fermentation

Review

# In Pursuit of Understanding the Rumen Microbiome

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**Abstract:** The rumen plays an indispensable role in ruminants to utilize ligno-cellulosic material and convert non-protein nitrogen into nutrients otherwise unavailable for human consumption. Recent advancements in the sequencing technology and omics approach have provided profound insights into the rumen world, wherein a consortium of archaea, bacteria, protozoa, fungi, and viruses exist and interact. These ruminal microbes alter the ruminal environment and execute several interlinked metabolic cascades that produce substrates for the host's energy and body requirements. Methane is emitted as a by-product during this complex fermentation process in ruminants leading to a loss in productivity while negatively impacting the environment. As ruminants play an ever-increasing role in our food supply chain, manipulating the rumen is the critical step towards maximizing the ruminant product's nutritional value while reducing its carbon footprint. Diet is the most straightforward way to alter the rumen microbiome, possibly in conjunction with phytobiotics and probiotics as feed supplements. Early life interventions allow the manipulation of microbial population structure and function that could persist later on in adult life. It has also been proven that the host exerts influence on the rumen microbiome as a heritable trait. The goal of this review is to provide a better understanding of the rumen, its key organisms, and its development to better identify, characterize, and engineer the rumen microbiome for efficient feed conversion and methane reduction.

**Keywords:** ruminants; rumen microbiome; rumen fermentation; methane; global warming

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## 1. Introduction

Ruminants are cloven-hoofed mammals of the *Artiodactyla* order, with domesticated cattle, sheep, and goats comprising 95% of the total ruminant population [1]. They do not produce cellulolytic or hemicellulolytic enzymes, but rely on the cooperative works among rumen microbes to degrade complex plant polysaccharides [2]. The rumen microbiome is the most diverse gut ecosystem in the animal kingdom and is composed of bacteria, protozoa, fungi, archaea, and phages [3,4]. Anaerobic rumen fermentation by a complex group of rumen microbes converts indigestible forages, food by-products, and non-protein nitrogen into high-grade meat and dairy products for human consumption [5]. The primary products of the rumen fermentation are short volatile fatty acids (SVFAs; predominantly acetate, propionate, and butyrate) and microbial crude protein. The SVFAs serve as an essential energy source, providing up to 70–80% of the host energy requirements [6] and, in the process, generates ATP for the synthesis of microbial cellular protein. Upon digestion, this microbial protein supplies 60% to 85% of the amino acids reaching the small intestine [7].

However, methane is generated during this fermentative process, which is then eructed into the environment, increasing methane concentration in the atmosphere [8]. This gas by-product is a short-lived climate pollutant with a lifetime of only 12.5 years in the atmosphere and 80 times more potent than carbon dioxide over 10–20 years [9,10]. According to

Environmental Protection Agency (EPA) and National Oceanic and Atmospheric Administration (NOAA), atmospheric concentrations of the major greenhouse gases such as carbon dioxide and methane have increased since 1950 from 350 to 410 ppm (28%) and 1100 to over 1900 ppb (70%), respectively [11,12]. Hence, methane emission into the atmosphere causes the earth a surge in global average temperature and brings host animals a loss of 2–12% energy which could otherwise be used for meat and milk production [13,14]. Moreover, the world's population is set to reach 9.8 billion in 2050 [15], increasing the demand for dairy and meat products by 1.04 million tons and 465 million tons, respectively. In addition, population growth and rapid urbanization can further intensify the challenges to tackling food insecurity, causing the food system to face increased demands for animal source foods [16]. Hence, to ensure sustainable global food supply, it is highly critical to understand the rumen microbiome and its role in feed digestion as well as methane production (Figure 1). This review article aims to provide a brief overview of rumen fermentation, microbial communities, and how the rumen microbiome is affected by various factors.

