Chapter

Climate Change Mitigation in Livestock Production: Nonconventional Feedstuffs and Alternative Additives

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Abstract

Livestock production has widely contributed to increase global production of greenhouse gases (GHG), mostly through digestive fermentation in ruminants. Moreover, emissions derived from livestock are estimated over 14% of the total anthropogenic GHG emissions to atmosphere. In addition, methane emitted from ruminal enteric fermentation is responsible for 25% of the total global methane emissions, which turns livestock activity into a main promoter of the climate change effect. However, these emissions may be diminished by modifying livestock diets through alterations in forage-concentrate ratios, the supplementation of feed additives, and the inclusion of alternative feedstuffs not commonly used as forage and protein sources in farm animal feeding. Additionally, the use of nonconventional feedstuffs is highly recommended since their production does not compete with human feeding and may provide metabolites used as methanogenesis suppressors. Likewise, agricultural by-products should be considered as potential feedstuffs for animal production by increasing the livestock efficiency and reducing the energy losses due to methane synthesis.

Keywords: methanogenesis inhibitors, nonconventional forages, feedstuff additives, secondary metabolites, methanogens

1. Introduction

The world's population have substantially increased in the last decades, and it is expected to keep increasing for the next 30 years until the population reaches 9.8 billion in 2050 [1]. Consequently, there is a growing demand for food and natural resources for human surviving. Livestock represents a main source of protein and energy for human consumption, as well as an important basis of financial revenues for families at rural areas. However, this economic activity is positively correlated to the climate change (CC) effect [2]. In the last centuries, CO_2 and NO_2 emissions have increased 31 and 16%, respectively; whereas, methane has increased twofold. Approximately, 40% of the methane emitted to atmosphere is originated from natural sources [2]; the remaining 60% is originated from anthropogenic sources (livestock, rice crops, fossil fuel exploitation, and dump).

In this way, emissions derived from livestock are estimated over 14% of the total anthropogenic greenhouse gases (GHG) emitted to atmosphere, which account approximately 50 gigatons of CO_2 equivalents per year (GTons-CO_2 equiv./yr) [3]. In addition, livestock is a major non-CO_2 GHG producer (CH_4 and NO_2); these gases possess a higher trapping heat index compared to at least 25 times for CO_2 [3].

Climate change effect has risen the average planet temperature approximately 1°C. In fact, polar caps are melting rapidly, which have increased the sea levels as a consequence [3]. If these trends keep on going, the CC effect will reach a non-return point, causing irreparably damages to the planet [4]. In addition, the UN encouraged developing countries (mainly Latin American countries) to strengthen their efforts to avoid an increase over 1.5°C in the temperature of the planet. Nevertheless, since CO_2 emissions increased substantially in the latest years, a 3°C rise of the temperature is expected by the end of the century [5].

Due to the latter, worldwide researchers and governments attempt to mitigate livestock gases production by changing livestock diets and offering alternative feedstuffs as an important strategy to mitigate GHG emissions and CC effect.

2. Ruminal enteric fermentation and methanogenesis

Methanogenesis was once considered a singular type of fermentation. However, in some respects, a very unique biochemistry is involved. The process is carried out by strictly anaerobic bacteria, all of which belong to the phylum *Euryarchaeota* in five orders that include mesophiles and thermophiles: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanopyrales*, and *Methanosarcinales*. Methanogens can be found in freshwater and marine environments, cold

