Chapter

Ruminal Microbiome Manipulation to Improve Fermentation Efficiency in Ruminants

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Abstract

The rumen is an integrated dynamic microbial ecosystem composed of enormous populations of bacteria, protozoa, fungi, archaea, and bacteriophages. These microbes ferment feed organic matter consumed by ruminants to produce beneficial products such as microbial biomass and short-chain fatty acids, which form the major metabolic fuels for ruminants. The fermentation process also involves inefficient end product formation for both host animals and the environment, such as ammonia, methane, and carbon dioxide production. In typical conditions of ruminal fermentation, microbiota does not produce an optimal mixture of enzymes to maximize plant cell wall degradation or synthesize maximum microbial protein. Well-functioning rumen can be achieved through microbial manipulation by alteration of rumen microbiome composition to enhance specific beneficial fermentation pathways while minimizing or altering inefficient fermentation pathways. Therefore, manipulating ruminal fermentation is useful to improve feed conversion efficiency, animal productivity, and product quality. Understanding rumen microbial diversity and dynamics is crucial to maximize animal production efficiency and mitigate the emission of greenhouse gases from ruminants. This chapter discusses genetic and nongenetic rumen manipulation methods to achieve better rumen microbial fermentation including improvement of fibrolytic activity, inhibition of methanogenesis, prevention of acidosis, and balancing rumen ammonia concentration for optimal microbial protein synthesis.

Keywords: microbial manipulation, rumen, feed additives, phytochemicals, fiber degradation, microbial protein, acidosis

1. Introduction

Rumen inhabits several microbial populations, that is, bacteria, protozoa, fungi, bacteriophages, yeasts, and methanogens symbiotically, which are very dynamic, plastic, and redundant in function with the changes in diets though core microbiota persists, which has probably evolved by host-microbiota interaction in the evolutionary pressure over thousands of years [1]. A symbiotic relationship exists between rumen microbes and host animals in which both provide desirable substrates to

each other mainly through these ways—1) physical breakdown of feed particles by mastication and rumination expands their surface area for microbial attachment and degradation, and consequently, microbes secrete various enzymes for dietary substrate degradation, 2) ruminal movements bring microbes in contact with the dietary substrate by mixing of digesta and consequently produce fermentation products (e.g., H_2, CO_2, ammonia, short-chain fatty acids (SCFAs), and 3) utilization (absorption and consumption) of the fermentation products for keeping optimal ruminal conditions (e.g., pH) to maintain microbial growth and microbial protein synthesis [2]. Therefore, due to the interactive ecosystem of the rumen, any modification to one component of this system has several effects on other components. The fermentation end products of any diet are incorporated into the final animal products (meat or milk). Thus, manipulation of the ruminal fermentation pathways is the most effective approach to improve ruminant health and production efficiency without exaggerated increases in nutrient supply. This in particular should help the small livestock holders in developing countries for continued production.

The literature explored various manipulation strategies including enhancing or inhibiting the growth or the metabolic activity of specific rumen microbiota (e.g., archaea for methanogenesis) and/or altering the ruminal fermentation toward specific pathways (e.g, decreasing H_2 production and increasing short-chain fatty acids (SCFAs) production [3, 4]. Extensive literature supports the supplementations of various rumen modifiers; however, efforts are still underway to find appropriate methods to simultaneously improve livestock production while reducing greenhouse effects on the environment. Through the following aspects, the most common methodologies for modifying the ruminal microbiome and fermentation characteristics are discussed in this chapter.

2. Enhancing fibrolytic activity and short-chain fatty acid production

Lignocellulose (complex polymers of cellulose, hemicellulose, pectin, and lignin) makes up the majority of the ruminant diet. Generally, forages, including crop residues, provide the main source of nutrition to ruminants that contribute to the food security and primary source of income of smallholder farmers in the developing countries [5–7]. This is also true where grazing animals are common in the developed countries. Hence, forage is virtually the only source of nutrition in the main beef-producing northern Australia, North and South America [8].

Although ruminants can digest fibrous feedstuffs, dietary cell wall polysaccharides are rarely completely degraded in the rumen. Less than 50% of the plant cell wall of most forage grasses are digested and utilized. This is attributed to the combination of the biochemical and physical barriers present in the ingested fibrous feedstuffs and retention time limitations of the ingested dietary substances in the rumen [9], resulting in excessive nutrient excretion, low nutrient intake, and a significant loss of dietary energy in the form of CH_4 emission [10]. Therefore, enhancing the rumen microbiota to degrade plant cell walls usually leads to improve animal productivity.

Ruminants cannot degrade lignocellulose themselves. An involved community of fibrolytic microorganisms catalyzes the degradation of the plant cell walls in the rumen. The major classical fibrolytic bacteria involved in fiber degradation are *Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens, Butyrivibrio,* and *Prevotella* spp. [11]. Anaerobic fungi also contribute to degrade cell wall components and play a special role in degrading low-quality forages. Fungi are able to

penetrate the plant tissue as a result of their filamentous growth and can degrade up to 34% of the lignin in plant tissues [12]. Fungi (i.e., *Neocallimastix* sp.) have a broad range of highly active fibrolytic enzymes and are the only known rumen microorganisms with exo-acting cellulose activity [11]. Cellulolytic activity is present in many rumen protozoa species, and the most efficient cellulose degraders are *Epidinium ecaudatum, Eudiplodinium maggii,* and *Ostracodinium dilobum* [13].

