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A Review

Methane Production in the Rumen and its Influence on Global Warming

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Abstract

In the last decades greenhouse gases emissions are a great concern globally. It is well known that from the agricultural sector, ruminant livestock due to natural fermentation process contributes substantially to the increase in methane production. Methane emissions represent the second contributors to global warming but it has a potential of 23 times more than carbon dioxide. Ruminal methanogenesis represents an energy loss to the animal besides contribution to greenhouse gases emissions. Many attempts have been initiated to reduce ruminal methane productions in an ecologically and sustainable way, such us: immunization against ruminal methanogenesis, defaunation, uses of chemicals additives, ionophore antibiotics, plant extracts or diet changes. This paper presents the methanogenesis process in rumen, its impact on climate change and a number of mitigation strategies that can be effective *in vivo* and *in vitro*.
Keywords: global warming, methane, rumen, feed additives, defaunation, immunization.

1. Introduction

In the global worming terms, methane is a particularly potent greenhouse gas (GHG) which has a global potential 21 times that of carbon dioxide [19], and accounts for 16% of total global GHGs emissions. From anthropogenic sectors arise approximately 70% of methane production and agriculture accounts for about two-third [21] enteric fermentation, a natural process produced by ruminant animals, being responsible for one-third of methane from agriculture [32].

The enteric methane produced by ruminants has its origin in the rumen [29].

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Ruminal digestion of feed by the microorganisms, under anaerobic conditions, results in the production of acetate, propionate and butyrate (volatile fatty acids) which are used by the animal as energy source, and the production of ruminal gases such as CO_2 and CH_4, eliminated through eructation [29]. Since many years, national governments and international organisations have therefore put much effort in mapping the soil. Soil maps are also increasingly used to derive spatially distributed soil inputs to environmental and ecological process models. For instance, soil maps provide important information about physical, chemical and biological soil properties needed by acidification and groundwater flow models [1].

Methanogenesis process besides its negative impact on the environment represents a loss of 2-15% of gross energy intake [14] for the animal, leading to an unproductive use of dietary energy [22]. Techniques to manipulate this process include elimination of protozoa [15, 30], use of antibiotics (such Monensin) and bacteriocins such as Nisin [7], use of lipids sources (fatty acids, oils, and seeds) [42; 11], organic acids [33] and ionophores [13] or change in dietary composition [27]. Another attempt was immunization and biological control.

These techniques were used by Wright *et al.* (2004) and implied the production of a vaccine against three rumen methanogens [44]. The results from the study showed a decrease in CH_4 production by nearly 8% in Australian sheep. Another vaccine prepared with a different set of methanogens species and tested in other geographical region did not achieve a positive result, maybe because the community of methanogens species differs under different conditions [44].

Cook *et al.* (2008) used passive immunization with antibodies produced by laying hens against three common methanogens present in the digestive tract of ruminants. These treatment decreased CH_4 production *in vitro*, but the effect was lost after 24 hours of incubation [9].

Plant extracts are a new, safe and inexpensive way to reduce methane emission from ruminants [22] since several plant secondary metabolites have shown antimicrobial activity [6], as they can modify ruminal fermentation in a way that the efficiency of utilization of feed energy is enhanced and methane production is decreased [12].

2. Methanogens and ruminal methanogenesis

Methanogens represent a distinct group of microorganisms [31] which belong to the domain *Archaea* and the phylum *Euryarchaeota* [38]. They possess unique cofactors such as coenzyme M, HS-HTP, F_{420} and lipids [31] important for methanogenesis process. The F_{420} cofactor is necessary for the activity of hydrogenase and formate dehydrogenase enzymes and allows them to fluoresce blue-green at 420 nm [1].

Coenzyme M acts as terminal methyl carrier in methanogenesis process and represents the smallest organic factor [40]. The cell wall of methanogens contain nonrigid surface layers [40] consisting of pseudomurein in *Methanobrevibacter* and *Methanobacterium* [3], heteropolysaccharide acid in *Methanomicrobiales* order [4] and protein in *Methanomicrobium* [3].

Among methanogens, the cell shape and characteristics vary as well. The most important methanogen found in rumen, *Methanobrevibacter ruminantium* is rod shaped [3] with pseudomurein in the cell envelope and requires coenzyme M [31], hydrogen, carbon dioxide and formate for methane production [3]. From the same order (*Methanobacteriales*) such *M. ruminantium*, *Methanobacterium formicicum* [38] is nonmotile rod or filament shaped with pseudomurein in the cell wall [31]. The species that belong to *Methanobacteriales* and *Methanomicrobiales* orders are methanogens without cytochromes and their energy source is represented by hydrogen and formate [31]. The species from *Methanosarcinales* order are coccoid shaped without motility [3] and they have cytochromes [38]. Cytochromes or membrane bound electron carriers, play a role in the oxidation of methyl group to carbon dioxide [38]. *Methanosarcina* spp. can use a large range of substrate such as H_2, CO_2, methanol, methylamines and acetate [3].