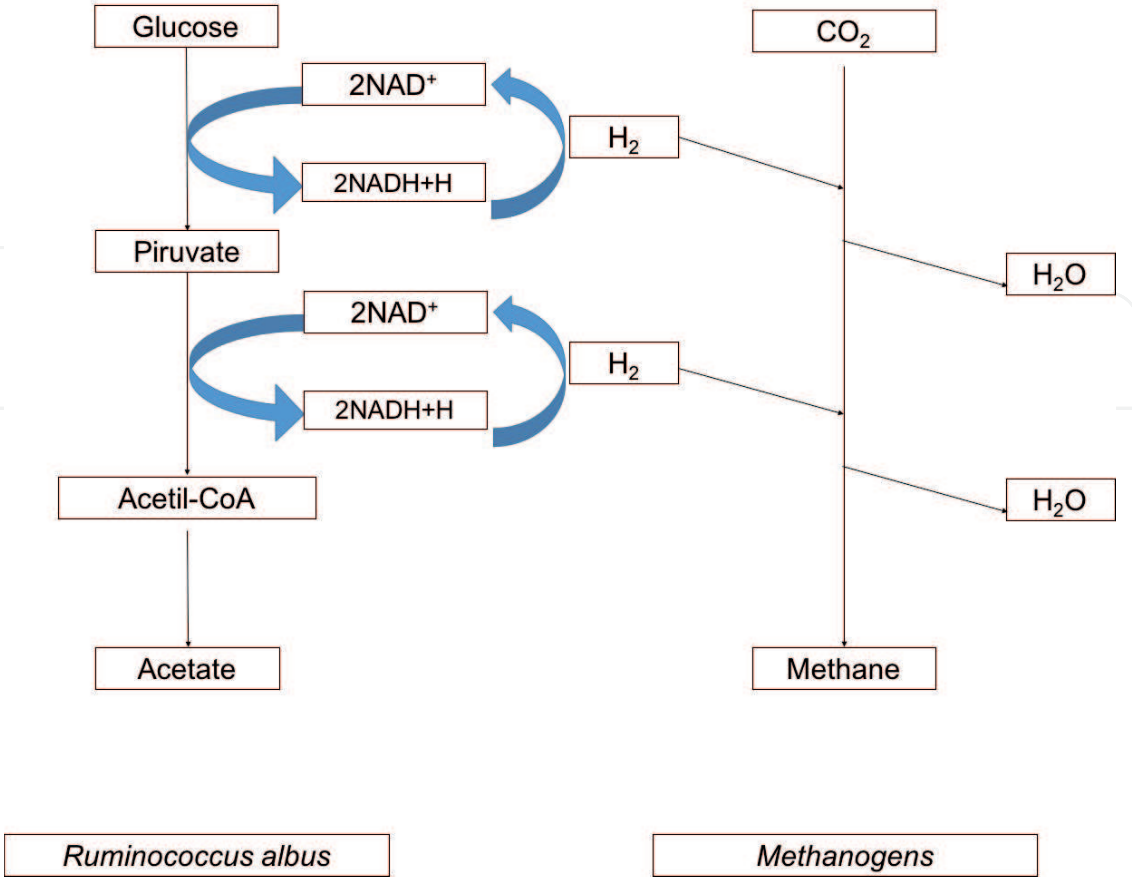

Figure 1.
Synergic relationship between Ruminococcus albus and methanogens (adapted from [10]).

sediments, and hydrothermal vents as free cells living in symbiosis within animals which produce methane as well as in symbiosis with anaerobic methane oxidation-promoting bacteria [6].

Ruminal degradation of fiber and starch generates hexoses which later are fermented through the glycolysis pathway. Pyruvate, as a final product of the glycolysis, is converted into volatile fatty acids (VFA), mainly acetic, propionic, and butyric acid, through different metabolic pathways. These VFA are rapidly absorbed by the animal and are used as energy source, while other products such as H_2 and CO_2 are generated. However, the hydrogen produced in the glycolysis inhibits NADH+H^+ ferredoxin oxidoreductase enzyme, which impedes NAD regeneration when a low H_2 pressure is present [7]. Therefore, methane production is essential for obtaining a high-performing rumen ecosystem, because H_2 accumulation is avoided, which could then inhibit dehydrogenase activity in later re-oxidation cofactors. An efficient H_2 capture in the rumen contributes to increase the rate of fermentation by the lack of its inhibitory effect on the microbial degradation of vegetative material [8, 9]. Hence, thermodynamically methane synthesis is favored. **Figure 1** represents the synergic relationship between *Ruminococcus albus* and methanogens, as an example of the expressed earlier [10].