There are various well-established procedures that can be used to improve forage utilization including modifying ruminal microbial fermentation toward more fiber degradation. These include mechanical and chemical processing of forages and genetically engineering of plants for cell wall composition. However, we will focus on ruminal fibrolytic microorganisms and their products in the following sections of the chapter.

2.1 Genetically engineered fiber-degrading bacteria

The manipulation of genes in genetically engineered organisms can produce a product with novel specific characteristics that may have significant value. This concept was exploited in developing genetically modified fiber-degrading bacteria to optimize their activity by producing the correct mixture of fibrolytic enzymes to maximize plant cell wall degradation. *Ruminococcus* and *Fibrobacter* strains were the most targeted fiber-degrading bacteria for genetic modifications because they cannot produce exocellulases that are active against crystalline cellulose. Therefore, altering this activity would make them more potent [11]. The genome sequences of *F. succinogenes, R. albus,* and *Prevotella ruminicola* strains are available [11].

As early as 1995, Miyagi et al. [14] suggested that inoculation of genetically marked *R. albus* into a goat rumen might be of benefit to rumen function, but they found that the inoculant usually disappears from goat rumen after 14 days. One of the reasons for this is that bacteria reproduce within the physiological and ecological limits of the rumen ecosystem in which cooperative networks exist among ruminal microorganisms; since some organisms cleave specific bonds, others utilize particular substrates, while others produce inhibitors [11]. The scientists' sights were turned to *Butyrivibrio* species because they are among the most rumen bacteria capable of hemicellulose degradation and are regarded as being ecologically robust [15]. Gobius et al. [16] reported the successful transformation of a diverse range of eight strains of *Bu. fibrisolvens* with xylanase (family 10 glycosyl hydrolases) from rumen fungus *Neocallimastix patriciarum*. Glycosyl hydrolases family 10 was selected because it is different from family 11, which typically exists in *Bu. Fibrisolvens* and this family is characterized by high specific activity and resistance to proteolysis. The transformation was functionally successful and the *in vitro* fiber digestibility measurements revealed an improvement in plant fiber degradation by the recombinant xylanase; however, this still does not allow them to compete with the far more fibrolytic species *Fibrobacter* and *Ruminococcus* [11]. Another genetically engineered bacteria, *Bacteroides thetaiotaomicron* was inoculated at approximately 1% of the total population into *in vitro* dual-flow continuous culture fermenters and persisted for at least 144 h with relative abundances of 0.48–1.42% and increased fiber digestion, particularly hemicellulose fraction [17]. Generally, most of the experiments that used modified fibrolytic bacteria were *in vitro* trials. However, it should be taken into consideration that the *in vitro* fermenters did not express the full complement of rumen microorganisms (particularly protozoa). Moreover, this microbial manipulation application seems to be costly, especially for the small livestock holders in developing counties.

2.2 Direct-fed microbials

The concept of direct-fed microbials is different from the term probiotics. Probiotics were identified by any live microbial feed additive that may beneficially influence the host animals upon ingestion by improving microbial balance in the intestine [18]. Viable microbial communities, enzyme preparations, culture extracts, or combinations of those products were included in the concept of probiotic supplements [19]. The DFM has a narrower definition than probiotics as it is defined as a source of life, naturally occurring microorganisms alive, naturally occurring microorganisms that improve the digestive function of livestock. The DFM includes three main categories; bacterial, fungal, and a combination of both [20]. DFM must be alive to impact ruminal fermentation; thus, the viability and number of organisms fed must be ensured at the time of feeding. Lactic acid-producing and utilizing bacterial species of *Lactobacillus, Bifidobacterium, Streptococcus, Bacillus, Enterococcus, Propionibacterium*, *Megasphaera elsdenii* and *Prevotella bryantii,* and yeasts such as *Saccharomyces* and *Aspergillus* were the significant microbes of most of the DFM for livestock production [21].

DFM can grow under ruminal conditions and manipulate the microbial ecosystem. Various factors may affect the activity of DFM including microbial strains, time of feeding, feeding system, treatment period, physiological conditions, and dosages [20, 22]. The microbial strains seem to be the main influencer—DFM containing mainly lactic acid-producing and utilizing bacteria can manipulate the growth of microorganisms adapted to lactic acid in the rumen while preventing the drastic pH drops, for example, *M. elsdenii* [19]. DFM of *Propionibacterium* species can manipulate the fermentation pathways toward a more molar portion of propionate production [20, 23]. *Propionibacterium* is naturally found in high numbers in the rumen ecosystem and known to ferment lactate to propionate, providing more substrates for lactose synthesis in early lactation dairy cows, improving energy efficiency for the growing ruminants by reducing methane emission [20, 23].