The major part of methanogenesis in ruminants occurs in the large fermentative chamber known as rumen [36]. In here methanogens utilize hydrogen and carbon dioxide to produce methane but the *Methanosarcina* spp. are an exception because they grow slowly on these two substrates [18] and therefore these species utilize methanol and methylamines to produce methane [37]. The methanogenesis process in the rumen is the last step in the anaerobic conversion of organic matter to methane. This entire course involves a large number of microorganisms **(**fig.1**).**

Bacteria species, fungi and protozoa hydrolyze the proteins, starch and plant cell wall polymers in amino acids and sugars [31].

The amino acids and sugars are then fermented to volatile fatty acids (VFAs), hydrogen and carbon dioxide [1]. Methanogens species using both hydrogen (80%) and formate (18%) [18], produce the methane gas.

During ruminal methanogenesis, the hydrogenotrophic methanogens that use CO_2 or acetate as their carbon source and H_2 the donor electron source have an important role [2] (fig. 2). Carbon dioxide is carried by methanofuran (MFR) and is reduced to formate [26]. This first step involves the electrons donated by ferredoxin (Fd) reduced with H_2 [26].

The formyl group created is transferred to tetrahydrometanopterin (H_4MPT) forming formyl-H_4MPT [26] which is reduced to methenyl- H_4MPT and then to methylene- H_4MPT [26]. These reactions are catalyzed by 5, 10-methenyltetrahydrometanopterin cyclohydrolase and methylene- H_4MPT: coenzyme F_{420} oxidoreductase [26]. In the next step, the reaction catalyzed by methyl- H_4MPT: HS-CoM methyltransferase (Mtr) transfers the methyl group to H_4MPT forming methyl - H_4MPT.

The last step involves methyl-CoM which is reduced to CH_4 by the reaction of methyl- coenzyme M reductase [26].