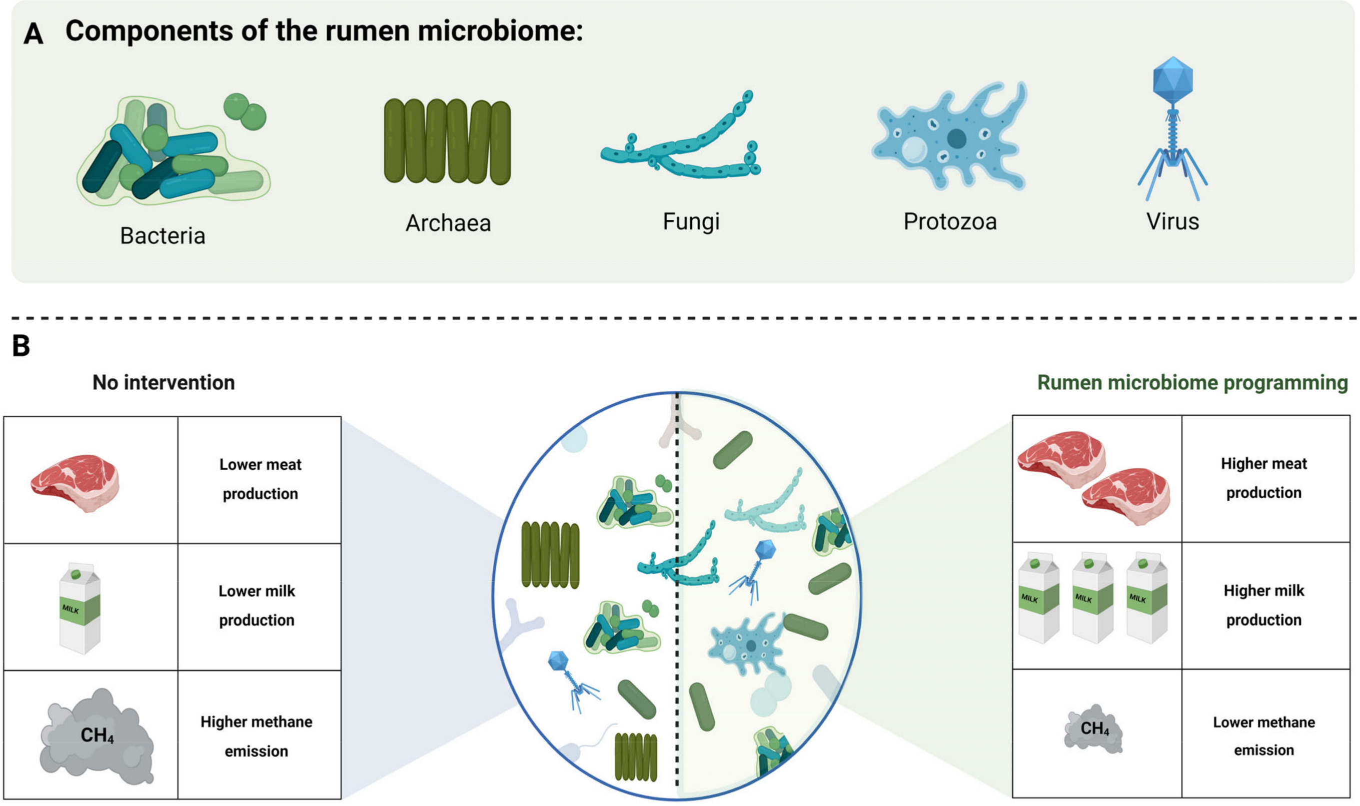

**Figure 1.** (**A**) Components of the rumen microbiome: bacteria, archaea, fungi, protozoa, and virus. (**B**) Improvement in the milk and meat production and reduction in methane emission through rumen microbiome programming.

## 2. Rumen Development

The digestive system of an adult ruminant consists of four compartments: rumen, reticulum, omasum, and abomasum [17]. At birth, all four compartments except the abomasum are anatomically undeveloped and metabolically non-functional [18]. The development of rumen is greatly affected by the nature of diet or feeding method. Hence, special care should be taken when transitioning from a liquid (milk) to a solid diet, as improper development of the rumen can impair critical functions, such as immune system, absorption, transportation, and metabolism of short-chain fatty acids [19,20]. As stated by Heinrichs [21], a smooth metabolic and physiological transition from a monogastric to a ruminant animal requires the development of the reticulorumen and its associated microorganisms. As a result of the rudimentary state of the reticulorumen and omasum, the presence of the esophageal groove, and the development of intestinal enzymatic state, newly born ruminants function as monogastric animals until these systems are fully developed [21,22]. When the calves are born, the weights of reticulorumen, omasum, and abomasum make up 38%, 13%, and 49% of the entire stomach weight, respectively. When the digestive system fully develops, their weight proportions will change to 67%, 18%, and 15% of the stomach weight, respectively (Figure 2) [23].

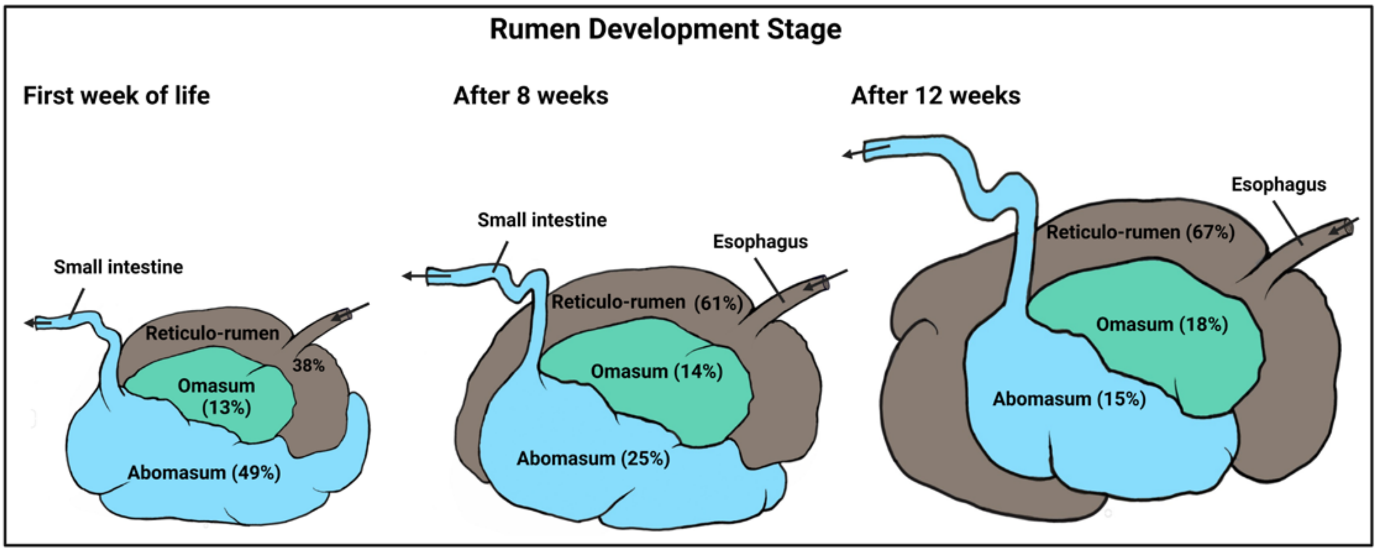

**Figure 2.** Transitioning of ruminant digestive system from early life to maturity [23].

Rumen development can be categorized into three phases: (i) non-rumination phase (from birth to 21 days), (ii) transitional phase (from 21 to 56 days), and (iii) rumination phase (from 56 days onward) [24]. During the rumen development, specific physiological or functional events occur, such as anatomical development, where the rumen mass and papillae grow; functional achievement, where the rumen achieves its enzymatic and fermentation activity; and establishment of rumen microbiota during which microbes begin to colonize [25]. Colonizing the gastrointestinal tract (GIT) by rumen microbiome is critical for normal neonate health, development, and intestinal mucosal immunity. There is a consensus that microbial colonization in the rumen occurs immediately after birth [26,27]. However, recent studies have demonstrated that microbes exist in the GIT of ruminant animals even before their birth and provided more profound insights into the dynamic fluctuations in the microbial community from fetus to adulthood [28,29].

## 3. Rumen Microbiome

Enormous efforts have been made to study the composition of the rumen microbial community and its dynamics with parameters such as diet, age, and host species. Since the 1940s, Robert Hungate, the father of rumen microbiology [30], pioneered this field and laid out many fundamental tenets in understanding rumen fermentation using culture-based techniques [31]. Recent advancements in culture-independent high-throughput sequencing technologies have greatly expanded the scope of the rumen microbiome enabling better analysis of the structure and function of the rumen ecosystem.