Direct-fed microbials, based on fungal cultures, mainly contain *Saccharomyces cerevisiae* and *Aspergillus oryzae*, which can remove oxygen from the surfaces of freshly ingested feed particles to maintain the ruminal anaerobic conditions for the growth of cellulolytic bacteria [22, 24]. Moreover, the end metabolites of yeasts in the rumen can provide the ruminal microbiota with growth factors (i.e., rumen acetogens, digestive enzymes, anti-bacterial compounds, organic acids, and vitamins), resulting in stimulation of ruminal cellulolytic bacteria and maintenance of pH for optimal fiber degradation, and consequently greater production performance [21, 22]. Due to the low cost of DFM compared to other commercial feed additives, it can be included among the suitable solutions to manipulate the ruminal fiber degradation for the smallholder livestock sectors.

2.3 Exogenous fibrolytic enzymes

Products of exogenous fibrolytic enzymes (EFE) that contain primarily cellulolytic and xylanolytic activities can manipulate the ruminal fiber degradation, and improve feed conversion efficiency and thus lead to enhanced productive efficiency of ruminants [9]. Published literature suggests that the mode of actions of EFE products are likely different than that of DFM products. The activities introduced to the rumen by EFE are not novel to the ruminal ecosystem as they would act upon the same sites of the feed substrate particles as endogenous fibrolytic enzymes [25]. The

release of reducing sugars by EFE is probably an essential mechanism by which EFE operates [26]. The degree of sugar release is dependent on the substrate types as well as the type of enzymes. The released sugars can attract secondary ruminal microbial colonization, or remove barriers to the microbial attachment to substrate feed particles by cleaving the linkage between phenolic compounds and polysaccharides [9]. As a result, the most significant effects of EFE probably occur in the interval between the arrival of the feed particles into the rumen and its colonization by ruminal microorganisms, as only the rate, not the extent, of cell wall degradation, has been improved [25]. EFE can also manipulate the rumen fibrolytic microorganisms by enhancing their endogenous fibrolytic activities.

Genes from ruminal fungi encoding cellulases, xylanases, mannanases, and endoglucanases have been successfully isolated. Protein bioengineering has been employed to improve the catalytic activity and substrate diversity of fibrolytic enzymes from ruminants. This has resulted in fibrolytic enzymes with up to 10 times higher specific activity, pH and temperature optima, and enhanced fiber-substrate binding activity than the original enzymes [27]. This, together with the low manufacturing cost, has led to more recent developments in the enzyme production industry, and as a result, a wide range of commercial EFE products is now available. Frequently the manufactures' recommended doses of most commercial EFE products have been measured under wide ranges of pH (4.2–6.5) and temperatures (40–57°C), which are not always close to typical ruminal conditions. Moreover, most of the commercial EFE products for ruminants are often referred to as xylanases or cellulases. However, none of these products comprise single enzymes; secondary enzyme activities are invariably present, namely, proteases, amylases, or pectinases [9]. A wide variety of feed substrates can be targeted by a single EFE product. Thus, the random addition of these products to ruminant diets without consideration for specific rumen conditions (pH 6.0–6.5 and 39°C) and the not yet tested efficiency for specific substrate will result in unpredictable effects and thus discouraging the adoption of the EFE technology [28, 29].

In general, enhancing the rumen microbiota to degrade the dietary fibers through the above-discussed strategies may lead to accelerating the energy production in the forms of short-chain fatty acids (SCFAs) and/or microbial protein synthesis. At the same time, it may also produce high amounts of CO_2 and CH_4.

3. Decreasing methanogenesis and increasing propionate production

The ruminal fermentation is the primary source of CH_4 emission from livestock; it is one of the most potent greenhouse gases featured by short atmospheric mean lifetime. Furthermore, a significant proportion of the ingested feed energy is also lost as CH_4 [40]. Methane is produced by methanogens mainly by reduction of CO_2 through the hydrogenotrophic pathway. Formic acid and methylamines produced by other ruminal bacteria are also reduced to CH_4 by some methanogens. Therefore, methanogens interact with other ruminal microorganisms (e.g., protozoa, bacteria, and fungi) through interspecies H_2 transfer [4]. Thus, maximizing metabolic H_2 flow away from CH_4 toward SCFAs production could improve production efficiency in ruminants and decrease environmental impact. There are various direct and indirect strategies to manipulate rumen methanogenesis; among these options, inhibiting the growth or the metabolic activity of methanogens seems to be the most effective approach. The efficiency of these strategies mainly depends on where methanogens reside. It can be seen from the smaller number of archaeal 16S rRNA gene sequences (461 vs. 8162)

recovered from protozoa than from ruminal content or fluid [4]. Free methanogens are mainly integrated into the biofilm on the surfaces of feed particles where H_2-producing bacteria actively produce H_2. These methanogens protected by the biofilm may not be inhibited to an extent similar to the free-living peers by anti-methanogenic inhibitors [4]. Also, methanogens can be inhibited indirectly through inhibiting rumen ciliate protozoa. Based on fluorescence *in situ* hybridization analysis, about 16% of the rumen ciliate protozoa contained methanogens inside their cells [30]. Most rumen ciliate protozoa have hydrogenosomes, unique membrane-bound organelles producing H_2 by malate oxidization; therefore, these organelles can attract some species of methanogens as endosymbionts [4].