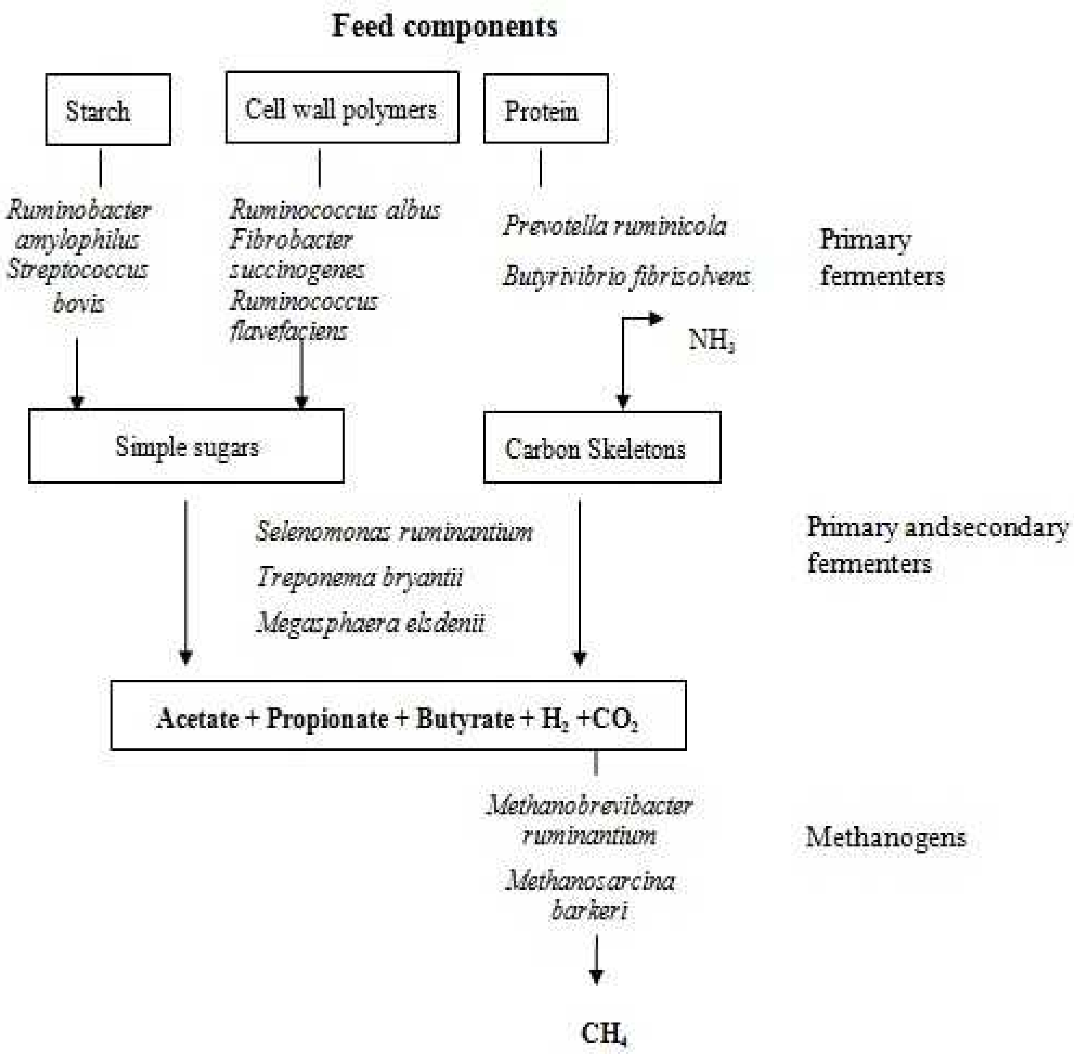

Figure 1. Microbial fermentation in the rumen. Primary digestive microorganisms digest feed to simple monomers which are in turn utilized by both primary and secondary fermenters. Methanogens prevent the accumulation of hydrogen by reducing carbon dioxide to methane [31]

Methanogens species have been classified into 28 genera and 113 species but in the nature can be expected to occur many more [20]. From the rumen few methnaogens have been isolated. The cultured methanogens have been assigned to seven species: *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanobacterium formicicum*, *Methanobacterium bryantii* *Methnaomicrobium mobile* and *Methanoculleus olentangyi* [20].

Also, *Methanobrevibacter smithii* and *Methanosarcina* spp. have been isolated from the rumen lately [20]. To analyze the total methanogens in the rumen some molecular techniques need to be used, such as PCR to amplify the 16S rRNA genes of archaea [20]. In some studies, Nicholson *et al.* (2007) analyzed the diversity of methanogens in the rumen using temporal temperature gradient gel electrophoresis [34]. The results showed that 66 methanogens sequences revealed the presence of methanogens species belonging to *Methanobacteriales*, *Methanosarcinales* and to the uncultured archaeal lineage [34]. The remaining 25 sequence were similar to *Methanobrevibacter ruminantium* and some were similar *M. smithii* [34]. *Methanobrevibacter ruminantium* and *Methanomicrobium mobile* were found in the bovine rumen to be the most important population by Yanagita *et al.* (2000) [45].

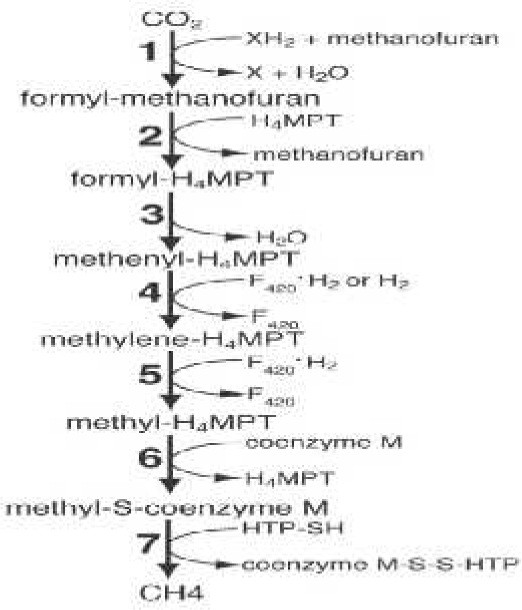

Figure 2. Methanogenesis pathway from H_2 and CO_2. The seven enzymatic pathways in the formation of methane in hydrogenotrophic methanogens are shown [2]

Clones similar with the methanogens from *Methanobacteriales* order were found in ovine rumen by Wright *et al.* (2004) [44]. Whitford *et al.* (2001) detected *Methanobrevibacter rumiantium* as the largest group of methanogens in lactating dairy cattle [43].

