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Literature Review

# Rumen Microbiota Mysteries: Nutrition, Function, and its Impact on Livestock Productivity

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**Abstract:** Ruminants are important herbivores in the global food system, serving as primary producers of meat and milk. The productivity of these animals is influenced by the functionality of their digestive organs, namely rumen. The digestion, metabolism and nutrients absorption in the rumen epithelium enhance the host defende mechanisms mechanisms, promoting health and productivity in ruminants. Therefore, this review aimed to examine the significance of rumen microbes in nutrition and their influence on ruminant productivity through an exploratory method based on literature obtained from Web of Science, Scopus, and PubMed. The results showed that rumen microbiota, comprising bacteria, fungi, protozoa, archaea, and viruses, played a significant role in the acquisition and use of nutrients through fermentation activities. The fermentation activities that occurred within the rumen led to the production of various end products, including Volatile Fatty Acids (VFA), Hydrogen ($H_2$), methane ($CH_4$), carbon dioxide ($CO_2$), vitamins (specifically B and K), microbial crude protein, and other bioactive molecules. The end products of fermentation synthesized by microbes were considered essential for host function and rumen epithelial development, including feed efficiency, immune system formation, and maintaining the balance of fermentation patterns. This showed the importance of rumen microbiota for survival, health, and ruminant productivity.

**Keywords:** Microbiota, Nutrition, Productivity, Rumen, Ruminants

## Introduction

Ruminants are herbivores capable of converting energy stored in plants into protein food products such as milk and meat consumed by humans globally (Mayulu *et al*., 2022; Sanjorjo *et al*., 2023; Muñoz-Tamayo *et al*., 2023; Wang *et al*., 2023). The ability to use biomass is correlated with the digestive system (Silva *et al*., 2024). The digestive system possesses unique and complex characteristics indicating fermentation activity. It comprises a stomach divided into four compartments (namely rumen, reticulum, omasum, and abomasum) (Susilo *et al*., 2019; Kailang *et al*., 2023). Rumen fermentation is a metabolic activity of a complex microbial community found in the reticulorumen (Aguilar-González *et al*., 2023). The fermentation function of the rumen allows ruminants to degrade forage (Sha *et al*., 2024), playing a major role in providing the main energy to support metabolic processes (Xu *et al*., 2021; Zhao *et al*., 2024).

Rumen of ruminants is rich in microbiota including anaerobic bacteria, protozoa, anaerobic fungi, bacteriophages, and methanogenic archaea. According to Choi *et al.* (2023) there are also viruses that have co-included with the host to influence the phenotype playing important metabolic and nutrient digestion roles for the host (Matthews *et al*., 2019; Sha *et al*., 2024). Microbiota can degrade complex polysaccharides in forage including hemicellulose, cellulose, and lignin. These included other substances that cannot be synthesized in the host body such as Short-Chain Fatty Acids (SCFAs) (Pokhrel and Jiang, 2024; Xu *et al*., 2021), microbial crude protein (Liu *et al*., 2021), ammonia, and metabolites. According to Sha *et al.* (2024) these substances function in immune system regulation, preventing disease, energy balance, and physiological development, which has an impact on ruminant productivity.

Essential enzymes are generally absent in the rumen for the digestion and fermentation of the main components of forage structural biomass, suggesting the importance of microbes for the survival and performance of the host (Cammack *et al.*, 2018). Close and constant interaction between microbiota and the rumen is essential to ensure good health and optimal productivity of the host (Liu *et al.*, 2021). Despite the significant benefits, the relevance of rumen microbes to nutrition and their impact on ruminant productivity is still limited. Therefore, this review aimed to explore the relevance of rumen microbes to nutrition and their impact on ruminant productivity.

## Materials and Methods

A review regarding the relevance of rumen microbes to nutrition and their impact on ruminant productivity used an exploratory method through literature sourced from Scopus, Web of Science, PubMed, and MDPI which are scientific databases capable of storing high-quality articles. The keywords are "rumen anatomy and morphology", "rumen microorganisms", "rumen and ruminant productivity", "rumen fermentation and efficiency", and "nutrition and rumen performance". Inclusion criteria are publication within the last seven years (2018-2024) to ensure the review is up-to-date, full-text written in English, and discussion of rumen characteristics, ruminant microbiota, rumen dysfunction as well as the correlation of rumen performance to ruminant productivity. These articles are excluded when considered not fit for the topic of discussion.

## Literature Review

### *Rumen Characteristic*

The stomach morphology characteristics of ruminants consist of four compartments (namely rumen, reticulum, omasum, and abomasum) (Na and Guan, 2022; Mayulu, 2023; Pokhrel and Jiang, 2024; Silva *et al.*, 2024). The rumen is a natural bioreactor for degrading and fermenting complex carbohydrates from plant biomass (Wang *et al.*, 2020; Andersen *et al.*, 2021) and producing SCFAs or Volatile Fatty Acids (VFA), gas ($CO_2$, $NH_3$, $CH_4$) and microbial crude protein as final fermentation products (Na and Guan, 2022; Sanjorjo *et al.*, 2023; Pokhrel and Jiang, 2024). It is located to the left of the abdominal cavity and anatomically has a surface lined with papillae for surface expansion and increased nutrient absorption. The rumen has five sacs, Namely *Cranial Sac* (CS), (CDBS), (CVBS), (CVBS), *Dorsal Sac* (DS) and *ventral sac* (VS), which are divided by pillars, grooves and folds, as shown in Fig. (1).

Rumen and reticulum are often considered as a unit separated by a reticulorumen fold (Xu *et al.*, 2021). The folded partition allows the contents of the rumen and reticulum to mix easily. As shown in Figure (2), the content of the rumen is divided into four zones, including the gas zone, as a place for gas accumulation, the pad zone (floating fiber), the fluid phase, and the high-density phase (Matthews *et al.*, 2019). The acidity level (pH) of rumen fluctuates ranging from 5.8 to 6.5 and has a temperature of approximately $39^{O}$C (Fregulia *et al.*, 2021), which allows optimal microbial growth and activity (Andersen *et al.*, 2021).

