet significantly influences rumen bacterial and archaeal communities. De Menezes et al. [46] found significant differences in bacterial communities between complete and pasture feeding, as well as between solid and liquid rumen contents. For example, higher abundance of members of the *Fibrobacteraceae* family was found in solid samples of total ration, and members of the propionate-producing *Veillonellaceae* family were found in samples from pasture-fed diets [46].

The metabolome of the rumen and reticulum is different, although both of them are the lower compartments of the rumen. Studies of the metabolome and metabolism in the five rumen compartments can contribute to improved cattle husbandry efficiency by providing the understanding how feed is processed in all compartments of the rumen.

The fermentation compartments and gastric compartments include the rumen, reticulum, abomasum, and reticulum-rennet. In a study of beef bulls, the majority of operational taxonomic units (hereinafter referred to as OTUs) related to tissue samples were found in the chyme. From 38 to 49 % of the OTUs found in the reticulum, rumen, abomasum, and reticulum-rennet of this bull were consistent across the samples of its tissue and chyme. The combination of physiological, anatomical, and microbial differences between rumen compartments highlights the need for analysis of the entire rumen to obtain a more comprehensive picture of the microbiome [47].

Despite significant progress, some aspects of the rumen microbiome still remain poorly researched. Key gaps in rumen microbiome data relate to the technical challenges of sequencing and analyzing eukaryotic genomes [1]. The role of eukaryotic microorganisms (protozoa, fungi) in the rumen ecosystem also requires further investigation.

Future research should aim to better understanding the interactions between various microbial communities, their impact on animal health and yield, and the development of microbiome modulation strategies to improve livestock husbandry efficiency.

Rumen bacteria

Bacteria are the dominant microorganisms in the forestomachs of the ruminants, with their total count reaching approximately 10^{10}–10^{11} cells/mL of rumen fluid, and their species diversity counts for over 200 species [34]. The composition of rumen bacteria is determined by a number of factors, including energy requirements, substrate preferences, and their tolerance to metabolic end products [48].

Previously, studies of rumen fluid in dairy cows were conducted during two lactation cycles [36]. The most common phyla were *Bacteroidetes* (49.42 %) and *Firmicutes* (39.32 %), and the most common genus was *Prevotella* (40.15 %). The study concluded that the rumen bacterial community is dynamic in terms of its diversity and composition, while specific community members are responsible for high and low milk yield [36]. Differences between the rumen microbiota and fecal microbiota can account for approximately 60 %. Most studies are conducted using the rumen rather than feces. In the other research of fecal microbiome, the majority of bacterial isolates were *Firmicutes* (51.9 %) and *Bacteroidetes* (45.8 %) [49].

Sequencing technologies have revealed that *Prevotella*, *Butyrivibrio*, and *Ruminococcus* are the most common bacteria in the rumen. Variations in host cattle diet provide a significant impact on the structure of the microbial community. In particular, dietary diversity has proved to increase microbial diversity [1].

Dairy cows primarily consume vegetation-based diets. There carbohydrates (starch and cellulose) serve as the main source of energy. Digestion of these carbohydrates occurs in the rumen due to digestive enzymes and microorganisms. These bacteria are in charge for the breakdown of cellulose and hemicellulose, which are the main components of plant fiber [50].

The capability to degrade cellulose depends largely on some factors like feed type, crop maturity, and the availability of cellulolytic bacterial communities [34]. Cellulose consists of β-1,4-linked glucose residues, whereas hemicellulose consists primarily of xylans, which are composed of β-1,4-linked xylose residues. Xylan is substituted with acetyl, arabinose, and glucuronic residues. The degradation of this complex plant polymer matrix requires the synchronous action of a wide range of hydrolytic enzymes [50].

During the study by Hungate in 1950 [51], cellulolytic bacteria were identified, the most common of which were classified as *Bacteroides succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*. Six years later another study identified another microorganism — *Butyrivibrio fibrisolvens* — but it was weak cellulolytic [52].