### *3.1. Bacteria*

Bacteria is the most abundant, diverse, and metabolically active group [32] among other rumen microbes, accounting for approximately 50–70% of the rumen microbial population with $10^{10}$–$10^{11}$ bacterial cells per gram of rumen content [4,13]. Their diversity and abundance in the rumen have been studied through a meta-analysis of 16S ribosomal RNA (rRNA) gene sequences [13]. Most studies have used genus-level identification for taxonomic assignment of 16S rRNA sequencing data from rumen samples primarily due to the short-length sequencing reads and lack of reference genome, making the resolution for the species level identification difficult and unreliable [33,34]. Guo et al. could identify slightly over 1% of the total OTUs to species level in Holstein cows ranging in age from 1 week to 5 years old [35]. However, it is estimated that there are over 7000 species of bacteria representing over 19 diverse phyla in the rumen [36]. In the studies reported to date, *Firmicutes* and *Bacteroidetes* are consistently the most predominant phyla, followed by *Proteobacteria* in the ruminants from cattle, such as dairy cows [37,38], buffalo [39], sheep [40], and yaks [41] to non-domesticated ruminants, such as Cervids [42]. The Hungate 1000 project in 2012 produced 480 bacterial genomes from diverse rumen samples. It revealed that members of the *Firmicutes* and *Bacteroidetes* phyla predominate in the rumen, contributing 68% and 12.8% of the Hungate genome sequences, respectively [43]. More recently, the Global Rumen Census report covered 742 samples across 32 different species from different geographical regions. It revealed that the 30 most abundant bacterial groups were all found in almost all samples accounting for 89.4% of all sequence data. Moreover, the structure of these core bacterial groups is strikingly similar in all parts of the world [44]. Several groups of bacteria belonging to phyla, such as *Actinobacteria, Acidobacteria, Tenericutes, Spirochaetes,* and *Verrucomicrobia,* have been found in the rumen in lower populations. Several species from genera, such as *Ruminococcus, Butyrivibrio, Prevotella, Fibrobacter, Coprococcus, Porphyromonas,* and *Butyrivibrio,* constitute the core rumen microbiome. They have also been found across different GIT segments (abomasum, duodenum, and rectum) [27]. Many rumen bacteria harbor several genes encoding various carbohydrate-active enzymes (CAZymes) that act synergistically to degrade plant lignocellulose [45]. For instance, metagenomic analysis revealed that the majority of the CAZyme-encoding gene fragments detected in sheep rumen samples belonged to the genera, such as Prevotella, Bacteroides, Fibrobacter, Ruminococcus, and Alistipes. CAZyme-encoding gene fragments found in this study were carbohydrate binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyl transferases (GT), and polysaccharide lyases (PL) families [46].

### *3.2. Archaea*

Members of the archaeal domain make up less than 3.3% of the total rumen rRNA (both 16S and 18S), of which the majority belong to a group of methane producers known as methanogen [47]. Despite their low population, many groups have made an overwhelming effort for decades to suppress the growth of this specific group of microbes by using numerous biological and chemical additives [48–50]. Methanogens are classified as free-living, epithelial, and protozoa-associated depending on their growth type [51]. The population of metabolically active methanogens associated with protozoa was found to be the highest because protozoa house an active archaeal population on both their interior and outer surface [52]. Methanogens utilize $H_2$ as an energy source [53] to reduce methane derivatives (methylotrophic), carbon dioxide (hydrogenotrophic), and acetate (acetoclastic) to $CH_4$, which is essential to prevent the accumulation of reducing equivalents and the overall inhibition of ruminal fermentation [47]. Of all three, hydrogenotrophic methanogen remains the most abundant member constituting about 78% of the total archaea, followed by methylotrophic methanogens accounting for up to 22%, while acetoclastic methanogen is the rarest among all [54]. Similar to bacteria, the structure of the archaeal community is surprisingly similar across ruminant species and all regions of the world [44,55]. However, the rumen archaea are much less diverse than bacteria, probably due to the limited number of substrates they can use [56]. The archaeal domain consists of seven orders: *Methanobacteriales, Methanosarcinales, Methanomicrobiales, Methanococcales, Methanomicrobiales, Methanopyrales,* and *Methanomassiliicoccales* [57]. *Methanobrevibacter* was consistently found to be the most dominant and ubiquitous genus across all rumen samples, followed by *Methanosphaera, Methanomicrobium,* and members from *Thermoplasmatales* [58,59]. A report on the initial colonization of methanogens in the rumen has revealed that metabolically active methanogens, such as *Methanomicrobiales mobile, Methanoccocales votae,* and *Methanobrevibacter* spp., were detected in the rumen of calves from as early as 20 min after birth [60]. Wang et al. observed active members of the genera *Methanobrevibacter*, *Methanomethylophilus*, and *Methanosphaera* colonizing the rumen of goats one day after birth [53]. Hence, these findings suggest the existence of alternative hydrogen providers in the rumen to support the growth of methanogens during these early stages of postnatal development [60].