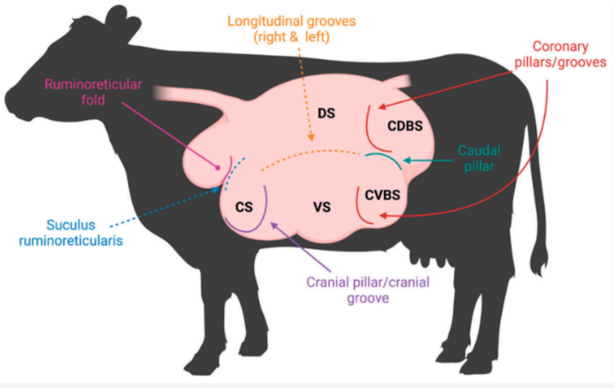


**Fig. 1:** Representation of Muscle and Rumen Sacs (Soltis *et al.*, 2023)

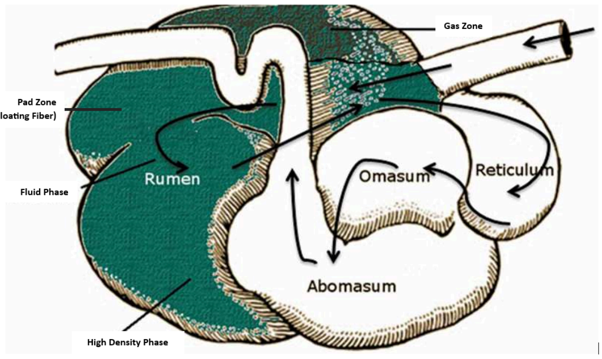
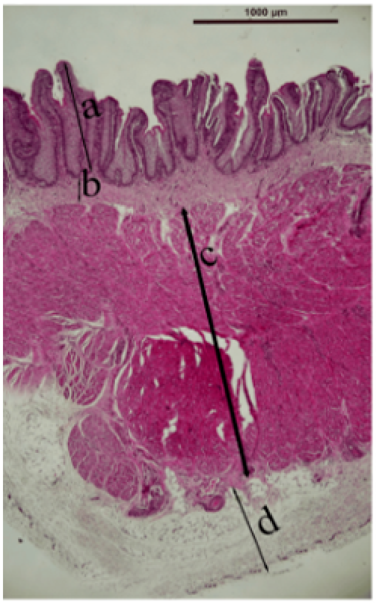


**Fig. 2:** Rumen Parts (Reconstructed from Matthews *et al.*, 2019)

Histologically, the rumen wall is composed of the tunica serosa, tunica muscularis, tunica mucosa, tunica submucosa, and lamina propria as shown in Figure (3). The tunica mucosa is the most important part, which is a non-glandular layer and has superficial stratified squamous epithelium. The epithelium consists of four layers or cellular levels that have different morphologies and functions, namely stratum corneum, stratum granulosum, stratum spinosum, and stratum basale, as shown in Figure (4) (Na and Guan, 2022; Wu *et al.*, 2022; Kailang *et al.*, 2023). Stratum basale cells are columnar in shape and play a role in nutrient absorption, energy metabolism, ketogenesis, and immunoglobulin secretion. The cells in the stratum spinosum and granulosum layers have tight and adherent connections. There are desmosomes that provide strong adhesion, thereby enhancing resistance to mechanical pressure and maintaining structural integrity. The stratum corneum and basale do not express junctional proteins and haven't barrier function junctional proteis and have no barrier function. The stratum granulosum cells are located perpendicular to the stratum spinosum cells (Pokhrel and Jiang, 2024). Furthermore, stratum corneum is the keratinized outer layer of cells, which is directly opposite the lumen (Pokhrel and Jiang, 2024). It functions to prevent microbes and exogenous toxic compounds including endotoxins and biogenic amines from penetrating rumen epithelium, serving as a physical barrier similar to the intestinal mucus layer (Na and Guan, 2022). The outermost layer of the rumen is the tunica serosa or serosal layer. The tunica serosa is a thin layer that forms a fine tissue that functions to minimize friction between the rumen and surrounding organs (Pokhrel and Jiang, 2024).



(a) Rumen Papilla; (b) Mucosal and Submucosal; (c) Muscular Layer; (d) Serosal Layer

**Fig. 3:** The histology of the Rumen Wall (Pokhrel and Jiang, 2024)

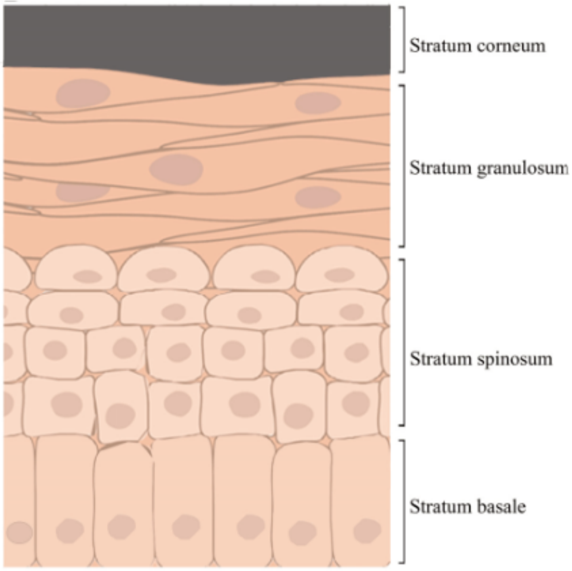


**Fig. 4:** Schematic Representation of Epithelium Layer in Rumen (Pokhrel and Jiang, 2024)

**Table 1:** Variations of Microbiota Species in Rumen; Source Reconstructed from Xu *et al.* ( 2021); Silva *et al.* ( 2024)