3. Fermentation modifiers

The rumen is an anaerobic bioreactor which contains a great diversity of microorganisms, such as bacteria, fungi, protozoa, and archaea. From all of these, just a few have been cultivable and virtually identified. However, the newer molecular biology techniques have widely contributed to the identification of ruminal microorganisms, as well as the activity from each consortium in the ruminal fermentation. Feedstuffs' degradation in the rumen is effectuated by microorganisms with different goals and at different proportions. In addition, the enzymatic and degradative activity of every consortium may be affected by several factors, such as diet, season, inherent characteristics of the ruminant's breed, geographic zone, feeding strategies, physiological conditions, intake, etc. [11]. Hence, modification in the ruminal fermentation can be achieved by alterations on the previously mentioned variables, showing positive changes in efficiency and productivity of the animal. Therefore, diverse targets have been defined through modification in the ruminal fermentation: (a) to decrease the ruminal methane synthesis through the increase of propionate production; (b) to improve fibers' ruminal digestion; (c) to increase undegradable rumen protein in order to increase the bypass protein to lower tract which later will be absorbed by the animal through the intestine walls; and (d) to reduce rapidly degradable carbohydrates in rumen [12]. According to the latter, diverse options have been studied to cover two or more targets.

3.1 Nonfibrous carbohydrates

Carbohydrate fermentation is the main source of energy for the ruminant. Quantity and quality of rapidly degradable carbohydrates, usually known as nonfibrous carbohydrates (NFCs), depends on the feedstuff. Thus, NFCs contained in corn (*Zea mays*) are mostly starch, whereas, in molasses, NFCs are mainly composed by mono- and disaccharides. Depending on the NFC type and the supplied feedstuff, certain pathways for synthesis may be favored. For example, whether increases in the structural carbohydrates are observed, the propionate synthesis pathway is enhanced. This pathway is beneficial to the animal since it reduces methane synthesis [13]. Otherwise, an increase in mono- and disaccharides decreases microbial protein synthesis through reductions in the abundance of ammonia-utilizing cellulolytic bacteria [14]. Moreover, high NFC concentrations tend to increase VFA production which could cause ruminal acidosis.

3.2 Fibrous carbohydrates

It has been demonstrated that increases in dry matter intake reduce methane production [15]. Moreover, increases in digestibility is expected in fibrous material whether it is fine ground, as well as augmentations in the passage rate through increases in the turnover rate. Therefore, if turnover rate is increased, the passage rate would also increase. Hence, through augmentations in the passage rate, microorganisms that possess a lower growth rate, such as protozoa and archaea, will defaunate, thus decreasing methane production [16]. Otherwise, digestibility and methane production could be increased by increasing the retention time [17]. Additionally, by increasing the intake above the minimum for maintenance, the animal methane production will arise proportionally. This phenomenon will provoke a reduction in methane production per production unity [18]. Therefore, an animal fed under a pasture basis will produce less methane as part of the GHG produced compared to an animal fed with a high-concentrate or high-fiber proportion diet.

3.3 Bypass protein

The protein contained in ruminants' feedstuffs could be divided into two groups: degradable rumen protein (DRP) and undegradable rumen protein (URP). The first is degraded in rumen, and it is used as a nitrogen source in the microbial protein synthesis; the second escapes from ruminal degradation and is transported to the lower tract where it is susceptible of being absorbed by the animal in the form of amino acids [19]. In spite of several reasons to name it bypass protein, one of the main characteristics is its low retention time in rumen or, the inverse action, the high passage rate. In the case of high passage rates, microorganisms which possess a low growth rate will tend to defaunate; this is the case of the methanogens. Thus, methanogenesis will be affected and methane production will be reduced. Nowadays, some secondary metabolites are identified as protein protectors, by forming complexes with proteins and avoiding their degradation in rumen. The latter allows proteins to go through the low tract and to be absorbed after liberating complexes due to the acidic pH in the intestine [20].