### 3.3. Fungi

Orpin first discovered the presence of anaerobic rumen fungi in 1975. He revealed multiple aspects of the rumen fungi, including their anaerobic nature, the presence of chitin in the cell wall, and their colonization of the plant fiber [61]. Orpin also successfully isolated the anaerobic fungus *Neocallimastix jrontalis* from the rumen of sheep and suggested that it was closely related to chytrid fungi, which was later formally assigned to a new family *Neocallimastigaceae* [62]. Since then, much attention has been given to this unique phylum, mainly due to its role in efficiently degrading recalcitrant plant material [63]. The fungal community represents about 10–20% of the rumen microbiome based on rRNA transcript, with significant fluctuation depending on the diet [64]. Host species affect the structure of the fungal community, as Guo Wei et al. showed that alpha-diversity indices in Tibetan yak are significantly greater than in sheep [65]. Phyla, such as *Neocallimastigomycota*, *Basidiomycota*, and *Ascomycota,* are known to exist in the rumen, and *Neocallimastigomycota* is the most dominant phylum across ruminant species, including cattle, sheep, yak, and camel [65–67]. Many fungal genera, such as *Neocallimastix, Piromyces, Anaeromyces, Caecomyces, Orpinomyces,* and *Cyllamyces,* have been described and characterized in detail based on their thallus morphology and zoospore ultrastructure. Even though microscopic data have been critical for fungal taxonomic classification in the past, this approach resulted in numerous complications due to the wide morphological variations, the absence of zoosporogenesis in some polycentric species, and similarities in morphological features. Hence, recent studies have adopted the rRNA operon-based analysis (using internal transcribed spacer 1 or D1/D2 region of the large 28S rRNA subunit) as a phylogenetic marker or a broader, whole-genome phylogenomic approach [68,69]. Rumen fungi synthesize high levels of cellulases, hemicellulases, and xylanases and perform a critical role in the initial colonization of lignin-containing tissues of forage and degradation of the plant cell wall with the help of hyphae [61,70]. It is increasingly becoming clear that anaerobic rumen fungi express CAZymes that allow fungi to become key players in digesting the plant cell wall in a strictly anaerobic rumen ecosystem. These crucial sets of enzymes have been preserved throughout the evolution of the rumen fungi, especially the *Neocallimastigomycota* phylum, facilitating this group of microorganisms to survive in the competitive rumen environment [63,71,72]. Similar to bacteria and archaea, the abundance, richness, and activity of the rumen fungi in the rumen depend heavily on the diet, host phylogeny, host genetics, and other rumen microorganisms [69].

### 3.4. Protozoa

Rumen protozoa are primarily ciliates, strictly anaerobic, and constitute the eukaryotic portion of the rumen microbiome along with fungi. Ciliate protozoa were the first microorganisms to be documented from rumen samples [73], and microscopy has been the chosen method in identifying the rumen protozoa for many decades [73]. The role of ciliate protozoa in rumen fiber degradation still needs to be better understood, as it has proven impossible to maintain rumen protozoa in axenic culture [74]. The population of protozoa in the rumen varies between $10^4$ and $10^6$ cells per mL of ruminal content and represents up to 50% of the ruminal biomass [74]. In several reports on protozoal community structure, *Entodinium* is the most dominant genus ($1.17 \times 10^6$ cells/mL of rumen liquor) in the rumen accounting for up to 90% of the total protozoal population across different ruminants. Other protozoal genera, such as *Dasytricha, Ostracodinium, Diplodinium, Diploplastron, Eudiplodinium, Epidinium, Ophryoscolex*, and *Polyplastron,* have also been found in the rumen [73,75–78]. Depending on their morphological features, ciliate protozoa can be classified into Holotrich and Entodiniomorphid protozoa. Entodiniomorphid protozoa contain a firm pellicle and cilia on the peristome, whereas the Holotrich protozoa are entirely covered with cilia [79]. Ciliate protozoa are characterized by their ability to harbor both epi- and endo-symbiotic methanogens [52]. The robust functional association between methanogens and protozoa community via interspecies hydrogen transfer significantly contributes to the overall $CH_4$ production in the rumen as demonstrated by the elimination of certain protozoa or defaunation [78]. The defaunation in the rumen could increase microbial protein supply by up to 30% and reduces methanogenesis by up to 11% [74]. These ciliates have a specialized organelle, known as hydrogenosome, that can metabolize pyruvate, synthesizing acetate, hydrogen, and carbon dioxide. Acetate produced during this fermentation reaction is then used by the ciliate protozoa as a carbon and energy source, while the methanogen uses $H_2$ and $CO_2$ for methanogenesis [47,80]. Ciliate protozoa contribute to feed degradation directly by breaking down plant fibers as they are known to produce a significantly large quantity of hydrolytic enzymes and indirectly by associating with other rumen microbes [81–83]. According to metagenomic and phylogenetic analysis of over 4000 Expressed Sequence Tag libraries, there is an extensive horizontal gene transfer (HGT) from bacteria and archaea to rumen ciliate protozoa involving numerous essential genes encoding for enzymes integral in carbohydrate metabolism and transportation. Hence, this suggests the close interspecies interaction in the rumen and evolutionary response by the ciliate protozoal to the carbohydrate-rich environment of the rumen [82,84].

### 3.5. Virus

The presence of viruses in the rumen was first discovered in the 1960s using rumen fluid from cattle and rumen isolates of *Serratia* and *Streptococcus* as bacterial hosts [85]. Another early pioneer study in 1969 showed six distinct morphological types of bacteriophages found in bovine rumen contents using an electron microscope [86]. Now it is known that the rumen harbors a dense ($10^7$–$10^9$ particles per gram) and diverse population of viruses co-existing with other rumen microbes [87,88]. However, rumen viral populations are still the least explored and understood compared to other rumen microbial populations. This could be due to various challenges involved in the isolation and characterization process as the isolation of viruses requires the availability of susceptible microbial hosts [89]. Viral sequencing is limited because it requires intact viral particles from environmental samples. A relatively low number of available virus sequences and a high percentage of uncharacterized viral genes further limit genomic/transcriptomic studies. This makes it difficult to annotate gene functions and viral taxonomy [87]. Nevertheless, phages particularly bacteriophages and archaeaphages are becoming an increasingly prominent focus of study due to their potential role in microbial lysis, gene transfer, animal production, and health [90,91]. Based on the recent comprehensive metagenomic analysis by Berg et al., 28,000 diverse viral genotypes were identified and reported that prophages were significantly more abundant than lytic phages (approximately 2:1) in the bovine rumen virome. The metagenomic study also revealed that *Siphoviridae* was the most dominant viral family, followed by *Myoviridae* and *Podoviridae* [92]. Several studies have reported that the most dominant bacterial hosts belong to phyla, such as *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* [92,93]. Since phages can directly integrate into the host genome, they are generally considered to be involved in the mechanism of genetic exchange. This facilitates HGT with other groups of rumen microbes, such as bacteria and archaea [94]. It has been shown that there is a high degree of sequence similarity in almost all putative mobile elements detected in rumen microbial genomes with rumen virome [92]. Viruses are a crucial component of the rumen microbiome and have impacts on substrate availability, nutrient cycling, and genetic exchange with other rumen microbes through HGT [95]. However, more research efforts are still needed to provide insights into the overall significance of phage-host interactions, their activation mechanisms in the rumen, and the biological and physiochemical properties of rumen viruses.