| Rumen Microbiota | Species | Criteria |
|---|---|---|
| Bacteria | *Methanobacterium phage ψ M1, Methanobacterium phage ψ M2, Methanobacterium phage ψ M10, Methanobacterium phage ψ M100, Methanothermobacter phage ψ M100* | Highly anaerobic and produces methane from $CO_2$ and $H_2$ |
| Protozoa | *Entodinium longinucleatum, Entodinium dubardi, Entodinium bovis, Entodinium bubalum, Entodinium caudatum, Entodinium bursa, Entodinium exiguum, Entodinium chatterjee, Epidinium caudatum, Isotricha intestinalis, Isotricha prostoma, Diplodinium dendatum, Dasytrica ruminantium, Diplodinium indicum, Oligoisotricha bubali, Polyplastron multivesiculatum, Eremoplasron bubalis, Eremoplastron asiaticus, Ophryoscolex inermis, Ophryoscolex purkynjei, and Entodinium dentatum, Buetschlia spp* | Digests lignocellulose and degrades complex compounds into reducing sugars |
| Archaea | *Methanobacterium bryantii, Methanobacterium formicicum, Methanobacterium mobile, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanosarcina bacteria, and Methanoculleus olentangyi* | Highly anaerobic and produces methane from $CO_2$ and $H_2$ |
| Fungi | *Orpinomyces intercalaris, Orpinomyces joynii, Neocallimastix variabilis, Neocallimastix hurleyensis, Neocallimastix frontalis, Piromyces mae, Piromyces commuis, Piromyces minitus, Piromyces rhizinflatus, Piromyces dumbonicus, Piromyces citronii, Piromyces polycephalus, Anaeromyces mucronatus, Anaeromyces elegans, Caecomyces communis, Caecomyces aqui, Caecomyces sympodialis, Cyllamyces aberensis, and Cyllamyces ucaris* | Degrades cellulose and lignin, forms formate, hydrogen, succinate, lactate, and acetate |

### *Rumen Microbiota*

According to Na and Guan, (2022), the rumen is essential for ruminants, serving as a habitat for microbial rumen that plays an important role in the acquisition and use of ruminant nutrients. These nutrients are used through fermentation to produce VFA, microbial crude protein, as well as vitamins (B and K) from the degradation of forage fiber and other organic materials (Liu *et al.*, 2021). Rumen microbiota is indispensable for the survival, productivity, and overall health of ruminants (Qi *et al.*, 2024). Furthermore, it is classified into three large groups, namely solid phase (70% of the microbial mass), free-floating in the liquid phase (25%) and microbiota associated with rumen epithelial cells (5%) (Matthews *et al.*, 2019; Na and Guan, 2022; Wu *et al.*, 2022). As shown in Figure (5), microbiota comprises thousands of species of microorganisms including protozoa ($10^4$-$10^6$ cells/ml), anaerobic bacteria ($10^{10}$-$10^{11}$ cells/ml), anaerobic fungi ($10^3$-$10^6$ cells/ml), bacteriophages ($10^9$-$10^{10}$ cells/ml), and methanogenic archaea ($10^7$-$10^9$ cells/ml) (Choi *et al.*, 2023; Khairunisa *et al.*, 2023) as well as viruses (Sanjorjo *et al.*, 2023). Microbiota species in the rumen are diverse and have specific criteria and/or functions (Table 1). The following are variations in microbiota species in the rumen.

**Fig. 5:** Components of Rumen Microbiota (Source: Sanjorjo *et al.*, 2023)

Rumen microbiota functions synergistically in hydrolyzing hemicellulose and cellulose into sugar monomers and fermenting into VFA (Khairunisa *et al.*, 2023) for energy metabolism (Liang *et al.*, 2024). The VFA produced consists of acetate (20%), propionate (10%), butyrate (5%), and succinate, which is absorbed by the host through the epithelial wall (Mayulu *et al.*, 2018; Andersen *et al.*, 2021). As the final product of fermentation, VFA play an important role in ruminant metabolism such as fatty acid synthesis and gluconeogenesis (Xu *et al.*, 2021), providing approximately 70-80% of the host energy needs (Mayulu *et al.*, 2018; Sanjorjo *et al.*, 2023). In addition to fermenting complex carbohydrates into VFA, rumen microbes can synthesize crude protein for growth (called microbial crude protein) by using energy and nitrogen obtained from feed (Liu *et al.*, 2021). Microbial crude protein supplies 60-85% of amino acids (Sanjorjo *et al.*, 2023) which is digested in the small intestine and absorbed by the host, thereby contributing to nutrition and health (Liu *et al.*, 2021).

Rumen bacteria are the most abundant microorganisms ($10^{10}$-$10^{11}$ bacteria per ml of rumen content) in rumen microecology, capable of secreting hydrolases that efficiently degrade lignocellulose (Matthews *et al.*, 2019; Liang *et al.*, 2024). Bacteria represent the largest number of more than 7000 species representing 19 phyla and are the most important microbial community in the rumen (Matthews *et al.*, 2019; Sanjorjo *et al.*, 2023; Silva *et al.*, 2024). The composition of bacteria is determined by several factors including preference for certain substrates (ratio), energy requirements, and resistance to metabolic products that are toxic in some species (Matthews *et al.*, 2019). *Prevotella*, *Butyrivibrio*, *Fibrobacter*, *Lachnospiraceae*, *Saccharofermentans*, *Succinivibrio*, and *Ruminococcus* are the most dominant bacterial species in the rumen and their community structure is influenced by changes in host diet (Mayulu *et al.*, 2020; Matthews *et al.*, 2019; Wei *et al.*, 2022). Gram-positive bacteria are often found in the rumen of ruminants given high amounts of forage. Meanwhile, ruminants fed by grain feed are dominated by gram-positive bacteria such as Lactobacillus (Matthews *et al.*, 2019). This shows that complexity in diet supports increased microorganism diversity.