3. Methane mitigation strategies

Uses of ionophore antibiotics

The most affective antibiotic in ruminant fermentation is monensin, although other such as nigercin, gramicidin and lasalocid are available [24]. Monensin is produced by *Streptomyces cinnamonensis* and it is known to increase milk production [39] and they do not alter the diversity and quantity of rumen methanogens [17]. They shift the bacterial population from gram-positive to gram-negative and this means a change in rumen fermentation from acetate to propionate [36]. This is the reason why monensin does not affect methane production by altering the methanogens population, but instead inhibits the growth of bacteria and protozoa [38]. Beauchemin and coworkers (2009) included monensin in diets at a dose of <20 mg kg^{-1} but this dose did not affected the methane production [5]. In higher doses such as 24-35 mg kg^{-1} diet, monensin decreased methane production by 4-10 % [35]. A decrease by up to 30% was been reported by Guan and coworkers in 2006 when they used a dose level of 33 mg kg^{-1} diet of monensin [13]. These studies suggest that ionophore antibiotics, in special monensin, can be used for short-term decrease of methane production and also can improve the feed utilization [36].

Plant extracts as feed additives

Some feed additives from plant extracts have been analyzed for their ability to reduce rumen methane production [25]. Such plants extracts are saponins, tannins and essential oils, but in the last years many other feed additives were studied. Tavendale and coworkers (2005) used condensed tannins from *Lespedeza cuneata* against rumen methane production and found that reduced methane emissions by up to 57% in terms of g/kg DMI [41]. Other authors found that sheep consuming 41 g of tannin containing *Acacia mearnsii* per kg DM produced methane with 13% less than sheep feed normal forage [8]. Saponins containing *Sapindus saponaria* reduced methane emissions by up to 20% without affecting methanogens number in Rusitec studies [16]. In other studies, saponins was found to inhibit protozoa number *in vitro* and to limit hydrogen availability for methanogenesis [14]. It was found that essential oils present the same effect such as monensin by inhibiting gram-positive bacteria [38].

Uses of lipids as feed additives

Lipids are an option for feed supplementation that has been studied for their effects on methanogenesis process [38]. Oils, such as coconut oil, was used in RUSITEC simulators against rumen fermentation and showed that the main component (lauric acid) inhibited methanogenesis [10]. Lauric acid may have the same mechanism such monensin by inhibiting gram-positive bacteria including cellulolytic ruminococci [23]. Eugenè and coworkers (2008) made a meta –analysis of methane output with lipid supplementation in lactating dairy cows and found that 1% of lipids decreased methane production with 2.2 % [11]. In some studies on sheep and cattle, addition of lipids on diet, decreased methane production with 5.6 % [4]. Martin et al. (2009) made an excellent review on *in vivo* experiments with lipids to investigate their effect on rumen methanogenesis [29].

Defaunation

Defaunation represents the process that eliminates protozoa population from the rumen. This treatment has been used to investigate the role of protozoa population in rumen and to study the effect on methane production [38]. It is known that methanogens in rumen are attached on protozoa and they share a symbiotic relationship with participation in hydrogen transfer [28]. Methanogens species that are associated with protozoa are responsible for 9 to 37 % of the methane production in the rumen [28] and for this reason treatments that affect protozoa population in rumen may have an effect on methanogenesis process [38]. Hegarty et al. suggested that defaunation treatment reduced methane with 13% but this impact varied with the diet [15]. Other authors suggested that defaunation had an effect on rumen methanogens for more than two years and diet supplementation with ionophore reduce methane production in short-term [13].

Immunization against rumen methanogens

In the last years researchers tried to found a way to inhibit methanogens actions without affecting other ruminal microorganisms. For this, it is essential to evaluate methanogens-specific targets. The genome sequence of *Methanobrevibacter ruminantium*, strain M1 by Leahy et al. (2010), provided new perspective on the lifestyle of the most important methanogen found in rumen [25]. This is also essential in evaluation of a vaccine against methanogenesis process, which can be a long-term methane mitigation technology [25]. Researchers from Australia demonstrated that the vaccination against rumen methanogens can be a method in methane mitigation strategies [44]. They immunized sheep with a mixed whole-cell preparation from three methanogens and it was observed that these vaccine reduced methane production with 7.7 %. Cook et al. (2008) used IgY avian antibodies against rumen metanogens [9]. The results showed a reduction of methane production after 12 h of incubation but after 24 h the effect was lost [9].

After all the studies on vaccination against rumen methanogens, it was found that methanogens populations can be influenced by diets and geographic position and this fact can be a challenge to prepare an incolum which can be effective across different production systems [36].

4. Conclusion

The strategies discussed above and many others can have a potential effect on ruminl methane production but these strategies have been tested experimentally, and thus need more research to confirm that they are effective. Researchers found many aspects about rumen methanogens and these can contribute to further evaluations in order to improve ruminants productivity and to decrease ruminal methane emissions. Maybe farmers are unlikely to adopt some strategies and technologies unless they are cost-effective and they cannot improve animal productivity. Uses of feed additives and uses of high-quality forages may be more likely to encourage the farmers but a strategy that is effective in long-term and that has not an indirect effect on methanogenesis is needed.

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