## 4. Rumen Fermentation-Metabolic Cascades

The microbial community of rumen executes complex metabolic cascades in a coordinated fashion, through which continual cross-feeding among the rumen microbiota occurs [96]. Due to sizeable functional redundancy, Moraïs & Mizrahi proposed a concept of functional groups by combining several groups of microbes with similar metabolic activity [97]. Rumen metabolic events begin with the microbial degradation of plant polymers into smaller soluble sugars. They yield vast arrays of metabolites, some channel into the host animal, serving up to 70% of its energy needs [6,98]. A symbiotic relationship between the microorganisms that produce fibrolytic enzymes and the host animal that provides an anaerobic fermentation chamber results in the effective digestion of a fiber-rich diet [2,99]. The entire rumen fermentation is categorized into three stages by the cognate functionality of microbial communities according to the assortment of their input and output metabolites (Figure 3) [96,97]. During the first stage, adherent microbes colonize the plant macromolecules, breaking down the cell wall using enzymes and releasing carbohydrate polymers, such as cellulose, hemicellulose, and starch [100,101]. The multienzyme complex molecular structure, Cellulosome, synthesized by many fibrolytic rumen organisms, facilitates the adherence of microorganisms to plant cells [102]. Since cellulose is the most abundant carbohydrate polymer accounting for up to 40% of the total dry matter and over 50% of the plant cell wall, cellulose degradation is the most crucial process for providing energy to ruminant animals [103]. Degradation of cellulose and hemicellulose into soluble sugars (hexose and pentose) is facilitated by prokaryotic (bacteria) and eukaryotic (fungi and protozoa) microorganisms [61]. In the second stage, rumen microbes metabolize the soluble sugars via various pathways, such as Embden-Meyerhof-Parnas (EMP) or pentose phosphate pathway (PPP), resulting in the excretion of SVFAs, organic acids, other metabolites, and gases, such as carbon dioxide and hydrogen. This crucial fermentative process is conducted by various bacteria (*Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, and *Ruminococci albus*), protozoa (*Entodinium caudatum*, *Enoploplastron triloricatum*, and *Eudiplodinium medium*), and fungi (*Neocallimastix frontalis*) [2]. The host absorbs volatile fatty acids via monocarboxylate transporters (MCTs), which is a principal feature of the evolved synergistic relationship between the microbes and host [98,104]. Electron disposal is the final rumen fermentation process, where methanogenic archaea consume most $H_2$ generated from the second stage of fermentation. During the oxidation of sugars to metabolites, such as acetyl-coA, $NAD^+$ is reduced to NADH. The reduced NADH must be reoxidized to $NAD^+$ to allow continuous fermentation. In the rumen's anaerobic condition, electron acceptor, such as oxygen, is absent and hence, methanogens primarily use $CO_2$ (hydrogenotrophic methanogens) as an electron acceptor. Methanogenic archaea can also reduce methyl compounds (methylotrophic methanogenesis), and acetate (acetoclastic) to methane [105,106]. This fundamental metabolic process is situated at the end of rumen electron flow and conducted predominantly by a few genera of the *Methanobacteriales* and *Methanomicrobiales* orders from the archaeal domain [107]. Moreover, intercellular $H_2$ transfer between methanogens and the fermentative community of bacteria, fungi, and protozoa regulates the $H_2$ concentration in the rumen, as traces of $H_2$ can significantly affect the rumen fermentation. Slight increase in the hydrogen partial pressure can inhibit the ability of many rumen microbes to generate electron carriers and disrupt the microbial metabolism and growth [108,109]. Other electron disposal pathways include nitrate and sulfate reduction to ammonia and sulfide, respectively [107].