4. Feed additives

Some strategies are focused on providing feed additives to modify the presence or absence of methanogens, protozoa, or the direct or indirect inhibition of ruminal methanogenesis [21]. By supplementing feed additives, good results are observed in methane production and productive performance. These strategies imply the use of high nutritive quality forages, organic acids, ionophores, probiotics, vegetable extractives, and secondary metabolites from different plants [22]. However, the most used are presented and briefly discussed:

- *Ionophores*: Ionophores are additives which possess a proved antimicrobial effect on some ruminal and cultivable strains, especially gram-positive bacteria [23]. Ionophore compounds like monensin and lasalocid have demonstrated to modify rumen fermentation and decrease methane emissions. The latter can be elucidated due to the fact that ionophores, as mentioned earlier, present affinity to hydrogen- and formate-producing, butyrate-producing, lactate-producing, and ammonia-producing bacteria, all of them gram-positive. However, succinate- and propionate-producing bacteria are resistant to ionophores [24]. Hence, it is assumed that reductions in the methanogenesis pathway are due to the hydrogen capture by propionate-producing bacteria, limiting methanogenesis through the restriction of hydrogen availability in the CO_2 reduction pathway. Unfortunately, prolonged use of monensin in steers has shown a loss of methanogenesis inhibition action and a resistance of bacteria to these antibiotics [25].

- *Homoacetogens*: Homoacetogens are a group of acetate-producing bacteria which can convert carbon dioxide into acetate using hydrogen [26]. The acetogenesis is a competitive pathway against methanogenesis for hydrogen use. Additionally, the production of ruminal acetate can be used as an energy source for the animal [27]. However, the thermodynamics of the reactions are more favorable to methanogenesis, and the use of ruminal homoacetogens as additives did not suppress methanogenesis in all the studies [28, 29].

- *Essential oils*: The effect of the addition of some essential oils into methanogenesis is through the capture of hydrogens in the biohydrogenation process of unsaturated fatty acids in the rumen [30]. Likewise, some medium-chain fatty acids contained in vegetable oils have demonstrated suppression of methanogenesis through the reduction of methanogens and ciliate [31]. In addition, some authors stated that the methanogenesis suppression with coconut oil was due to a change in methanogens population [32].

- *Yeast cultures*: The most used yeast culture in livestock research is *Saccharomyces cerevisiae*, and it has been used as a fermentation modifier [23]. Additionally, yeast cultures have been used as rumen fermentation modifiers and promoters of microbial growth [33]. In fact, rumen fibrolytic bacteria have a clear preference for a nitrogen source for ammonia production, and this is enhanced by yeast cultures for microbial protein synthesis [34]. Moreover, recent reports have suggested the stabilization of pH through a decrease in lactate production when using in vitro yeast cultures [35]. Thus, the antimethanogenic action is suggested through the improvement of fiber digestion and increasing ammonia-utilizing bacteria [36].

- *Others*: Vaccination and the use of bacteriophages are a different alternative for methane mitigation. Hence, vaccines against methanogens like *Methanobrevibacter* spp. have been applied to sheep presenting methane reductions of 7.7% [37]. Likewise, the use of phagaes against rumen archaea has been suggested by other authors as a strategy for methane abatement [22].

5. Conventional and nonconventional forage sources

As expressed before in this chapter, the increasing global population demands for a higher feed production, converting animal feeding production into a natural competitor for human feeding production in the search for arable lands. Consequently, diverse researches have focused into trying different forage sources which were not conventional as animal feeding before but now could be considered as alternative forage sources [38, 39]. Nonconventional forages include a wide variety of perennial plants and agriculture and commercial by-products which do not compete with human feeding. Therefore, diverse advantages can be observed when utilizing alternative forages such as (a) a considerable reduction in the feeding source costs; (b) exploitation of nutrients contained in agriculture by-products which otherwise would not be used (these by-products could be high in rapidly digestible carbohydrates or in fiber, both suitable for ruminants); and (c) an increase in the by-product cost which eventually will create economic benefits for producers and the productivity chain supply. Moreover, some agricultural by-products do not need any processing to be offered as animal feeding, hence the desirability of these by-products. Some of the ruminants feeding produced under this basis are:

a. Crop by-products such as garlic leaves, onion leaves, cocoa husks, coconut meal, cracked rice, sugarcane bagasse, molasses, tapioca discards, oat straws, and some aquatic crops like water hyacinth and azolla [38–41]

b. Perennial crops, seeds, and leaves of shrubs and trees like *Leucaena*, guamuchil, mesquite, mango, ebony, etc. [42–44]