Methane formation pathways comprise of three main steps; transfer of methyl group to coenzyme M (CoM-SH), reduction of methyl-coenzyme M with coenzyme B (CoB-SH), and reusing heterodisulfide CoM-S-S-CoB [4, 31]. Thus, obstruction of any of these steps may manipulate CH_4 production. A wealth of literature on rumen CH_4 manipulation strategies in ruminants have been published recently, but relatively very few have emphasized the suitable mitigation strategies at the farm level [32]. Each method has some potential advantages and limitations. The principal interest for animal producers is income, as they usually do not take CH_4 mitigation strategies or climate changes into account. Thus, any strategy to mitigate greenhouse gasses emission would only be of practical interest if achievements on the efficiency of animal production can be obtained. This can be obtained through rumen CH_4 modifiers that enhance the production of SCFAs and/or reduce proteases. The following part addresses some of these microbial modifiers.

3.1 Ionophores

Ionophores are polyether antibiotics that act as inhibitors to hydrogen-producing bacteria. They are widely used as successful growth promoters in the livestock industry due to their ability to modulate rumen fermentation toward propionate production, thereby decreasing CH_4 production. Since propionate and CH_4 are terminal acceptors for metabolic H_2, any increase in propionate production may accompany reduced CH_4. In addition, ionophores positively affect ruminal fermentation through inhibition of deamination compared to proteolysis, inhibition of hydrolysis of triglycerides, and biohydrogenation of unsaturated fatty acids, while enhancing the trans-octadecenoic isomers (cited from [33]).

From the literature, monensin and lasalocid are the most well-known ionophore-type antimicrobials used as rumen modifiers. Mainly, they inhibit Gram-positive bacteria; however, they can also inhibit some Gram-negative bacteria. Ionophores decrease CH_4 production by inhibiting H_2 producing bacteria by penetrating the bacterial cell wall membrane. They act as H^+/Na^+ and H^+/K^+ antiporters, dissipating ion gradients required for the synthesis of ATP, transport of nutrients, and other essential cellular activities in bacteria, resulting in retardation of cell growth and cell death [4, 34]. Monensin can decrease total methanogens number in cattle, and also alter the community composition of methanogen species, for example, monensin decreased the population of *Methanomicrobium* spp. while increasing that of *Methanobrevibacters* spp. [4].

Unfortunately, ionophores present a temporary impact on ruminal manipulation effects due to the adaptation of the microorganisms of these inhibitors. Ionophores are now restricted due to the possible resistance of pathogenic microorganisms to antibiotics [33]. Recently, the global scenario has shifted the interest toward plant

natural feed additives with potential abilities to modulate CH_4 emission [35, 36]. Moreover, the type of the dietary feeds affects the efficiency of ionophores with the better effect of ionophores observed in high starch diets [33]. Thus, this approach seems to be less effective for the small livestock holders in most developing countries since the forages are the main ingredient in the diets.

3.2 Natural feed additives as rumen modifiers

3.2.1 Plant secondary compounds

Numerous plant secondary compounds (PSC), including tannins, flavonoids, saponins, essential oils (EOs), organosulfur compounds, have been recognized as having the potential to modulate ruminal microbial fermentation [37–39]. Plant secondary compounds are natural phytochemicals with the potential ability to manipulate rumen fermentation without causing microbial resistance or residual noxious effects on animal products [3]. Unlike ionophores, the different active components found in plant extracts may manipulate ruminal microbiota through more potent mechanisms of action (e.g., antimicrobial and antioxidant), which may avoid the risk of losing activity over time [40].

3.2.2 Tannins

Tannins are polyphenolic compounds with different molecular weights ranging from 500 to 5000 Da [41]. Tannins are classified into two major groups, that is, condensed (CT) and hydrolyzable tannins (HT). CT are proanthocyanidins consisting of oligomers or polymers of flavan-3-ol subunits. They act through binding with dietary proteins and carbohydrates by making strong complexes at ruminal pH [41–43]. Therefore, they are the most plant secondary metabolites studied in terms of rumen modulation pathways.

The literature reported quite various effects of CT supplementations regarding CH_4 mitigation [38]. Some studies suggest a direct effect of CT on methanogens by binding with the proteinaceous adhesin or parts of the cell envelope, which impairs the establishment of methanogens-protozoa complex and decreases interspecies H_2 transfer, and inhibits growth [44]. Other studies suggest an indirect effect of CT through the anti-protozoal effect. However, the effects of CT on rumen protozoal activity are varied in the literature, probably because some of the CTs have a direct effect on rumen methanogenic archaea, which are not associated with the protozoa. Tannins also can indirectly inhibit CH_4 per unit of the animal product through tannin–protein or organic matter complexes under ruminal conditions, while protein from these complexes is released post ruminally, making it available for gastric digestion at abomasum and small intestine conditions, leading to enhancing the animal productivity [43]. Another theory is that tannins can act as H_2 sink reducing the availability of H_2 for CO_2 reduction to CH_4, implying that 1.2 mol CH_4 is produced per mol of catechin [44].