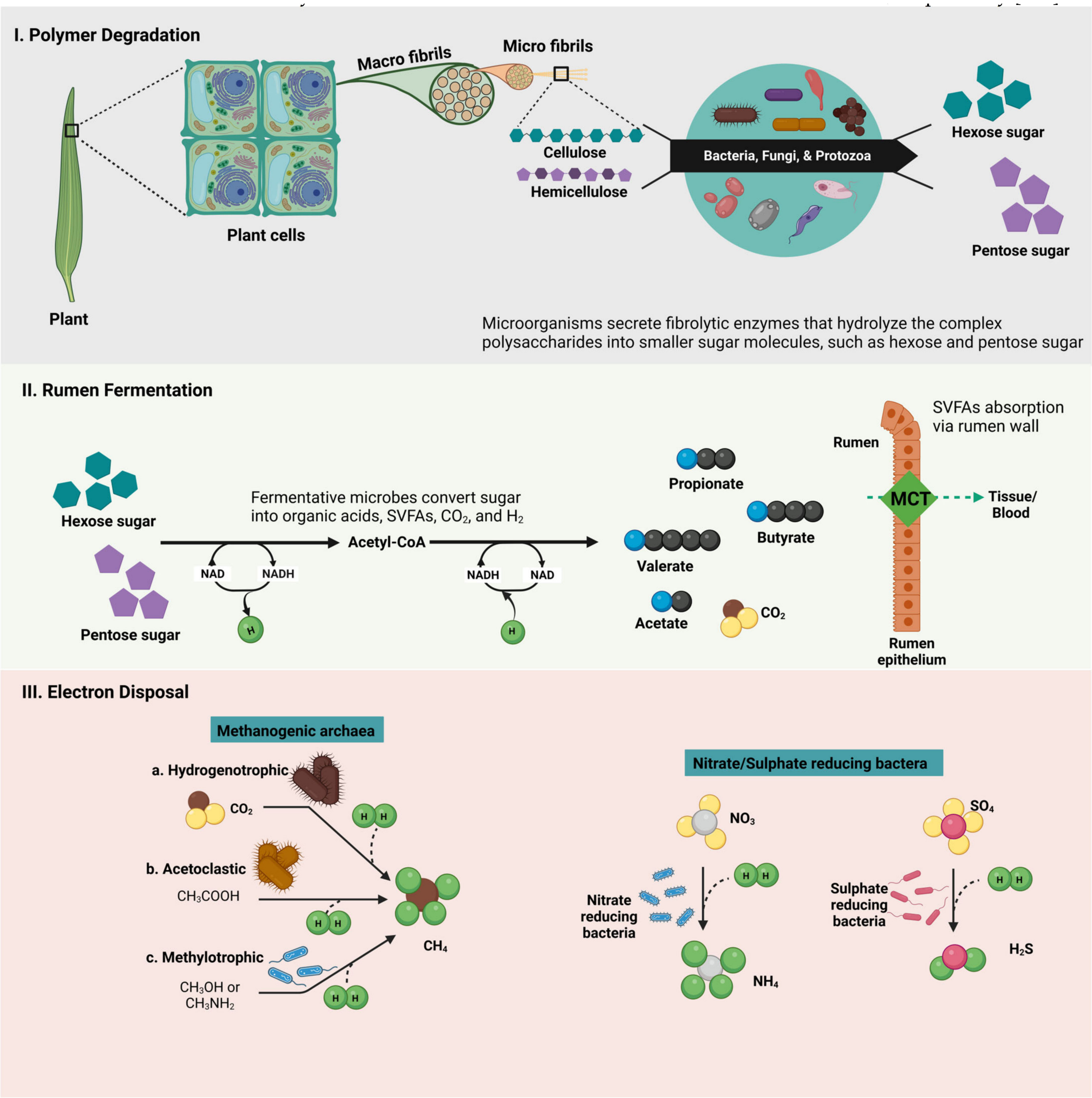

**Figure 3.** Rumen metabolic cascades represented by three stages of metabolic events: polymer degradation (**I**), rumen fermentation (**II**), and electron disposal (**III**). Blue hexagon and purple pentagon represent hexose and pentose sugar, respectively. Dark grey circle and blue circle represent methyl and carboxyl group, respectively. Circles with different color represent different elements: brown (carbon), yellow (oxygen), green (hydrogen), light grey (nitrogen), and pink (sulfur). SVFA: Short volatile fatty acids. MCT: monocarboxylate transporter.

## 5. Factors Affecting Rumen Microbiome

Several key factors that influence rumen microbiota and fermentation, including diet, feed additives, the host, and other early life interventions, are discussed below (Figure 4).

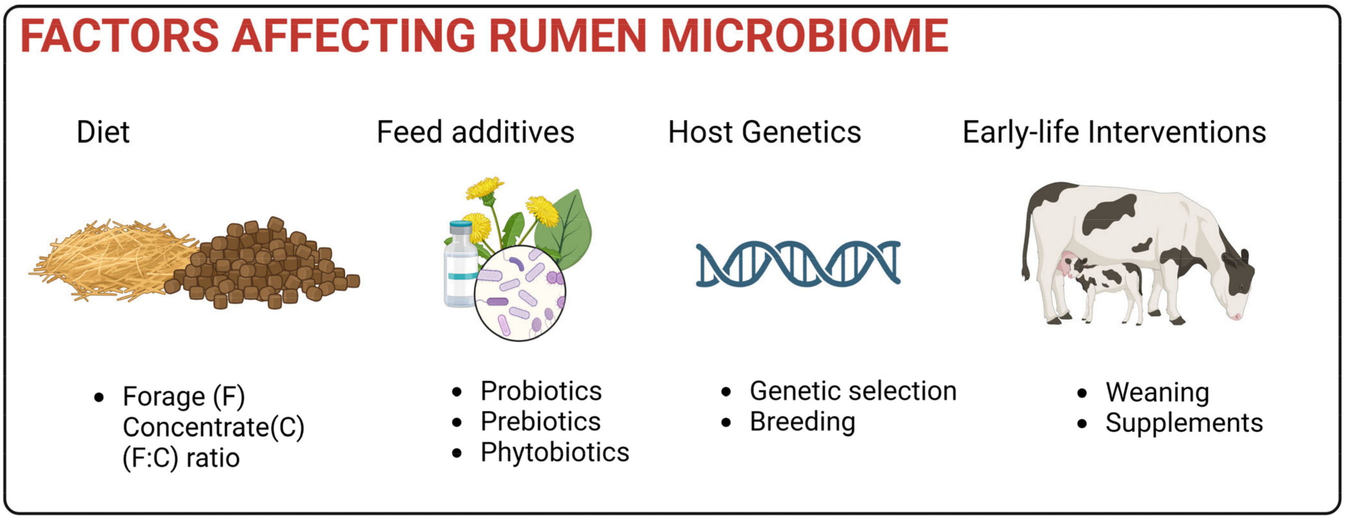

**Figure 4.** Factors affecting the rumen microbiome: Diet is the major factor affecting rumen microbiome. Other factors include supplementation of feed additives, host genetics, and early life interventions.

### *5.1. Impact of Diet*

Diet is one of the major factors affecting ruminal microbial activity [44], influencing the host's feed efficiency and nutrient supply. Hence, an appropriate diet is essential for a healthy rumen environment that improves rumen microbiota and promotes ruminant growth and development. At 28 days of age, calves fed with milk and concentrate had a higher relative abundance of methanogens and bacteria known to degrade readily fermentable carbohydrates than milk fed-only calves [110]. Forages, which mainly consist of neutral detergent fiber (NDF), form the basis of the adult ruminant diet [111]. The amount of NDF provides an approximation of the concentration of cellulose, hemicellulose and lignin [112,113]. The ruminants are usually fed with a mixture of forage, and starch-rich concentrate feeds to balance their energy, protein, minerals, and vitamin requirements. When adult dairy cows were fed an NDF-rich diet than a starch-rich diet, there was an increase in the biodiversity of bacteria and fungi, and the rumen concentrations of protozoa, anaerobic fungi, and methanogens in the cows. This was attributed to NDF-rich diets providing less acidic conditions which microorganisms can grow faster and generating a more comprehensive range of cellulosic and heteropolysaccharide substrates than starch diets [114]. Conversely, in diets with an increasing proportion of starch-rich concentrates, ruminal pH decreases linearly due to the excessive production of SVFAs by rumen microorganisms as there was a larger amount of fermentable carbohydrates [115]. This subsequently causes ruminal acidosis leading to dysbiosis affecting bacterial richness and diversity negatively [114–117]. However, propionate concentration increased along with total protein in blood serum, which led to higher animal productivity [118,119]. In yaks, amylolytic *Bacteroidota* and cellulolytic *Firmicutes* decreased with increasing starch-rich concentrate levels [117]. In another study by Zhang et al., Holstein heifers fed with a high concentrate diet negatively impacted bacterial and archaeal richness and diversity but not anaerobic fungi richness and diversity [115]. Overall, an increase in the proportion of fiber in the diet results in a corresponding increase in the abundance and diversity of ruminal bacteria, fungi, and protozoa [120], but decreases when dietary F:C ratio is lowered. These findings showed how rumen microbiota changes according to the diet provided.