Some farmers are still not aware of the nutritional value contained in by-products or in the form to be included into the productivity chain of animal feeding in an efficient way. In this regard, Asia and Africa are heavily focused on attempting to reach this goal. Due to the nature of some agricultural by-products, these tend to decompose in a short time. Hence, some techniques should be used to preserve and increase their shelf life. Therefore, some of the preservation techniques commonly used are listed as follows:

I. *Silages*: Many of the agricultural by-products are obtained in huge quantities due to the nature of the crops. However, the high humidity contents contribute to a short lifetime due to the rapidly appearance of fungi and, eventually, a decomposition. Therefore, the silage elaboration is a recommended preservation method due its large periods of storage, and it can always be offered fresh and with certain aroma provided from the fatty acids synthetized in the lactic fermentation which will add palatability for ruminants [45].

II. *Chemical treatments*: Some agricultural residues obtained from cereal crops are treated with chemicals to increase their digestibility. Thus, by-products with high lignocellulosic complexes could be treated with ammonia in anaerobic conditions to enhance lignin and fiber hydrolysis, which will improve their digestibility [46]. However, this process requires special plastic sheets that increases costs and could become an unaffordable process for small producers. In this way, previous researches have reported diverse alternatives using mud and eliminating the use of plastic sheets [47].

III. *Multi-nutritional blocks*: Another conservation technique which involves the utilization of high humidity agricultural by-products is the elaboration of multi-nutritional blocks [48]. This technology is very flexible and allows the producer to use ingredients considered as indispensable in animal feeding. Additionally, important nutrients could be available for longer periods of time since the useful life of these blocks is very extensive. Although in dry seasons, drought decreases considerably the nutritional quality of forages. These blocks are generally offered as supplementation in livestock feeding in rangelands as part of an extensive feeding system, and they are commonly elaborated to supplement vitamins and minerals, such as zinc and copper [49]. However, recent investigations are using more ingredients to improve their nutritional value and turn this into a more versatile practice.

IV. *Supplementation*: Supplementation is extensively used, especially if there is a deficient feed due to poor nutritional quality of some ingredients. By using this technique, some essential nutrients will be delivered to the livestock which otherwise could not be obtained by the animal itself. Nevertheless, the acquisition of ingredients for supplementation is unaffordable for some small producers. On the other hand, there are certain agricultural by-products which could be offered to the livestock and contain certain important nutrients at very low or even null cost. In this way, shrub and tree seeds could be a very good option. *Leucaena*, guamuchil, and ebony seeds are rich in protein and unsaturated lipids; these are being used without any affections in productive performance in small ruminants at very low cost. In this way, shrubs and trees seeds could be a very good option. Leucaena, ***Vicia faba***, ebony and other seeds are rich in protein and unsaturated lipids; these are being used without any affections in productive performance in small ruminants at a very low cost [50–53].

6. Plant metabolites

In the last years, ruminants have been target of several feeding strategies aiming to reduce ruminal methane production and emissions; most of them have been stated earlier in this chapter. However, the use of secondary metabolites arises as a viable and newer alternative in this concern. There is evidence which proves certain secondary metabolites, such as condensed tannins, saponins, and alkaloids, reduce methane production in in vivo and in vitro assays [54]. Generally, the mechanisms of action of these compounds point out to certain metabolic pathways:

I. **Tannins**: Tannins are water-soluble polyphenol polymers with a high and diverse molecular weight. They can form complexes with proteins, mainly, and metal ions, amino acids, and polysaccharides in a lesser extent. These metabolites are normally synthetized in shrubs, trees, legumes, fruits, cereals, and grains [55]. Tannins are divided into two groups: condensed tannins (CTs) and hydrolysable tannins (HTs).

Hydrolysable tannins: These are complex molecules attached to a polyol group as a central core which are partially or fully esterified with a phenolic group (e.g., gallic acid). The remaining phenolic groups could be later esterified or oxidized to produce more complexes with HTs [55].