Fibrobacter succinogenes and *Ruminococcus albus* [53] bacteria demonstrated their efficiency in breaking down cellulose. Their ability to digest cellulose is much higher than that of other cellulolytic bacteria. This is likely due to the fact that *F. succinogenes* and *R. albus* feature several genes which encode enzymes involved in cellulose breakdown. The end products of fermentation produced by these cellulolytic bacteria are CO2, propionate, acetate, and butyrate. Additionally, lactic acid, succinic acid, hydrogen, ethanol, and formic acid are also fermentation products but are quickly utilized by other bacteria [53].

Strain of *R. albus 7*, and some others with bright yellow-lemon color of its colonies (on a complex medium with addition of 0.4 % cellulose) break down cellulose better than strain 8, which has white colonies [54].

Starch is an important component of ruminants' diets, and high-grain content diets significantly increase its presence in the rumen. Low amount of *Streptococcus bovis*, an amylolytic bacterium, is most often found in cows fed with high-forage diets or in cows adapted to grain-based diets for some time. However, its amount increases significantly in non-adapted cows fed with high-grain content diets [55].

S. bovis feature a lower optimal pH for growth than many other bacteria. The increase in its numbers after high-grain content diets is explained by a sharp increase in rumen glucose levels and the death of protozoa because of higher acidic environment created by such diets.

Some anaerobic bacteria are capable of obtaining energy by breaking down pectin. The most important pectolytic species include *Butyrivibrio fibrisolvens*, *Lachnospira multiparus*, and *Prevotella ruminicola*. These species are capable of breaking down pectin into oligogalacturonides, this way releasing large amounts of acetate, a volatile fatty acid important for cattle metabolism [56].

Lactic acid is formed from starch, and since it is not digested by the animal, it is absorbed through the rumen walls, thus resulting in increased lactic acid levels in the blood and pH decreasing. If an animal's diet changes too fast, volatile fatty acids accumulate in the rumen and negatively affect the microbiota and animal health. Those abrupt changes lead to a decrease in rumen pH and an increase in populations of *S. bovis* and *Lactobacillus* [34,57].

Ruminants are fed with citrus byproducts, which contain high amounts of pectin. They can serve as an alternative to grain-based feeds with high enzymatic activity, this way preventing overgrowth of *S. bovis* and associated rumen acidosis [34]. Furthermore, some studies suggest that the use of such products can improve feed efficiency for milk production [58].

Rumen bacteria play key role in the ruminants' digestion. Their diversity and functions are closely linked to the composition of an animal's diet. Understanding these correlations is crucially significant for optimizing ruminant nutrition and health, as well as for improving livestock husbandry efficiency.

Rumen fungi

Fungi are an important part of the rumen ecosystem because they serve as decomposers, for example, in the breakdown of fibrous feedstuffs. Recent studies have revealed a surprising abundance of both anaerobic and aerobic fungi within the rumen, with their population structure being significantly dependent on diet [4,59].

Rumen fungi (10^3–10^6 zoospores/ml of rumen fluid) are predominantly anaerobic and belong to the phylum *Neocallimastigomycota*, class *Neocallimastigomycetes*. This class includes six previously recognized genera (*Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces*) with twenty-one known species, as well as two newly discovered genera, *Oontomyces* and *Buwchfawromyces* [34].

Fungi in the rumen of the ruminants play a key role in the digestive process, particularly in the breakdown of complex plant polysaccharides. Anaerobic fungi belonging to the phylum *Neocallimastigomycota* are particularly efficient in producing enzymes such as cellulases, hemicellulases, and xylanases. These fungi are capable of completely degrading non-lignified plant walls and are more efficient at colonizing and degrading lignin-containing tissues in comparison with bacteria [60].

Recent study [61] demonstrated the availability of significant amounts of aerobic fungi in the rumen of buffaloes, with a predominance of ascomycetes (*Ascomycota*) and basidiomycetes (*Basidiomycota*) under various feeding conditions. These fungi, particularly those that form mycelium, are able to penetrate through rigid plant cell walls, this way facilitating the decomposition of resistant plant material [4].