In the rumen, bacteria efficiently degrade hemicellulose and cellulose which are components of forage consumed by ruminants, and convert them into VFA (Liang *et al.*, 2024). The ability to degrade cellulose depends on forage type, plant growth stage, and the availability of cellulolytic bacteria (Matthews *et al.*, 2019). *Ruminococcus albus* and *Fibrobacter succinogenes* are effective in digesting cellulose compared to other bacteria (Matthews *et al.*, 2019; Perez *et al.*, 2024). The final products of cellulose fermentation are acetate, butyrate, propionate, and $CO_2$, with some major pectinolytic bacteria including *Lachnospira multiparus*, *Prevotella ruminicola*, and *Butyrivibrio fibrisolvens* (Matthews *et al.*, 2019).

The size of protozoa ranges from 20-200 µm and they are surrounded by a pellicle or cuticle in the rumen. These organisms are anaerobic and non-pathogenic, representing some of the simplest eukaryotes (Xu *et al.*, 2021; Silva *et al.*, 2024). Protozoa are primarily parasitic and predatory feeding on microorganisms, organic matter, and cell debris (Yu *et al.*, 2024). The density of protozoa in the rumen is about $10^4$ to $10^6$ cells per milliliter of rumen fluid (Silva *et al.*, 2024), making up around 50% of the total rumen microbial population (Sanjorjo *et al.*, 2023; Yu *et al.*, 2024), showing their importance in the rumen microbiota. Protozoa are classified into two main groups, namely entodiniomorphids and holotrichids, which belong to the Entodiniomorphida and Vestibuliferida orders, respectively (Yu *et al.*, 2024). *Entodinium* is the most common genus comprising 90% of the protozoan population in the rumen, with approximately $1.17\text{x}10^6$ cells/ml of rumen fluid (Sanjorjo *et al.*, 2023). However, the abundance varies based on the host species and diet (Yu *et al.*, 2024). Other protozoa genera include *Diplodinium, Dasytricha, Eudiplodinium, Epodinium, Ostracodinium, Polyplastron, Ophryoscolex* (Sanjorjo *et al.*, 2023),(Silva *et al.*, 2024). Ciliated protozoa are classified into two groups based on their morphology features, namely holotrichs, with cilia covering their entire body, and entodiniomorphids, which have cilia and a strong pellicle around the peristome (Sanjorjo *et al.*, 2023).

Protozoa have special functions to survive in the rumen environment where the majority are anaerobic, but very few species absorb $O_2$ (Xu *et al.*, 2021). Absorption of $O_2$ is beneficial to the host for maintaining the anaerobic atmosphere of the rumen and degrading complex carbohydrates (Xu *et al.*, 2021). The ability of protozoa to degrade complex carbohydrates causes a buffering effect in the rumen, reducing the risk of acidosis when the host consumes a diet rich in grains and sugar (Silva *et al.*, 2024). Protozoa are also responsible for approximately 34-40% of degrading fibrous feed ingredients and are active in breaking down lipids to provide hydrogen through hydrosomes (Silva *et al.*, 2024). Degradation of carbohydrates, fats, and proteins is facilitated by direct engulfment. Furthermore, the digestive capacity of lignocellulose by protozoa is attributed to lateral gene transfer from the bacteria ingested (Xu *et al.*, 2021).

Cellulolytic and hemicellulolytic bacteria are produced by protozoa in lower numbers compared to entodiniomorphida (Xu *et al.*, 2021). Rumen ciliates also influence the production of ammonia and VFA. This is because ciliates in the rumen secrete proteolytic enzymes for the production of amino acids and $NH_3$ (Xu *et al.*, 2021). The type of microbiota ingested determines protozoan nitrogen metabolism, which affects nitrogen recycling (Xu *et al.*, 2021) and reduces the efficient use in feed (Yu *et al.*, 2024). Compared to bacteria, protozoa contain more Unsaturated Fatty Acids (UFAs), thereby serving as an important source of beneficial fatty acids (Yu *et al.*, 2024). Specifically, holotrichids contribute ~27% to total rumen lipids (Yu *et al.*, 2024). Rumen protozoa can reduce UFA biohydrogenation by preying on biohydrogenation bacteria and storing Conjugated Linoleic Acid (CLA) and vaccenic acid to protect UFA from bacterial biohydrogenation (Yu *et al.*, 2024).

Anaerobic fungi in rumen fluid ($10^3$-$10^5$ per ml) facilitate the degradation of complex carbohydrates in forage into simple sugars (Tamilselvan and Selwynraj, 2024). The fungi community represents approximately 10-20% of rumen microbial communities based on ribosomal RNA transcripts (rRNA) with significant fluctuations based on the feed consumed by the host (Xu *et al.*, 2021; Sanjorjo *et al.*, 2023). Moreover, the fungi genera that are often found in rumen fluid include *Rhizophus* spp (12.8%), *Aspergillus* spp (56%), *Paecilomyces* spp (7,1%), *Scedosporium* spp (6.3%), and *Trichophyton* spp (8.5%) (Silva *et al.*, 2024). Rumen fungi efficiently degrade lignocellulosic biomass due to their powerful pseudo-root and enzymatic system (Liang *et al.*, 2024). The byproducts of fungi metabolism include hydrogen, lactic acid, and ethanol (Tamilselvan and Selwynraj, 2024). Although fungi do not have mitochondria and cytochromes which are coplayers of oxidative phosphorylation, the presence of hydrogenosomes facilitates energy formation (Xu *et al.*, 2021). Hydrogenosomes are mitochondrial derivatives formed during evolution and are not limited only to fungi genera but various anaerobic eukaryotes and trichomonads possess these organelles (Xu *et al.*, 2021). Compared to conventional mitochondria, hydrogenosomes have pyruvate/ferredoxin reductase as well as space for ATP production and pyruvate conversion (Xu *et al.*, 2021).