Tree foliages are good feed resources for the small ruminants, which are rich in protein and perform catalytic functions in improving ruminal fermentation, especially in low-quality forage-based diets in developing countries [45]. The nutritionists have paid great attention to the tanniferous legumes and tree foliages as alternative cheap feed resources (especially in drought conditions and arid and semi-arid regions) and to achieve CH_4 mitigation goals in the developing countries [46]. Many plants were investigated in the literature; however, the results are highly variable

among studies. Soltan et al. [43] studied various tanniniferous browsers and found that some plants (i.e., *Prosopis* and *Leucaena*) similarly modulate ruminal fermentation as ionophores perform by decreasing the acetate to propionate ratio, CH_4 and NH_3-N, while *Acacia* reduced CH_4 through decreasing fiber degradation although it had similar CT concentration as *Leucaena*. Thus, it seems that not only does tannin concentration play a role in the modulation of the ruminal fermentation process, but also types, molecular weights are important in determining tannin potency in modulating rumen fermentation patterns. The presence of HT and other plant secondary metabolites (mimosine in *Leucaena*) together with CT can interact with the action of CT [44, 47].

3.2.3 Saponins

Saponins are a group of plant secondary metabolites with high molecular weight glycosides in which a sugar is linked to a hydrophobic aglycone. It can be generally classified as steroidal and triterpenoid [48, 49]. The effects of saponins on rumen fermentation modulation have been reviewed extensively [49]. The main biological effect of saponins is on the cell membranes of bacteria and protozoa. Saponins are highly toxic to protozoa compared with bacteria because saponins can form complexes with sterols present in the protozoal membrane surface, disrupting the membrane function [49]. Thus, it can indirectly affect the methanogenic archaea through their symbiotic relationship with rumen protozoa [38]. However, some literature assumed that the effects of saponins on rumen protozoa could be transient due to the ability of ruminal bacteria to degrade saponins into sapogenins. The sapogenin compound cannot affect protozoa [50].

3.2.4 Essential oils

Essential oils (EO) are volatile aromatic complexes obtained from different plant volatile fractions by steam distillation. They can be obtained from various plant parts including leaf, stem, fruit, root, seed, flower, bark, and petal. EO contains numerous bioactive substances; the most important ones are terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids. Due to the lipophilic properties of these components, EO act against various rumen bacteria through interacting with the cell membrane [3].

Several EO compounds, either in pure form or in mixtures, had antioxidant and anti-bacterial properties; therefore, they can modulate the ruminal fermentation pathways [51]. The EO, unlike ionophores, does not alter the ruminal microbial activities through a specific mode of action. Therefore, EO may have more potent mechanisms of action that may not likely lose their effectiveness over time. Soltan et al. [40] suggested two mechanisms in explaining how combination of phenylpropanes and terpene hydrocarbons components in EO mixtures work together to enhance additive antimicrobial activity—1) phenolic compounds may increase cell membrane permeability through the action of hydroxyl group, thus facilitating the transport of terpene hydrocarbons into the microbial cells, which then combine with proteins and enzymes inside the cells; 2) phenolic compounds could increase the size, number or duration of the existence of the pores created by the binding of terpene hydrocarbons with proteins in cell membranes.

The effects of EO on rumen fermentation are variable depending on concentrations, types, diet and adaptation period, but most EO are found to have anti-methanogenic

properties [35, 52]. Patra and Yu [52] studied various EO with different chemical structures (clove, eucalyptus, origanum, peppermint, and garlic oil) *in vitro* at three different concentrations (0.25, 0.50, and 1.0 g/L) for their effect on CH_4 production and archaeal abundance and diversity and they found that all these EO suppressed CH_4 production, but the extent of CH_4 inhibition and ruminal fermentation differed among the EO. Further studies are needed to understand the interactions of the active compounds with the dietary ingredients and their activity against specific methanogens should be identified without adverse effects on fermentation patterns and rumen fiber degradability, as well as the different doses for each EO. Also, attention needs to be paid to the palatability as some EO may adversely affect palatability and dry matter intake due to the aroma they add to the ration. Therefore, many products of encapsulated EO are available in commercial forms, but this raises the question of the suitability of these products as feed additives at the farm level in developing countries.