Fungi in the rumen produce enzymes that break down plant cell walls, providing nutrient access for other microorganisms. Although bacteria are the most common microorganisms in the rumen, fungi also act as effective degraders. Certain fungal species are thought to play a significant role in improving feed conversion and productivity in ruminants [62].

Research has shown that the composition of rumen microorganisms can vary significantly depending on diet. Diets rich in fiber promote the growth of microorganism populations with high cellulolytic activity, while diets high in starch can promote the growth of other microbial communities.

The higher diversity of microbial communities is observed in free-range ruminants in comparison with domesticated breeds, which observation proves the influence of lifestyle and diet on rumen microbial populations [5]. Further research is needed to elucidate the mechanisms regulating the dynamics and ecological roles of both aerobic and anaerobic fungi in the rumen microbiota. Using omics approaches (genomic, transcriptomic, and proteomic) provides insight into the unique cellular processes, evolutionary history, and metabolic capabilities of these fungi [60].

Rumen fungi are an important component of the ruminant microbiome and play a key role in the breakdown of complex plant polysaccharides. Their ability to degrade lignocellulosic materials makes them indispensable in the digestive process in ruminants. Understanding the complex interactions between fungi and other rumen microorganisms, as well as the influence of various factors on fungal communities, can lead to improved nutrition and health of ruminants, as well as the optimization of feeding strategies for increased feeding productivity.

Rumen methanogenic archaea

Methanogenic archaea play a vital role in the rumen ecosystem of ruminants. These unique microorganisms possess specific metabolic processes that enable them to survive in the anaerobic conditions of the rumen. Methanogens are the only microorganisms capable of producing methane and exist at concentrations of 1010–1011 cells/mL of rumen fluid, constituting less than 4 % of the rumen microbial community [34,63,64]. Methane, produced by these organisms through microbial fermentation in the ruminants' digestive tract, is a potent greenhouse gas, thus making the research of methanogens important from both a scientific and environmental point of view [65].

Archaea are divided into two kingdoms: *Euryarchaeota*, consisting of methanogens and extreme halophiles, and *Crenarchaeota*, consisting of hyperthermophiles and non-thermophiles [34].

Current research confirms that the genus *Methanobrevibacter* dominates among the rumen methanogens, accounting for approximately 74 % of the total methanogen rRNA gene sequences. Members of the order *Methanomassiliicoccales*, along with the genus *Methanosphaera*, form the second-largest group, accounting for approximately 16 % of the sequences [33]. *Methanobrevibacter gottschalkii*, *Methanobrevibacter ruminantium*, the species of the genus *Methanosphaera*, and two members of the family *Methanomassiliicoccaceae* (formerly known as "Rumen cluster C") together account for approximately 90 % of the total rRNA sequences of rumen methanogens genes.

The primary function of most methanogens in the rumen is to reduce CO2 with the help of hydrogen gas (H_2) to produce methane (CH_4). This process plays a key role in maintaining low hydrogen levels within the rumen, which promotes the growth of other microorganisms and increases feed fermentation efficiency [34].

However, it is worth to note that there are exceptions as well. For example, *Methanosphaera stadtmanae* produces methane by reducing methanol with H_2 rather than CO_2. This species feature one of the most stringent energy metabolisms of all methanogenic archaea. This diversity of metabolic pathways highlights the complexity and adaptability of rumen microbial communities.

Development of strategies to reduce methane emissions from farm cattle is of great scientific and ecological importance. These strategies may include: (1) targeting methanogens or microorganisms that produce substrates for methanogenesis; (2) studying the correlations between methanogens and other rumen microorganisms; (3) using information obtained from sequencing the genomes of rumen microorganisms to develop vaccines, inhibitors, and other methods to reduce methane emissions; (4) breeding animals with low emissions; and (5) modifying the rumen microbial community [65]. These approaches are aimed to comprehensively address the issue of methane emissions in cattle farming.

The research [66] showed that selective breeding ruminants for low methane emissions can significantly impact their digestive physiology.