Methanogenic archaea in the rumen are responsible for the reduction of carbon molecules to methane, using electron donors and driving the flow of electrons across the microbial food web. The domain of archaea comprises several orders including Methanobacteriales, Methanosarcinales, Methanomicrobiales, Methanococcales, Methanopyrales, and Methanomassillicoccales (Xu *et al.*, 2021; Sanjorjo *et al.*, 2023). Methanobrevibacter is the most dominant, followed by Methanosphaera, Methanomicrobium, and members of Thermoplasmatales (Sanjorjo *et al.*, 2023). Archaea members constitute 0.6-3.3% of the total rumen microbiota which function as methane producers or anaerobic methanogens (Xu *et al.*, 2021; Sanjorjo *et al.*, 2023). The process is supported by bacteria and ciliated protozoa, including *Methanobrevibacter* spp. (63%), *Methanosphaera* spp. (9.8%), *Methanomicrobium* spp. (7.7%), *Thermoplasma* spp. (7.4%) and *Methanobacterium* spp. (1.2%) (Silva *et al.*, 2024). Hydrogenotrophic methanogen populations are abundant comprising approximately 78% of the total archaea, followed by methylotrophic methanogens (22%) and small amounts of acetoclastic methanogens (Sanjorjo *et al.*, 2023). The archaeal community structure is similar to all ruminant species globally and is more diverse than bacterial species (Sanjorjo *et al.*, 2023).

Cytochrome-based methanogen has higher growth rates and require approximately 10 times greater hydrogen concentrations for optimal growth. However, non-cytochromic methanogen requires lower hydrogen concentrations for growth and produce methane (Xu *et al.*, 2021). The use of hydrogen by methanogens is very important because it lowers pressure, which allows the conversion of endogenic mrtabolic reactions to exergonic reactions. This makes bacterial fermentation more energetically beneficial (Xu *et al.*, 2021). The coculture of rumen methanogen and fungi has a significant influence on cellulolytic and fermentation activities (Xu *et al.*, 2021). The interaction of commensal methanogens with protozoa and other microbiomes facilitates the degradation of complex plant polymers (Xu *et al.*, 2021), with an increase in energy production to maximum levels. However, gas production has an inhibiting effect on the overall energy obtained from digested feed (Xu *et al.*, 2021).

The presence of a virus in the rumen has an impact on nutrient cycling, substrate availability, and genetic exchange with other microbes through Horizontal Gene Transfer (HGT). The population of the virus reaches $10^7$-$10^9$ particles per gram and coexists with other microbes. However, the presence of viruses in the rumen is still the least explored and understood compared to other microbial populations (Sanjorjo *et al.*, 2023). This is due to various challenges such as the virus isolation and characterization process which requires the availability of a microbial host. The sequencing process is also limited, requiring intact virus particles from environmental samples. Furthermore, the number of available viral sequences is relatively low. The percentage of uncharacterized viral genes is high, thereby limiting genomic or transcriptomic studies (Sanjorjo *et al.*, 2023). These challenges also hinder the annotation of gene function and virus taxonomy. Generally, virus genotypes based on comprehensive metagenomic analysis are highly diverse (28,000 genotypes) and prophages are significantly more abundant than lytic phages (approximately 2:1) in the bovine rumen virome (Sanjorjo *et al.*, 2023). The *Siphoviridae* virus family in the rumen is very dominant, followed by *Myoviridae* and *Podoviridae*.

A virus has two different life cycles, namely the lytic and lysogenic (Yu *et al.*, 2024). Lytic virus lyses host cells to release progeny types for the next round of infection along with cellular components and increases the nutrient cycle in the rumen (Yu *et al.*, 2024). A virus in the lysogenic cycle inserts its genome into the host genome as a prophage leading to lysogeny that provides the host with new metabolic abilities, showing ecological fitness and potentially facilitating host evolution. Therefore, a virus can influence rumen microbiome and function which impacts ruminant productivity (Yu *et al.*, 2024). Virus shows variability in species infected, ranging from specific strains to multiple phyla, as shown in Table (2). The formation of biofilm can be facilitated by releasing extracellular DNA. However, this process potentially disrupts biofilms by lysing the host and depolymerizing exopolysaccharides (Yu *et al.*, 2024). In the Rumen, the presence of a virus also mediates the nutrient cycle including carbohydrates, lipids, proteins, and microbial protein metabolism (Yu *et al.*, 2024). The ability of the virus to lyse hosts causes an increase in the availability of microbial proteins for bacterial proteolysis, contributing to intraruminal microbial protein recycling (Yu *et al.*, 2024).

**Table 2:** Rumen microbiome which is based on substrate; Source Yu *et al.* (2024)

| Substrate | Rumen Microbiome |
|---|---|
| Fiber | *Prevotella, Ruminococcus, Fibrobacter* |
| Starch | *Streptococcus, Ruminobacter* |
| Protein | *Prevotella, Butyrivibrio* |
| Sugar fermentation | *Selenomonas* |
| Methanogen | *Methanobrevibacter* |
| Protozoa | *Entodinium* |

### *Ruminants Manipulation*

Ruminants do not have the necessary enzymes to digest fiber, leading to dependence on microbes in the digestive system. Therefore, most methods to increase feed utilization in ruminants are based on genetic manipulation of rumen fermentation or through the incorporation of fibrolytic microorganisms or enzymes in ruminant feed (Almassri *et al.*, 2024). Rumen manipulation generally aims to increase useful processes such as feed conversion and VFA production or reduce inefficient stages including methane generation. This method increases energy availability for ruminants to achieve higher efficiency in meat and milk production (Aguilar-González *et al.*, 2023). The following rumen manipulation strategies can be carried out include.