3.2.5 Propolis

Propolis is a mixture of resinous substances collected from buds of deciduous trees and crevices in the bark of coniferous and deciduous trees and secretions by honeybees [53, 54]. The bees use propolis to fill cracks, cover hive walls and embalm invading intruder insects or small animals [55, 56]. The literature reported that the chemical composition of propolis is highly variable by bee collection site since geographical location plays an important role [54]. The most bioactive components are belonging to groups of isoflavones, flavonoids, and fatty acids that have been reported to be biologically active [53]. Recently, bee propolis has been recognized as a natural alternative feed additive to antibiotics in ruminant diets [54]. Compared to ionophores (e.g., monensin), different propolis sources can reduce CH_4 production while improving the organic matter digestibility and total SCFAs *in vitro* and *in vivo* [53, 57]. Morsy et al. [58] reported that CH_4 reduction caused by propolis supplementation is accompanied by increasing urinary allantoin, total purine derivatives, and enhancements of individual and total SCFAs. Thus, they suggested that propolis can help in the redirection of ruminal organic matter degradation from CH_4 production to microbial synthesis and SCFAs. From a practical view, propolis can be a promising feed additive in the vegetation places where it is produced in a large amount such as Brazil.

3.3 Plant oils

Fats are usually used as energy sources for dairy cattle. The addition of fats is a promising approach for modulating rumen microbial communities and the fermentation process. Fats are known to inhibit microbial activity; however, supplementing fats up to 6% of dry matter has shown no adverse effects on total nutrient digestibility and total SCFAs [59]. A meta-analysis study suggests that methane emissions can be declined by 0.66 g/kg DM intake with each percentage increase in dietary fats, within dietary fat concentrations of 1.24–11.4% [59]. Fats containing high levels of C12:0, C18:3, and polyunsaturated fatty acids up to 6% of the dietary diet may be considered for CH_4 mitigation without compromising the productivity in dairy cattle [59].

Plant oil supplements can modulate CH_4 directly by inhibiting rumen protozoa and methanogens while enhancing biohydrogenation of polyunsaturated fatty acids (PUFA) to act as ruminal hydrogen sink for hydrogen produced by rumen microorganisms and reducing fiber degradation with less H_2 production in the rumen [60].

The literature showed variable effects of plant oils on CH_4 emission and rumen fermentation; this might be related to the oil type (free oil or whole seed), diet composition (forage to-concentrate ratio), and fatty acid type (short-chain or PUFA) present in diets [59]. Generally, consideration of vegetable oils supplementation to lower CH_4 emission may depend upon the cost and expected outcome effect on animal productivity.

3.4 Chitosan

Chitosan is a natural polycationic polymer, nontoxic, biocompatible, biodegradable; thus, it is safe for human as well as animal consumption [61]. It is a linear polysaccharide composed of two repeated units—D-glucosamine and N-acetyl-D-glucosamine linked by β-(1–4)-linkages [61]. It can be found in the structural exoskeleton of insects, crustaceans, mollusks, cell walls of fungi, and certain algae, but it is mainly obtained from marine crustaceans [62]. It is characterized by anti-inflammatory, antitumor, antioxidative, anticholesterolemic, hemostatic, and analgesic effects. Moreover, it has a high antimicrobial affinity against a wide range of bacteria, fungi, and protozoa; therefore, it has been recently tested as a rumen fermentation modulator and considered as a promising natural agent with CH_4 mitigating effects [61]. The antimicrobial mechanism of chitosan can include interactions at the cell surface and outer membranes through electrostatic forces, the replacement of Ca^{+2} and Mg^{+2} ions, the destabilization of the cell membrane, and leakage of intracellular substances, and cell death. The antimicrobial properties of chitosan can also include chelating capacity for various metal ions and the inhibition of mRNA and protein synthesis [61].

It seems chitosan activity depends on the diet type as well as the ruminal pH. The literature reports suggest that the maximum effect of chitosan is noted when grain (starch) is incorporated in the ration at low pH values, shifting the fermentation pattern to a more propionate production pathway, which could be explained by the higher sensitivity of Gram-positive bacteria than Gram-negative bacteria against chitosan [61, 63]. This type of change in ruminal fermentation by chitosan results in reductions in CH_4 production. Moreover, supplementation of chitosan alters the rumen bacterial communities related to fatty acids biohydrogenation, that is, *Butyrivibrio* group and *Butyrivibrio proteoclasticus* that lead to increases in concentrations of milk unsaturated fatty acids and cis-9,trans-11 conjugated linoleic acid [64].

3.5 Chemical feed additives

Numerous chemical additives were used to modulate the rumen microbial activity for optimizing animal productivity, namely, defaunating agents, and anti-methanogenic agents to reduce CH_4 emission. Patra et al. [4] reported the most promising anti-methanogenic agents that effectively lower CH_4 without adverse effects on rumen degradability or producing SCFAs and each of which works through different modes of action when added together to additively decrease CH_4 production. These include halogenated sulfonated compounds (e.g., 2-bromoethanesulfonate, 2-chloroethanesulfonate, and 3-bromopropanesulfonate), 3-nitrooxypropanol (3NOP), nitrate, and ethyl-3NOP are used to inhibit methyl-CoM reductase activity, the final limiting step to complete the methanogenesis pathways. Halogenated aliphatic compounds with 1 or 2 carbons can impair the corrinoid enzymes function and inhibit cobamide-dependent methyl group transfer in methanogenesis or may serve as terminal electron (e^-) acceptors. Some agents, namely, lovastatin and mevastatin were found to inhibit

3-hydroxy-3-methylglutaryl coenzyme, which is essential in the mevalonate pathway to form isoprenoid alcohols of methanogen cell membranes [4]. The addition of nitrate has two benefits—it can inhibit methanogenesis and acts as a nonprotein nitrogen source, which could be useful in low-quality base diets [65].