The Food and Agriculture Organization of the United Nations (FAO) estimates that in 2015, greenhouse gas emissions from livestock agricultural food systems amounted to approximately 6.2 billion tons of CO_2-equivalent, which is approximately 12 % of all anthropogenic greenhouse gas emissions [67].

Our current understanding of rumen methanogens is insufficient to develop effective strategies for reducing methane emissions while maintaining rumen function. Despite extensive research, the dominance and role of *Methanobrevibacter spp*. still remain unclear. To gain new knowledge, more culture isolation studies are required, particularly for poorly studied groups (e.g., *Methanomassiliicoccales*), as well as genomic data of better quality [68].

In light of projected global population growth and an expected 20 % increase in demand for animal products by 2050 [67], developing effective strategies to reduce greenhouse gas emissions from the global livestock sector is becoming a matter of paramount importance. A balance must be found between meeting growing food demand and minimizing the negative impact of livestock farming on the planet climate. This requires innovative approaches to managing agricultural systems and the implementation of new technologies capable of significantly reducing the carbon footprint of the livestock breeding industry.

Ruminal phages

As it was mentioned earlier, rumen microorganisms play a crucial role in the ruminants' vital functions. Bacteriophages (phages) are the most numerous and well-studied group of viruses in the rumen. Their density varies between 107 and 1011 particles/mL of rumen fluid. Bacteriophages are an integral part of the rumen microbiome, significantly influencing microbial dynamics, nutrient recycling, and overall rumen function. These viruses play a critical role in the complex rumen ecosystem by infecting bacterial populations and causing lysis of microorganisms [12,34]. In the rumen, bacteriophages infect bacteria, archaeophages are targeted to archaea, mycophages infect fungi, and protozoan viruses [12]. Morphological studies have identified phages from the *Myoviridae, Siphoviridae, Podoviridae*, and *Mimiviridae* families.

Viruses are simple infectious particles (virions or free viruses) consisting of genetic material (DNA or RNA in single or double strands) and a protein shell. Some viruses have an additional outer membrane, and are called as enveloped viruses. The totality of viruses in the environment is called the virome [29,69].

In the rumen, the density of free virus particles per milliliter varies in the range between 5×10^7 and 1.7×10^{10} [70,71]. In addition, viruses also parasitize other microorganisms, thereby modulating the microbiome and influencing the cattle itself and its productivity [72]. However, viruses in rumen fluid still remain poorly studied, despite their direct impact onto the rumen ecosystem [73].

A study in both beef and dairy cattle examining the relationship between diet and the rumen virome and its impact on animal performance showed an indirect rather than a direct interaction between diet and the rumen virome, as diet influenced the host to varying degrees. It was also found that rumen viruses can regulate microbial strains and communities through antagonistic and mutualistic interactions. There is a correlation between the cattle yield efficiency and dietary changes that alter the rumen virome. The virome of beef cattle fed with highly concentrated feed is less diverse than that of cattle fed with medium-concentrated feed. The virome of dairy cattle is more diverse when fed with high-lipid and high-starch feed. However, virome diversity decreased in cattle fed in grazing grass-lands, compared to a total ration fed to the cattle [74]. Recently, a comprehensive rumen DNA virome profile was compiled using NGS and HiFi sequencing technology [73] to expand the rumen database, and the authors also proposed mechanistic pathways by which viruses may influence feed efficiency. This is hypothesized to occur either by disrupting host cells or by inducing metabolic changes in the host.

Yoshiaki Sato [75] analyzed the genomes of viruses from the rumen of Japanese Black cattle and found that the virome differed between cattle fed with low-fat and high-fat diets, as well as between cattle with low and high slaughter weights. Possible maternal transmission of rumen viruses to the cattle offspring may also influence beef cattle productivity [76].