### *Use of Plant-Based Bioactive Compounds*

Bioactive compounds and secondary plant metabolites such as tannin, saponin, and essential oil have anti-microbial properties that can be used in ruminant production to reduce $CH_4$ emissions and improve efficiency. Phytochemicals have direct toxic effects on methanogens such as condensed tannins or protozoa including saponins. The types of phytochemicals that are often used in rumen manipulation include saponins and tannins. Specifically, saponin is a surface-active glycoside composed of aglycone-sapogenin and glycone-saccharide (Króliczewska *et al.*, 2023) which acts as a natural rumen modifier to manipulate the population, the composition of microbes, and fermentation (Kholif, 2023). The main sources of saponins in ruminant rations include *Quillaja saponaria, Yucca schidigera, Medicago sativa,* and *Camellia sinensis* (Króliczewska *et al.*, 2023). Saponins modify rumen fermentation directly by influencing the ecosystem, composition, and activity of microbiota such as bacteria, fungi, protozoa, archaea, and viruses (Kholif, 2023; Pepeta *et al.*, 2024).

The defaunation mechanism of saponins disrupts protozoan cell membranes in the rumen (Króliczewska *et al.*, 2023). According to Króliczewska *et al.* (2023), saponins indirectly affect methane production by slowing down methanogenesis, increasing certain gene expressions related to methanogenesis, and reducing methanogenic populations. The ability to reduce methane varies based on its chemical structure, source, dose, and given ratio (Króliczewska *et al.*, 2023). However, using saponins in ruminants has some limits since their antiprotozoal effects are temporary and their toxicity depends on the plant species, conditions, and growth stage (Króliczewska *et al.*, 2023). These antiprotozoal effects can be increased by changing the chemical structure to protect against microbial degradation. Meanwhile, the risk of toxicity is often minimized through physical, chemical, and biological treatment (fermentation) (Króliczewska *et al.*, 2023).

Tannin is a class of organic chemicals that can affect the rumen environment, serving as secondary polyphenolic metabolites of plants (Króliczewska *et al.*, 2023). Furthermore, tannin is classified into two types, namely hydrolyzed (gallic acid polyesters and various individual sugars) and condensed (flavonoid polymers) (Króliczewska *et al.*, 2023; Ramdani *et al.*, 2023). Hydrolyzed tannin when exposed to dilute acids in feed will break down into simpler phenolic and non-phenolic compounds (Króliczewska *et al.*, 2023). The content of several phenolic hydroxyl units in tannin allows the ability to configure protein complexes with metal ions, amino acids, and polysaccharides. The ability of tannin to bind protein can also protect against rumen degradation, thereby increasing flow and amino acid absorption in the small intestine (Ramdani *et al.*, 2023).

The condensed fraction affects feed digestibility, modifies rumen fermentation, and reduces methane emissions, showing a higher anti-methanogenic potential (Króliczewska *et al.*, 2023). Based on previous reports, tannin has been proven to reduce methane production in the ruminant's digestive system through indirect inhibition of hydrogen-producing microflora and direct hindering of methanogens (Króliczewska *et al.*, 2023). Tannin supplementation with an average dose of 10-20 g/kg dry matter can reduce methane production in rumen fermentation by 50%. This reduction ability is based on the source, type, and molecular weight of the tannin and the methanogenic community in the rumen (Króliczewska *et al.*, 2023). Tannin also inhibits methanogenesis bacterially and bacteriostatically by acting on fibrinolytic bacteria based on chemical structure and bacterial species (Króliczewska *et al.*, 2023). Tannin's anti-methanogenic binds to protein, where the phenolic hydroxyl group interacts with amino acid residues using hydrogen bonds and hydrophobic interactions (Króliczewska *et al.*, 2023). It is also used in ruminants to reduce the solubility and rumen breakdown of most feed proteins (Ramdani *et al.*, 2023).

### *Chemical Intervention*

The most effective method capable of inhibiting methanogenesis is chemical, although with negative impacts on humans, animals, and the environment (Króliczewska *et al.*, 2023). Chemicals that are often used include 1,3-propanediol mononitrate and ionophores. The majority of chemicals used are analogs of methane or cofactors of the Methyl-coenzyme M, which is included in methyl transfer during methanogenesis. Inhibitors used include bromochloromethane, trichloroacetamide, chloral hydrate, nitroethane, 3-nitrooxypropanol also known as 3-nitrooxy-propan-1-ol or 1,3-propanediol mononitrate (3-NOP), and chloroform. In ruminant rations, 1,3-propanediol mononitrate is used to reduce methane emission by 20-35% at a dose of 60 mg 3-NOP/kg dry matter. It can be metabolized to 3-nitrooxypropionic acid, 3-hydroxypropionic acid, $NO_3^-/NO_2^-$, and $CO_2$. The 3-NOP metabolite contributes to endogenous compounds, such as lactose and glucose which are converted into $CO_2$ (Króliczewska *et al.*, 2023). Furthermore, the use of 3-NOP with fumarate can lower hydrogen buildup and better inhibit methanogenesis. Cammack *et al.*, (2018) identified ionophores as an additive to reducing methane in ruminants, thereby improving feed efficiency. These are polyether carboxylates made by *Streptomyces* spp. that make the gram-positive bacteria and protozoa cell membranes more open to ions, slowing bacterial growth and changing rumen fermentation. This improves the rumen's ability to process feed. Common ionophores include lasalocid, monensin, salinomycin, and Naracin, with monensin being the most widely used for increasing feed efficiency and controlling coccidiosis. The safe dose for monensin is between 20 to 50 mg/kg in complete feed (Króliczewska *et al.*, 2023). However, monensin does not significantly reduce methane emissions in ruminants (Króliczewska *et al.*, 2023).

### *Electro-Fermentation and Microencapsulation*

Electro-fermentation is a new technology that aims to control or redirect the fermentation pathway by applying an electric current to the culture medium. This method is considered a bio-electrochemical system or microbial electrochemical technology (Aguilar-González *et al.*, 2023). A bio-electrochemical system is a device or bioreactor where biological processes are carried out along with some components to produce several value-added products. These include electricity in microbial fuel cells, hydrogen in microbial electrolysis cells, or fermentation in electro-fermentation cells (Aguilar-González *et al.*, 2023). An electrochemical stimulation potential of 0.75 volts increases the production of acetate, propionate, and butyrate by 71, 86, and 63%, respectively (Aguilar-González *et al.*, 2023).