4. Control of acidosis

Diets containing high amounts of rapidly fermenting soluble carbohydrate result in pH drop due to excessive production of lactate or VFA or a combination of both, which may be of subacute ruminal acidosis (pH between 5.0 to 5.5) or acute acidosis (<5.0) type with acute or chronic in duration [66]. The consequences of acidosis range widely along with death and more importantly lower productivity, especially in subacute ruminal acidosis [66, 67]. Decreasing the ruminal pH leads to inhibition of rumen cellulolytic bacteria. Therefore, maintaining ruminal pH at the average level (5.8–7.2) is an essential factor to balance the rumen microorganisms between acid producers and consumers. In this context, buffering reagents and alkalizer (e.g., sodium bicarbonate, magnesium oxide, and calcium magnesium carbonate), direct-fed microbials, and malate supplementation may increase pH in the rumen and production when ruminants are fed with high-grain based diets [66, 68]. Malate supplementation can stimulate Selenomonas ruminantium that converts lactate to VFA [69]. Marden et al. [70] reported that the inclusion of 150 g of sodium bicarbonate increased total ruminal VFA concentration by 11.7% compared to the control diet fed to lactating cows. The addition of sodium bicarbonate, magnesium oxide, and calcium magnesium carbonate reduced the duration of time ruminal pH persisted below 5.8 in lactating dairy cows fed a high-starch (342 g/kg DM) containing diet and increased milk and fat yield, and milk fat concentration, but reduced milk *trans*-fatty acids isomers [71]. The efficacy of the acid-neutralizing capacity of the alkalizers depends upon physical and chemical properties that influence the solubility in the ruminal conditions. However, in developing country conditions, the acidosis problems are usually less severe as ruminants are mostly fed with roughage-based diets.

5. Enhancing ruminal microbial protein synthesis

Microbial protein in the rumen (RMP) accounts for between 50 and 90% of the protein entering into the duodenum and supplies the majority of the amino acids required for growth and milk protein synthesis [72]. Therefore, increasing RMP synthesis is important for improving animal productivity. Moreover, increasing the RMPS is an effective strategy to decrease protein (i.e., nitrogen) excretion in livestock since the dietary protein unless utilized properly by ruminal microorganisms is degraded to ammonia in the rumen, and ammonia is absorbed from the rumen, metabolized to urea in the liver, and excreted in urine causing environmental nitrogen pollution [10, 73].

There are many factors affecting RMP synthesis including dry matter intake, type of the ration fed (forage to concentrate ratio), the flow rate of digesta in the rumen, the sources, and synchronization of nitrogen and energy sources [74]. Among these, the amount of energy supplied to rumen microbes was found to be the main factor affecting the amount of nitrogen incorporated into RMP. Phosphorylation at the substrate level and electron transport level are two significant mechanisms of energy

generation within microbial cells [75]. Based on 10 reconstructed pathways associated with the energy metabolism in the ruminal microbiome, Lu et al. [75] found that the energy-rich diet increased the total abundance of substrate-level phosphorylation enzymes in the glucose fermentation and F-type ATPase of the electron transporter chain more than the protein-rich diet. Therefore, they concluded that energy intake induces higher RMP yield more than protein intake. In this context, any factor affecting the available amount of soluble carbohydrates to rumen microbes will affect the efficiencies of RMP synthesis. Therefore, most of the previously mentioned rumen modifiers (e.g., plant secondary metabolites, dietary oil) may affect the RMP synthesis; however, most of the studies have ignored the determination of RMP.

Maximizing RMP synthesis seems to be the most effective approach for the small livestock holders in most developing countries since microbial protein sometimes becomes the only protein source for the animals fed on poor quality forage diets with low or without concentrate supplementations. Balancing the diets of these animals by supplementing of leaves of legumes, urea-molasses multinutrient blocks, urea in the form of slow ammonia release, and other nonprotein nitrogen resources found to be favorable for RMP synthesis [8, 10, 29, 73]. It has been recognized that feeding high true proteins (the most expensive ingredients in the ruminant diet) can be utilized by ruminal bacteria in about the same way as the ammonia from nonprotein nitrogen (e.g., urea). The optimum concentrations of ammonia in the rumen for maximal RMP synthesis are about 50–60 mg/L and 27–133 mg/L from the *in vitro* and *in vivo* studies, respectively [73].