### *5.2. Impact of host*

There has been a growing consensus in recent mammalian host-microbial interaction studies [121–123] regarding the nature of the microbial composition of the gastrointestinal tract as a polygenic trait. Various genome-wide association studies have been conducted to identify host chromosomal regions that influence microbiome composition and function in the rumen. In a cohort study of 709 beef cattle, it was found that 19 single nucleotide polymorphisms (SNPs) located on 12 bovine chromosomes were associated with 14 rumen microbial taxa, of which five were known quantitative trait loci for feed efficiency in cattle [124]. Breeding has also demonstrated a significant correlation between host and microbes. In a unique multibreed of Angus-Brahman herd from 100% Angus (*Bos taurus*) to 100% Brahman (*Bos indicus*), about 30% of the microbial community was found to be significantly associated with breed composition. Especially, SNP markers located in or near mucin-coding were strongly correlated with breed composition and contributed to the differences in the relative abundance of mucin-degrading bacteria (*Clostridium, Rikenellaceae,* and *Akkermansia*). Mucin is a critical component of the GI tract defense system in which elevated presence of mucin-degrading bacteria can cause increased susceptibility to GI pathogens [125]. In a separate study, rumen metabolite and rumen microbiome patterns among sika deer (*Cervus nippon*) and elk (*Cervus elaphus*) hybrids showed strong correlation along with changes in the amount of SVFAs and amino acids. Pathways associated with alanine, arginine, proline, and phenylalanine were enriched, which correlated positively with the abundance in *Prevotella* spp., *Acetitomaculum* spp., *Quinella* spp., *Succinivibrio* spp. and *Ruminobacter* spp. The rumen microbiota in the hybrids differed from that in their parents as well. This suggests that there is a significant effect of host genetics on the rumen microbiome that may have resulted from vertical transmission [126].

Moreover, the ruminal features of the host include not just the host's genetic components but also the heritability of its rumen microbiota [127]. Li et al. found out that *Bacteroidetes*, the predominant bacterial phylum had low heritability estimates in cattle and were primarily affected by dietary interventions. On the other hand, the phylum *Firmicutes*, usually composed of fibrolytic organisms involved in starch hydrolysis that could produce acetate, formate, and succinate [128], had moderate heritability estimates. Major butyrate producers, such as *Butyrivibrio* [129] under the family *Lachnospiraceae,* were not heritable in the rumen [124]. Nevertheless, these suggests that some rumen microbial features are heritable. Therefore, genetic selection and breeding can be applied to modify the rumen microbial taxa, but it is unlikely to exert any effect if driven by external factors, such as diet.

As much as gut microbes are influenced by hosts, another key aspect of gut biology is the regulation of host physiology by intestinal microbes. In several studies, gut microbes whether indigenous or pathogenic, have been shown to influence intestinal stem cell (ISC) activity and change intestinal morphology [130]. In mice fed with *Bifidobacterium* and *Lactobacillus* spp., the diet significantly increased ISC proliferation, resulting in improved gut barrier function, while conferring protection against gut injury induced by radiation exposure and chemotherapy drug treatment [131]. The colonization of *Bacteroides thetaiotaomicron*, a prominent member of the intestine microflora in humans and mice, upregulates genes involved with intestinal transport and barrier function [132]. Intestinal microbes also regulate immune response as the ubiquitous *Bacteroides fragilis* corrected systemic Tcell deficiencies in germ-free mice [133]. Ruminants often experience microbiome dysbiosis including gastrointestinal associated diseases or metabolic disorders such as ruminal acidosis and ketosis [27]. These diseases reduce ruminal epithelial cell proliferation and threaten the integrity of the rumen [134], which can be repaired by supplementation of specific microbial strains. However, information regarding specific ruminal bacteria and their interaction with ISC remains to be further elucidated. Regardless, there has already been increasing evidence of continuous interaction between gut microbiota and intestinal stem cells forming a niche that is vital to maintaining a healthy epithelial environment [135,136].

### 5.3. Feed Additives

Feed additives, such as probiotics, prebiotics, and phytobiotics, have been increasingly used to promote the health of ruminants and minimize methane emissions from the rumen. These additives modulate the rumen microbial community and are essential to animal nutrition. Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host" [137], and act to outcompete and replace pathogenic bacteria in the gastrointestinal tract. They are involved in food digestion and the secretion of organic acids and several metabolites which regulate the rumen microbial community [138], leading to increased animal productivity [139]. The most commonly used probiotic preparations are the lactic acid bacteria (*Lactobacillus*, *Streptococci*, *Bifidobacteria*), *Propionibacterium*, *Enterococcus*, the fungi yeast *Saccharomyces*, and filamentous fungi *Aspergillus* [140]. In goats, the addition of *Lactobacillus rhamnosus* and *Enterococcus faecalis* favored the dominance of beneficial fibrolytic or cellulolytic bacteria regardless of dietary treatment [141], which is observed similarly in cattle using yeast as a probiotic [142]. A multispecies probiotic mixture of *Bacillus subtilis, Lactobacillus acidophilus,* and *Saccharomyces cerevisiae* was inoculated into newborn female calves, significantly leading to higher average daily gain in the first eight weeks after birth while improving immune function and decreasing the incidence of diarrhea. It also resulted in an increased relative abundance of fiber-degrading *Ruminococcaceae* and *Bifidobacterium,* which plays a vital role in immune support [143]. Nowadays, probiotics are supplemented with prebiotics to boost their effect. Prebiotics are non-digestible dietary substances that stimulate the activity of beneficial microbes in the gut [144]. These are usually oligosaccharides which, when fermented by probiotics, confer benefits on the host [145,146] by altering the gastrointestinal microflora [147].