The concept of microencapsulation can be categorized into chemical and physical processes that facilitate the isolation of rumen microorganisms from the environment. These processes reduce the impact of gastric acid, bile, enzymes, and other chemicals (Wei *et al.*, 2022). Microencapsulation also protects the encapsulated core ingredients (enzymes or other active ingredients) against rumen degradation by reducing their reactivity or transfer to the external environment and from unfavorable environmental conditions such as pH and microbial communities. This causes an increased bioavailability of the active ingredients in the gastro tract ruminant intestine (Almassri *et al.*, 2024). The method protects encapsulated core ingredients such as enzymes from environmental stress and ensures their targeted delivery, leading to increased bioavailability in the abomasum and gastrointestinal tract (Almassri *et al.*, 2024).

### *Rumen Dysfunction*

Rumen disorder is a major threat to the health and welfare of ruminants, with the most common type being digestive (Kinde and Asfaw, 2021). Disorder in the homogeneity of the gastrointestinal microflora has severe effects on the digestive system and various organs. This is because variation in communal relationships contributes to various metabolic disorders, including ruminal acidosis, bloat, diarrhea,ulcers in the gastrointestinal tract, hypoglycemia, and reticuloperitonitis.

Rumen tympany or bloat is a condition where excess gas accumulates in the rumen. This condition can occur in ruminants that are given rations with extremely high quantities of grain or forage (Xu *et al.*, 2021). Bloat in ruminants can be categorized into two types, namely free gas and frothy bloating (Xu *et al.*, 2021). Free gas bloating is associated with pathological/physical problems that inhibit the release of gas from the stomach. This condition requires manual action to remove gas when the bloating is caused by physical obstruction (Xu *et al.*, 2021). Esophageal obstruction, cysts, thoracic or cervical enlargement, tumors, hypocalcemia, and reticular dysfunction are the main conditions affecting eructation or gas belching (Xu *et al.*, 2021). Frothy bloating is caused by continuous feed consumption leading to foam that cannot be easily removed from the stomach, containing both hydrophobic and hydrophilic properties. Froth is obtained from the digestion of some polymer compounds including fatty acids, lipopolysaccharides, glycolipids, and glycans. The presence of these partially digested compounds increases rumen viscosity and inhibits gas elimination. Gas distension puts pressure on nearby organs, causing pain, edema, organ failure, and death. The strategies that can be applied to treat free gas and frothy bloating include using a stomach tube to remove gas and partially digested feed, administering anti-froth agents, and installing a fistula or cannula (Xu *et al.*, 2021).

High accumulation of organic acids in the rumen shows an imbalance between microbial production, use, and rumen absorption of organic acids (Golder and Lean, 2024). The phenomenon is caused by consuming feed ingredients that are more easily fermented and rich in carbohydrates such as molasses, sugar beets, and grains (Xu *et al.*, 2021). Fermentation of these compounds causes the production of high amounts of lactic acid so that the rumen pH decreases drastically. Moreover, a significant decrease in rumen pH can damage the epithelium causing ulcers and mucosal inflammation, as well as inhibiting cellulolytic bacteria to increase propionate-producing bacteria (Xu *et al.*, 2021). Changes in rumen microbiota cause improper metabolism, leading to liver dysfunction, lung disorders, and death (Xu *et al.*, 2021).

Hypoglycemia occurs when the rate of glucose absorption is very low compared to use. Carbohydrate deficiency causes hypoglycemia because glucose is needed in the gluconeogenesis process (Xu *et al.*, 2021). Disharmony in rumen microbiota activity causes disruption of polysaccharide degradation which affects glucose exchange. To overcome this problem, the provision of appropriate ration in terms of quantity and quality considering environmental cleanliness and adding cellulolytic bacteria has been proven effective (Xu *et al.*, 2021). A serious problem that commonly occurs in the stomach of ruminants, particularly in calves, is diarrhea. This disorder is associated with various symptoms including electrolyte imbalance, dehydration, and weakness/lethargy (Xu *et al.*, 2021). Bacterial infections (*Salmonella sp*, *Mycobacterium paratuberculosis*, *Enterobacter sp*, *Clostridium perfringens*, and *Staphylococcus*), virus *(Rotavirus* and *Adenovirus*), parasite (*Trichonema sp* and *Strongylus sp*), and protozoa *(Entamoeba sp*, *Trichomonas sp*, and *Giardia sp*) can also cause diarrhea (Xu *et al.*, 2021). In the gastrointestinal tract, ulcers often occur in the digestive tract of ruminants, specifically in the abomasum and duodenum. Gastrointestinal ulcers are caused by improper feed intake, stress due to overgrazing, malnutrition, and microbial infections. Perforated ulcers generally have a detrimental effect on the epithelium of the gastrointestinal tract when compared with non-perforated ulcers. Disorders of the outer epithelium of the digestive tract are caused by acid production and can be overcome through the administration of probiotics containing lactic acid. Antihistamines with iron injections can also reduce pain and bleeding in adult ruminants (Xu *et al.*, 2021). Reticuloperitonitis or reticulitis, is a disorder caused by unsystematic feeding. Irregular feeding habits cause disturbances in the disharmony of rumen microbiota (Xu *et al.*, 2021).