Condensed tannins: These compounds are also known as proanthocyanidins and are mainly polymers of the flavan-3-ol units which are bind by interflavonoids C3-C8 and C4-C6 linkages, such as catechin and epicatechin. The methanogenic activity conferred to tannins is mostly due to the condensed tannins; CTs attach to proteins and avoid their degradation in rumen. Additionally, CT decrease methanogenesis through a reduction in fiber digestion [56]. Some studies affirm that CT enhances acetate formation via acetogenesis; this metabolic pathway uses hydrogen for acetate synthesis and reduces methanogenesis [57].

II. **Saponins**. According to their chemical structure, they are divided into two groups: steroids and triterpenoids. Steroids are predominantly in plants and are composed of 27 carbon atoms in the central skeleton of its molecule (e.g., spirostanol and furostanol). Otherwise, triterpenoids are composed mainly of aglycones with 30 carbon atoms in its molecule (e.g., oleanane) [58]. These are the most common types of saponins, especially in legumes [59]. Methanogenic action of saponins occurs by protozoa defaunation which is associated to methanogens. Moreover, saponins enhance production of propionate, a natural competitor of methane in hydrogen capture [58]. Nevertheless, some studies affirm that methane inhibition action by saponins is dose and time dependent and not conclusive [59].

III. **Flavonoids**. Flavonoids are phenolic compounds (like tannins); however, these contain only 15 carbon atoms linked to 2 aromatic rings connected through a 3-carbon bridge [60]. These metabolites are particularly studied for human purposes, and their biological benefits to health correlated to their consumption [61]. Almost all flavonoids are conjugated to glycosides and are common to find hydroxyl groups in carbons with four, five, and seven positions [60]. In addition, flavonoids stimulate microbial metabolism and reduce methane production through enhancing acetogenesis pathway and increasing hydrogen capture in propionate anabolism, in a similar way as described earlier with saponins [28, 59].

7. Other feedstuffs

Since the 1970s, ruminal microbes and their effect on ingested nutrients have been subject of intensive research [27]. Ruminal microorganisms are crucial for the digestive performance of animals. Addition of feedstuffs in diets of ruminants has led to investigate their effects on the absorption and utilization of nutrients as well as the ruminal environment and conditions. Genetically modified *Escherichia coli* showed a ruminal methanogenesis mitigation effect in sheep [62]. Other researches [63] reported that *Lactococcus lactis* produces nisin, which has demonstrated antimicrobial activity against Gram-positive bacteria, resulting in a mitigation effect on ruminal methane emission.

β1–β4 galacto-oligosaccharides (GOS), along with glucose, fructose, and starch, present in the rumen are used by *Bifidobacterium* and *Lactobacillus* as substrates to produce lactate and acetate. Lactate is one of the main transitional compounds during propionate production, which competes against methanogens for available hydrogen. As a result, methane production can be decreased by GOS consumption [64].

7.1 Probiotics

Probiotics are commonly defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host." Other authors indicate that a probiotic food carries 106–107 CFU/g viable probiotic cells, until the shelf life of the product is reached [65]. Probiotic foods contain sensitive ingredients, such as probiotic cells that require protection against oxidative stress, high acidity, freezing, shear stress, and other undesirable factors. Although microencapsulation has been primarily used to protect bioactive ingredients due to its advantages [66], co-extrusion technology has become an emerging alternative to encapsulate probiotic bacteria.