Depending on their life cycle in the rumen, viruses can be divided into two types: temperate and lytic. Temperate viruses create a provirus by integrating their genetic material into the host genome. The provirus then becomes responsible for the symbiotic interaction with the host. Under the influence of a number of factors (physical or mechanical), or spontaneously, the prophage can then enter the lytic cycle. Lytic viruses act by infecting host cells, replicating, and causing cellular lysis. Subsequently, the progeny virions are released and infect other hosts [73]. Microbial lysis caused by the phage releases cellular components, such as proteins and enzymes, which can be used by other microorganisms or absorbed by the host animal. This process also promotes nutrient recycling, as the enzymes released during lysis enhance feed fermentation and improve nutrient absorption. Phages are involved in the control of microbial populations, regulating their numbers through “kill-the-winner” dynamics, where dominant bacterial species become the targets for their viral predators, thus allowing less competitive species to survive. Furthermore, phages facilitate genetic exchange, facilitating horizontal gene transfer between bacteria, which plays a crucial role in the adaptation and evolution of the rumen microbiome.

Phages can alter the rumen environment by influencing the composition and activity of microorganisms. For example, phage therapy has been proposed to reduce methane emissions in ruminants by targeting methanogenic archaea. Phage therapy in ruminants shows promise as an alternative to antibiotics, offering targeted solutions to key challenges in animal husbandry. This approach uses bacteriophages to address issues such as methane emissions, dysbiosis, and subclinical rumen acidosis. Studies have identified phage-derived lytic enzymes, such as PeiR from *Methanobrevibacter ruminantium*, which have the potential to reduce methane production by targeting rumen methanogens. Furthermore, phage therapy may help restore microbiome homeostasis in cases of dysbiosis and combat antibiotic-resistant microorganisms.

Rumen amoeba

Amoebas are single-celled eukaryotes found in a variety of environments, including the rumen of ruminants. Although generally less studied than other rumen microorganisms, they are an integral part of this complex ecosystem. Amoebas may represent an important reservoir for rumen bacteria, although their exact role is not fully understood. They are nonetheless part of the diverse microbial community in the rumen [34].

Similar to ciliates, amoebas in the rumen survive by engulfing bacteria through phagocytosis. This feeding behavior allows them to actively ingest bacteria as a protein source, which may influence overall protein dynamics in the rumen ecosystem [77].

Some bacteria can survive phagocytosis by protozoans, including amoebas, and survive as endosymbionts [34]. For example, *Campylobacter jejuni* has been shown to invade *Acanthamoeba polyphaga* and can replicate itself in the vacuoles. This relationship between bacteria and amoebae may have implications for the rumen and overall health of cattle, as *C. jejuni* and *C. fetus* can significantly impact fertility, immunity, and overall health condition of the cows [78,79].

Amoebae, along with other protozoa, may be involved in regulating prokaryotic diversity in the rumen [3]. Their predatory behavior could potentially mitigate the effects of competitive exclusion between bacterial taxa, thereby helping to shape the composition of the prokaryotic community.

The role of amoebae in the rumen is not as well studied as the roles of other microorganisms, like bacteria or ciliates. Further research is needed to understand their ecological significance and how they interact with other members of the rumen microbiome. Although amoebae are available in the rumen and is likely to play a role in its complex ecosystem, their specific contribution to rumen function and animal health still requires further studies [34].

Rumen protozoa

The most important protozoa in the rumen of ruminants are ciliates / infusoria, which comprise more than

50 % of the rumen's microbial mass. Based on morphological characteristics, ruderal protozoa are divided into two orders: endodiniomorphs (order: *Entodiniomorphida*) and holothrichs (order: *Vestibuliferida*). They are the biological indicators of proper rumen function. Ciliates break down the structural and non-structural carbohydrates, and participate in host digestive metabolism.

Depending on geography, diet, and a number of other factors, the protozoan profile of the rumen varies among the ruminants. In 2020, Cedrola et al. [80] analyzed the past and current studies of the rumen trichostomatids and in order to expand the phylogeny proposed using evolutionary models, such as the method of branch lengths (MBL). The same authors conducted a taxonomic review of ciliates among Brazilian livestock in Brazil and described a new morphotype of entodinid ciliates *Entodinium caudatum* m. *orbonuclearis* [81]. They found that urea supplementation did not affect the density of rumen trichosomatid ciliates, their relative abundance, or their diversity.