Reduction in CH_4 production can enhance the RMP synthesis. Soltan et al. [10, 29] observed that inclusion of *Leucaena* in sheep diet up to 35% with or without polyethylene glycol enhanced the RMP and the body nitrogen retention while reducingCH_4 emission; they suggested that optimizing microbial growth efficiency might help to redirect organic matter degraded from CH_4 formation to RMP synthesis. Plants or feed additives containing phytochemicals with high antioxidant activity can promote more nutrients for microbial uptake, enhancing RMP synthesis, while reducing CH_4 emission due to lessening the ruminal oxidative stress [36, 53].

6. Reduction of ruminal protein degradation and ammonia production

From an economic view, dietary protein concentrates increase production costs, especially for developing countries. Furthermore, the microbial population in the rumen has a high proteolytic capacity to degrade the dietary protein. Therefore, nutritionists are interested in formulating diets with ruminal undegradable protein sources. The protein degradation in rumen depends mainly on three processes—proteolysis, peptidolysis, and deamination. Many protein-degrading bacteria are naturally found under ruminal conditions, that is, *Ruminobacter amylophilus*, *P. ruminicola*, *Butyrivibrio fibrisolvens, S. ruminantium*, *Streptococcus bovis*, and *P. bryantii*. There are many amino acid-fermenting bacteria, that is, *Clostridium sticklandii*, *Clostridium aminophilum*, *M. elsdenii*, *B. fibrisolvens, P. ruminicola, S. bovis,* and *S. ruminantium* [73]. Increased ruminal ammonia concentration is an indicator of the high degradation of dietary protein. Many factors can affect ruminal protein degradation and ammonia concentration, such as the type of dietary protein, the energy sources, the predominant microbial population, the rumen passage rate, rumen pH [35]. The ruminal bacteria can utilize ammonia for the synthesis of amino acids required for their growth. The optimal ammonia concentration needed to maximize the RMP synthesis ranges from 88 to 133 mg/L [76].

Several inhibitors of ruminal microbial protein degradation and ammonia production were reported in the literature. Condensed tannins, slow-release urea products, encapsulated nitrate, clays (e.g., bentonite and zeolite that acts through cation exchange capacity), and biochar were found to reduce the rapid increase in ammonia production and maintained the ruminal pH. Urea pool in the rumen is contributed from urea in the diet and recycling of urea through saliva and ruminal wall. The urease enzyme produced by the ruminal microbiota rapidly degrades urea to ammonia causing ammonia toxicity and inefficient urea utilization when used in excessive amounts [73]. Inhibitors of urease may reduce the risk of ammonia toxicity and efficient utilization of urea and other nonprotein nitrogen compounds [77].

7. Enhancing functional values of milk and meat

Ruminant-derived foods (milk and meat) contain a high amount of saturated fatty acids, which are associated with human health concerns. Therefore, improving the functional value of ruminants' products by increasing the content of beneficial fatty acids (FAs) and decreasing detrimental ones, specifically, decreasing the content of saturated FAs and increasing n-3 FAs and conjugated linoleic acids (e.g., cis-9, trans-11 C18:2, also called rumenic acid) have been great interests among the researchers [78]. Manipulating ruminal biohydrogenation of polyunsaturated fatty acids (PUFAs) has been the target to increase meat and milk content of rumenic acid and vaccenic acid, as both compounds are major intermediates in the biohydrogenation. To elevate rumenic acid content in products, inhibiting the last step of biohydrogenation needs to be attempted without affecting lipolysis and isomerization and reduction of linoleic acid and linolenic acid to rumenic acid and vaccenic acid. Alternatively, to elevate PUFAs in meat and milk, in particular n-3FAs, inhibition of early steps of biohydogenation should be targeted. Secondary compounds such as tannins, saponins, or essential oils rich in terpenes present in plants and forages or supplementation of vegetable oil can improve some aspects of meat and milk quality including n-3 FAs, conjugated linoleic acids, antioxidant properties [73, 79–81].

8. Conclusions

The ruminal fermentation end products are typically the outputs of several interactive reactions among the rumen microbial populations. Manipulations of rumen microbial fermentation toward enhancing fiber digestibility, SCFAs production, and outflow of microbial biomass, while reducing ammonia and CH_4 emission are the most probable ways to improve animal productivity. Numerous rumen fermentation modifiers have been studied during the last few decades; however, their positive effects are sometimes associated with undesirable effects or highly significant costs (e.g., ionophore antibiotics, anti-methanogenic chemical feed additives, or essential oils). Moreover, most of these modifiers exhibited inconsistent efficacy in the literature mainly because of the variability in animal age, breed, diet formulation, physiological status, rumen microbial resistance, and adaptation. Despite the long history of studies on the rumen modifiers, most of the measurements are determined through the treatment period but knowledge is still limited on animal responses in later life or impacts on human health and growth. However, there is unanimous agreement that an ample array of drought-tolerant plants containing effective bioactive compounds,

DFM, fibrolytic enzymes, and nonprotein nitrogen sources would cost-effectively modify the ruminal fermentation. Therefore, a combination of two or more of these rumen modifiers with complementary modes of action may be a promising approach to optimize the productivity of ruminants in developing countries.

Conflict of interest

The authors declare no conflict of interest.

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