### *Correlation between Rumen Performance Towards Ruminant Productivity*

Ruminants and microbes in the rumen have a mutualistic symbiotic relationship (Perez *et al.*, 2024). This relationship is very important considering that ruminants do not produce the enzymes needed to break down consumed feed fiber, but have an abundant consortium of microbes in the rumen that generate a series of enzymes to digest feed, particularly plant polysaccharides (Terry *et al.*, 2019). Specifically, microorganisms produce fibrolytic enzymes to digest feed into energy that can be used for ruminants (Cammack *et al.*, 2018; Tapio *et al.*, 2023) and the host provides anaerobic fermentation space (rumen) (Sanjorjo *et al.*, 2023). The symbiosis between the rumen microbiota and the host depends on the balance of the host environment and microbial fermentation (Cammack *et al.*, 2018). The interactions between the rumen microbiota and their hosts contribute to variation in many host-expressed phenotypic traits (Cammack *et al.*, 2018) and are important prerequisites for ensuring host health as well as optimal ruminant productivity (Liu *et al.*, 2021; Sha *et al.*, 2024). Phenotypic traits mediated by microbes and expressed through the host include feed efficiency, daily body weight gain, as well as the ratio of body weight gain to feed (Na and Guan, 2022). Host shows some traits that are related to the presence of microbial taxa (*Prevotellaceae, Lachnospiraceae, Ruminococcaceae* family) suggesting the function of rumen microbiota in the ruminant digestive process (Qi *et al.*, 2024), health, nutrient use (Clemmons *et al.*, 2019), and production (Zhao *et al.*, 2024).

Various nutrients are produced by rumen microorganisms for the host such as organic acids that function as glucogenic precursors, protein, as well as vitamins (B and K) (Greenwood *et al.*, 2024). Pantothenate is among the nutrients made and it is important for breaking down fatty acids. It serves as a main part of coenzyme A (CoA) required to perform several functions in the metabolism of ruminant intermediates like moving fatty acids in and out of mitochondria (Clemmons *et al.*, 2019). Furthermore, pantothenate is made by some bacteria in the rumen, particularly Flavobacteria (Clemmons *et al.*, 2019). There is potential for rumen bacteria to degrade lignocellulosic biomass and convert it into VFA that are used in urea, ammonia metabolism, and microbial protein synthesis (Liang *et al.*, 2024). According to Liang *et al.*, (2024), rumen bacteria along with methanogens degrade lignocellulose and consume $H_2$ to support carbon, nitrogen, and hydrogen metabolism (Liang *et al.*, 2024).

Major decomposers of carbohydrate are rumen microbiota because of the ability to secrete carbohydrate-active enzymes (CAZyme) like glycoside hydrolase and glycosyl transferase (Liang *et al.*, 2024). In rumen, microbial activity that degrades feed ingredients anaerobically can meet about 70% of the energy needed by ruminants (Wang *et al.*, 2022; Khairunisa *et al.*, 2023). According to (Muñoz-Tamayo *et al.*, 2023), the fermentation process helps ruminants get nutrients from different types of feed. The rumen, as part of the digestive system in ruminants, is like a small ecosystem that lets them digest many kinds of feed, especially plant-based ones (Zhao *et al.*, 2024). Plant fibers consumed by humans will be fermented by microbes to become final fermentation products in the form of VFA, carbon dioxide ($CO_2$), hydrogen ($H_2$), water ($H_2O$), ammonia ($NH_3$) and methane ($CH_4$) (Cammack *et al.*, 2018; Susilo *et al.*, 2019; Andersen *et al.*, 2021; Tapio *et al.*, 2023).

The host absorbs VFA through rumen epithelium and uses it as an energy source (Mayulu *et al.*, 2023; Tapio *et al.*, 2023; Perez *et al.*, 2024). VFA plays an important role in muscle development (meat-forming components) or the synthesis of milk components (Zhao *et al.*, 2024) and basic ruminant metabolism such as fatty acid synthesis and gluconeogenesis. It also acts as host energy source (Xu *et al.*, 2021; Zhao *et al.*, 2024) to meet production performance (Wang *et al.*, 2020). After digesting food, ruminants produce microbial crude protein (Liu *et al.*, 2021; Tapio *et al.*, 2023), which is obtained by the small intestine and used as amino acids (Perez *et al.*, 2024). Lima *et al.*, (2023) reported that microbial crude protein constituted amino acids that ruminants required for making milk and meat protein (Lima *et al.*, 2023).

## Conclusion

In conclusion, this review showed that digestion and basic metabolism occurred in the rumen of ruminants. Rumen also served as an ecosystem of microbiota including bacteria, protozoa, fungi, archaea, and viruses that played a role in the sustainable ruminant production, environmental responsibility, and human welfare. Based on the results, it was discovered that optimizing rumen microbiota improved ruminant welfare and productivity, addressing the environmental impacts associated with ruminant production (methane emissions). In digestive processes, energy metabolism, and host immune regulation, microbiota influence the survival, production, and reproduction capabilities of ruminants in certain environments. To optimize rumen performance, various dysfunctions and manipulations could be performed, including plant-based bioactive compounds, chemical interventions, electro-fermentation, and microencapsulation. Rumen contained microbial taxa (*Prevotellaceae, Lachnospiraceae, Ruminococcaceae* families) related to feed efficiency traits, daily body weight gain, and the ratio of body weight gain to feed. Fermentation activity in the rumen produced various final products such as VFA, $H_2$, $CH_4$, $CO_2$, vitamins (B and K), microbial crude protein, and other bioactive molecules. The end products of fermentation synthesized by microbes were found to be essential for host function, the development of epithelium, the formation of the immune system, and maintaining the balance patterns. Rumen microbiota also influences intestinal metabolism, nutrient absorption, feed efficiency, and immune responses, and mediates host function, thereby determining the performance of ruminant productivity.

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## Author's Contributions

**Hamdi Mayulu**: Conceptualized the study, developed the methodology, and contributed to the original draft preparation.

**Merry Christiyato**: Contributed to writing, and was responsible for reviewing and editing the manuscript.

## Ethics

All references related to the enrichment of this scientific knowledge have been included in the bibliography of the journal.

### *Declaration of Competing Interest*

The authors declare no conflict of financial interests or personal relationships that could affect the work reported in this review.

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