Alternatively, natural additives of plant origin, such as essential oils or herbs, are used as candidates for reducing methane emissions, as antimicrobials and for improving animal productivity [148]. Recent issues with antimicrobial drug residues polluting the environment have highlighted the role of phytobiotics or phytonutrients as a replacement to modulate rumen fermentation and influence the microbiota structure [149]. These include tannins, flavonoids, and essential oils, which are plant secondary metabolites with anti-inflammatory, antioxidant, and antimicrobial properties [150]. Orzuna-Orzuna et al. performed a meta-analysis on the effect of tannins showing decreased methane production with no apparent effect on weight gain, feed consumption, and feed efficiency on beef cattle across 32 studies [151]. However, some sources of tannins, such as *Leucaena leucocephala, Acacia negra,* and *Uncaria gambir,* exert antimicrobial activity against the rumen protozoa and methanogenic bacteria [151].

On the other hand, essential oils can increase dry matter intake and daily weight gain of beef cattle. At the same time, it improves feed efficiency, likely attributed to its positive effects on rumen fungal and fibrolytic bacterial populations [152]. Phytobiotic-rich herbal extracts contain an extensive array of organic compounds that may be useful in animal nutrition. In dairy cows, rosemary extract influenced the microbiota of dairy cows, especially the abundance of the genus *Prevotella*. It not only exerted anti-inflammatory and antioxidative properties on the cattle, but also increased propionate production and maintained pH stability in the rumen [153]. The flavonoid-rich alcohol extract of a Mongolian medicinal herb *Allium mongolicum* Regel (AME), increased the relative abundance of fibrolytic bacteria, but decreased those bacteria associated with propionate production, such as *Prevotella, Succiniclasticum,* and *Selenomonas,* leading to a decreased propionate production in lambs. Furthermore, AME supplementation did not affect the palatability in the diet but promoted the secretion of an insulin-like growth factor and adrenocorticotropic hormone leading to a significant increase in the average daily weight gain [154]. In a separate study by Stefanska et al. a combination of phytobiotics and multi-strain probiotics containing *Lactobacillus* strains was used as a supplement in neonatal calves. The combination led to a robust rumen microbiome increasing total bacteria while enhancing calf health and growth performance in the process [155].

### 5.4. Early Life Interventions

The gastrointestinal tract (GI) of newly born calves has long been considered sterile and microbial colonization starts immediately after contact with the dam's vaginal canal, fecal material, saliva, skin, and colostrum milk [156]. However, it has been recently challenged by increasing evidence of vertical transmission from the placenta, umbilical cord, or amniotic fluid of dams during the gestation period [157–159]. Microbes were found in the GI tract of young Holstein calves as early as 20 min after birth, indicating microbial colonization before or during birth [60]. This suggests that the maternal calf can influence the bovine fetal gut microbiome as early as the pregnancy stage. Mineral supplementation of the dam did improve the richness and diversity of the fetal gut microbiome during the gestation period [160]. Similarly, supplementation of methionine enriched microbes and metabolites that regulate vital metabolic pathways typically associated with healthy calves [161]. This could be the first step in engineering the rumen microbiome, influencing the microbiota community composition that could persist for better health and enhance animal productivity later in life.

The rumen and its microbiota changes throughout the first year of the calf's life as it grows, with various microbial groups beginning to occupy and colonize quickly. This development stage includes the critical transition phase of weaning. As feeding shifts, the microbial diversity increases as it is weaned as early as 7 up to 17 weeks of age. As the ruminant is weaned, this decreases *Actinobacteria*, which are essential early colonizers for converting milk components in the neonate's gut. On the other hand, fiber degrading *Bacteriodetes* and *Fibrobacteres* increase along with the replacement of milk with a total mixed ration (TMR), including grass silage [162]. Even the mode of feeding (suckled vs. bottle-fed) can alter the microbiome. Bottle-feeding delayed the onset of an anaerobic environment in the gut along with higher levels of *Escherichia/Shigella* suggesting an increased number of potential pathogens [163].

This period of instability in the gastrointestinal tract during the process of weaning provides an opportunity for manipulation wherein supplements can be added to program the rumen microbiome development [22]. Palma-Hidalgo et al. inoculated fresh rumen fluid from adult Murciano-Granadina goats into 80 newborn goat kids from day one until 11 weeks of age, resulting in an accelerated rumen microbial development showing the greater presence of plant degraders *Rikenellaceae* and *Fibrobacter*. This is a sign of a highly matured bacterial community of strict anaerobes capable of degrading recalcitrant fiber facilitating the transition from liquid to solid feeding with minimized weaning stress [164]. This highlights that the manipulation of the microbial population of the rumen is achievable before birth and early life interventions can result in increased productivity and improved health that can last a lifetime.

## 6. Conclusions

The global food system relies heavily on the production of ruminant livestock, which generates copious amounts of methane. This results in lower productivity and higher costs due to a significant loss of feed energy. Rumen fermentation produces SVFAs, microbial crude proteins, and vitamins by digesting plant fibers, non-protein-nitrogen, and other organic matter in the diet. The rumen microorganism influence gut metabolism as well as nutrient absorption, immune response, and health of the host. Recent advances in molecular and sequencing technology have revolutionized the way in which microbial ecology is studied. In the past, 16S rDNA sequencing has been instrumental in understanding the taxonomic composition of the rumen microbiome. Next-generation sequencing (NGS) techniques have enabled scientists to analyze and understand complex microbial communities from wider and deeper perspective. Through the use of metagenomics and metatranscriptomics analysis, we can identify the key group of microbes that perform a significant role in various processes, including cellulose degradation, fermentation, acetogenesis, and methanogenesis. This will enable us to modulate the structure of the rumen microbiome. Metatranscriptomics has been extensively used to detect key enzymes, such as CAZymes, involved in the degradation of plant biomass. The identification of novel and efficient fiber-degrading enzymes through functional metagenomics and metatranscriptomics could provide insight into strategies to improve ruminal feed conversion.

Despite decades of rumen research, current culture collections do not represent the typical composition of a rumen. Several rumen microbes remain unidentified, and the cultivation of rumen microbes is not completely understood. The identification, isolation, and characterization of ruminal microorganisms, both taxonomically and biochemically, is still a matter of research, particularly in the area of culturomics. With advances in the omics approach, we will be able to improve animal feed efficiency and health, and reduce methane emissions from ruminants.

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