8. Extrudates and extrusion process

8.1 The use of the extrusion process in the supplementation of probiotics

Extrusion processing using oil and alginate solutions to create emulsions as core medium [67] has found a favorable survival of probiotic *L. acidophilus* at 4°C for 50 days. Over the years and because of technological advances, extrusion has become an almost unlimited cooking processing alternative due its inherent versatility. Multiple studies had focused on designing and evaluating the incorporation of biomass, distillery by-products, fruit pomaces, agro-industrial by-products, and dairy residues [68]. One of the main advantages of the thermal and pressure conditions during extrusion is the inactivation of antinutritional factors, elimination of pathogens, improved digestibility, reduced level of toxins, as well as the bitterness of some oil plants (flax, cotton, peanut, and sunflower) while achieving the desired organoleptic characteristics by properly adjusting residence times, specific energy absorbed, and pressure effects on the raw materials [67]. Other authors extruded rye whole meal to decrease microbial contamination and used it as cultivation medium for the evaluation of supplementation of dairy cow ration with *P. pentosaceus* BaltBio02 (9.6 log10 CFU g^{-1} $head^{-1}$ day^{-1}) [69]. Obtained results showed an increase ($P < 0.05$) of milk yield but did not affect milk composition or ruminal fermentation parameters. *Lactobacillus sakei* KTU 05-6 (9.6 log10 CFU g^{-1} $head^{-1}$ day^{-1}) was also analyzed but showed no significant impact on yield or ruminal parameters.

On the other hand, a different study evaluated the effect of different doses of probiotic containing 1.6 × 109 CFU/g of *Bacillus licheniformis* and 1.6 × 109 CFU/g of *Bacillus subtilis* on in vitro digestibility of concentrates and forages [70]. These authors concluded that 3 g $head^{-1}$ d^{-1} of probiotic increased by 10.9% starch digestibility after 12 h of incubation, indicating a promotion of NDF digestibility in roughages and starch in concentrates, although no significant changes were obtained of acetate, propionate, and butyrate molar ratios, possibly due to negligible changes on H^+ concentrations that affect the environmental pH of ruminal microorganisms [71]. An enhanced VFA production results in a pH reduction and growth inhibition of fermenting fibrous carbohydrate bacteria, which compromise NDF digestibility.

9. Current strategies

9.1 Methane reduction through improvement of the forage quality

There is a lot of information about supplementation of secondary metabolites, certain additives, and increasing concentrate fraction in the diet of livestock to abate methane emissions. However, some producers in developing countries are not able to afford these alternatives. Otherwise, methane production in ruminants in developing countries is directly correlated to a poor quality in feedstuffs offered to livestock, by decreasing the efficiency and productivity for productive unit [72]. In this way, the strategies that producers and researchers in developing countries use imply the production of improved forage sources which is cheaper than the acquisition of some supplements. Additionally, the use of these forage sources may increase the fertility in the soil which is desirable for nitrogen fixation. Consequently, by improving the quality and quantity of forage, the productivity will increase, and methane production will be reduced by productive unit.

9.2 Vaccination and chemical compounds

On the other hand, other researchers have focused their efforts on evaluating the inclusion of protected lipids and nitrate compounds [73, 74]. In addition, the use of some nitrate compounds showed no effect on organoleptic and nutritional properties in edible products for ruminants [75]. However, both strategies could be discarded by increases on fiber digestibility and a reduction of dry matter intake. Otherwise, the acquisition and use of these compounds in livestock will substantially increase production costs and market price. In the past decades, chemical compounds were used as inhibitors in methane synthesis through vaccination or the analogue supplementation. Nevertheless, methanogen defaunation is not a viable long-term alternative since microorganisms are easily adaptable to different environments. Additionally, the use of other additives, like ionophores, is forbidden in the USA. In this way, the use of plant extractives and especially metabolites arises as a sustainable alternative; however, there are not conclusive results which lead to a punctual design of dietary strategies. The latter is exposed since some of these metabolites may be present in edible products of ruminants affecting their organoleptic properties [76]. In addition, further studies are required to demonstrate the effectivity of extractable compounds of plants which are well perceived by the population as an alternative for chemical compound supplementation.

10. Conclusions

Methane and GHG mitigation in livestock is possible through different strategies, most of them as dietary alterations. However, it is necessary to carry out conclusive in vivo studies evaluating the use of metabolites and extractable plants' compounds, as well as the use of alternative forage sources which may provide directly these metabolites affecting the presence of ruminal methanogens and protozoa. Moreover, each region or geographic zone has different forage sources even perennial that can be produced locally. The incorporation of these into livestock feeding arises as a viable and sustainable alternative for mitigating GHG emissions, especially methane.

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