Cellulolytic activity of rumen-inhabiting protozoa was identified. Cellulolytic protozoa such as *Eudiplodinium maggie*, *Ostracodinium album,* and *Epidinium caudatum* are protozoa that degrade cellulose by absorption [50]. The study in 2003 of the rumen protozoan *Polyplastron multivesiculatum* revealed that these protozoa can digest fiber [82]. Devillard et al. isolated the xylanase XYN10B from GH10 from rumen protozoa and described its enzymatic properties [83]. It was also suggested that some rumen protozoans may have acquired genes for cellulases and xylanases through horizontal transfer. In a study by Andersen et al. [84], it was found that although some protozoan species are capable of digesting starch, they were less common in animals that produce little methane. Cysteine peptidases were also found to be beneficial for nitrogen assimilation in ruminants [85].

Omics studies have shown that predation of the protozoa *En. caudatum* includes endocytosis, phagosomes, lysosomes, and cells motility [86]. Compared to isotrichids, endodiniomorphids were found to feature higher predatory activity. This is indicated by the increased nitrogen concentration in the rumen and the higher abundance of endodinimorphs in goats compared to the bovine cattle being analyzed [87].

In vitro studies of the rumen have demonstrated the possibility of suppressing protozoan activity using specific lysozyme and peptidase inhibitors without disrupting fermentation processes [88]. The implementation of proteomics, genomics, and transcriptomics technologies can help identify specific targets for effective management of protozoa activity in the rumen.

Factors influencing the rumen microbiome

There are many factors that influence the rumen microbiome, including age, diet, genetics, geographic location, breed variety, and sex. Understanding and managing these factors is crucial for ensuring efficient nutrients utilization by the ruminants via influencing the microbial population in the rumen [1,62].

Dairy and beef cows have different nutritional needs, which are reflected in their diets and, consequently, their microbiomes. Dairy cows require the diets that optimize milk yield and milk quality. Their gastrointestinal tract is tailored to efficient processing of high-fiber diets. Changes in feed intake can alter the composition of the rumen microbiome and general production efficiency during lactation. Beef cows, in their turn, are targeted to weight gain and ensuring meat quality. Their gastrointestinal tract is optimized to convert feed into muscle mass. Similar to dairy cows, the rumen microbiota of beef cattle can vary depending on the diet type, thus impacting growth rate and feed conversion efficiency [89].

Although the rumen microbiome varies across various diets, it is able to adapt to these changes and remain efficient. Certain rumen microbiota species in dairy cows are associated with improved feed efficiency. Some anaerobic microbes promote cellulose breakdown, which improves nutrients absorption and overall productivity [90–92].

Researches confirm the influence of diet on the rumen microbiome. For example, one study in beef cattle found that *Prevotella* species count was higher in a group with inefficient consumption of residual feed. Feeding the cows with low-forage diet resulted in decreased abundance of *Fibrobacter succinogenes*, and increased abundance of *Entodinium* and *Prevotella* spp. [93].

The buffalo rumen fungal community is dominated by ascomycetes, with amount varies depending on diet, thus indicating a significant influence of feed composition on rumen fungal diversity and functionality.

Conclusion

The rumen microbiome is a complex and dynamic ecosystem that influences livestock health. The achievements in molecular techniques have significantly expanded our understanding of the diversity and functions of rumen microbial community participants. This knowledge is key for development of strategies to enhance livestock yield performance, improvement of feed efficiency, and mitigation of environmental impacts such as methane emissions. Future researches should focus on clarification of the complex interactions between the host and various microbial communities, and exploring the potential of rumen-derived probiotics for sustainable livestock husbandry. As our understanding of the rumen microbiome keeps on growing, it will undoubtedly lead to innovative approaches in livestock nutrition and management, thus contributing to more efficient and environmentally friendly ruminant livestock production systems.

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The authors declare no conflict of interest.