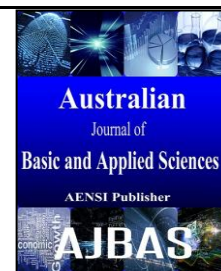




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Strategies to Mitigate Methane Emissions during the Digestive Process in Ruminants: A review

^{1,2}Samir Medjekal, ²Mouloud Ghadbane, ³Hacène Bousseboua

¹University Mentouri of Constantine, Department of Applied Microbiology, BP 360, route de Ain El-Bey, 25.017 Constantine, Algeria

²University Mohamed Boudiaf of M'sila. Faculty of Science. Department of Microbiology and Biochemistry. 28 000 M'sila. Algeria.

³Ecole Nationale Supérieure de Biotechnologie, Ville universitaire Ali Mendjeli BP 66E RP 25100, Ali Mendjeli/Constantine (Algérie).

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ABSTRACT

Methane emitted from ruminant livestock is regarded as a loss of feed energy and also a contributor to global warming. Methane is synthesized in the rumen as one of the hydrogen sink products that are unavoidable for efficient succession of anaerobic microbial fermentation. Strategies for reduction of methane emissions from the rumen have been proposed. Mainly through rumen microbial manipulation, by the use of agents including chemicals, antibiotics and natural products such as oils, fatty acids and plant extracts. A more recent approach is the development of vaccines against methanogenic bacteria. While ionophore antibiotics have been widely used due to their efficacy and affordable prices, the use of alternative natural materials is becoming more attractive due to health concerns regarding antibiotics. It was concluded that the most promising areas for future research for reducing methanogenesis are the development of new product/ delivery systems for antimethanogenic compounds in the rumen and reduction in protozoal numbers in rumen. Whatever the approach proposed, however, before practical solutions are applied in the field, the sustainability of CH₄ suppressing strategies is an important issue that has to be considered. The evaluation of different strategies, in terms of total greenhouse gas emissions for a given production system, is discussed.

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INTRODUCTION

Background:

Methane (CH₄) is one of the three main greenhouse gases (GHG), together with carbon dioxide (CO₂) and nitrous oxide (N₂O). Methane has a thermogenic affect some four to six times that of carbon dioxide and the one most associated with animal agriculture (Leng, 1993; Moss, 2000; Gworgwor, 2006). The agricultural sector contributes a total of 10-12% of global anthropogenic GHG emissions (McAllister, 2011). The livestock sector is one of the largest CH₄ producers, with total emissions from enteric fermentation estimated to be 70-100 Tg/year (Hegarty, 1999; Thorpe, 2009; Ramin, 2013).

Ruminants establish a symbiotic relationship with rumen microorganisms by which the animal provides nutrients and optimal environmental conditions for the fermentation of feeds, and microorganisms degrade fibre and synthesize microbial protein as an energy and protein supply for

the animal, respectively. However, this symbiotic relationship has energy (losses of methane) and protein (losses of ammonia N) inefficiencies (Van Nevel, 1988; Calsamiglia, 2007). The ruminal methane production is a by-product of the microbial digestive process and represents a loss of 2–12% of the feed energy (Calsamiglia, 2007). Furthermore, emission of methane is considered as one of the most important global environmental issues (IPCC, 2001). Therefore, decreasing methane production is desirable for reducing the greenhouse gas emission with improved efficiency of the digested energy utilization (Johnson, 1995; Goel, 2011).

Ruminant nutritionists have long been interested in modulating the competition among different microbial populations with the objective of improving the efficiency of energy and protein utilization in the rumen. Among the factors that influence ruminant methane production, changes in the rumen fermentation pattern resulting from altered diets is probably the most important. Furthermore, various feed supplements have been found to directly

Corresponding Author: Samir Medjekal, University Mentouri of Constantine, Department of Applied Microbiology, BP 360, route de Ain El-Bey, 25.017 Constantine, Algeria
Tel: (213) 0661 400 670 E-mail: medjekal.samir@univ-msila.dz

or indirectly reduce methane emissions, including halogenated methane analogues (Ungerfeld, 2004), bacteriocins (Lee, 2002), propionate enhancers, acetogens, immunization, genetic engineering, phage, fats and probiotics such as *Saccharomyces cerevisiae*, *Aspergillus oryzae* etc. (Moss, 2000; Gworgwor, 2006; McAllister, 2011; Hegarty, 1999; Thorpe, 2009; Ramin, 2013; Van Nevel, 1988; Calsamiglia, 2007; IPCC, 2001; Johnson, 1995; Goel, 2011; Ungerfeld, 2004; Lee, 2002; Boadi, 2004; Kumar, 2009).

Methane mitigation in ruminants is possible through various strategies. Today, the feeding management approach is the most developed. An integrated approach that considers the rumen microbiota, the animal and the diet seems the best approach to find a long-term solution for reducing enteric CH₄ production by ruminants.

Factors influencing methane production:

Factors that can be identified as influencing CH₄ production in ruminants include dietary characteristics as well as the fermentation conditions in the rumen. Diet has an important impact not only on methanogen numbers but also on methane production, as both the quantity and quality of feed can alter the rumen fermentation pattern. It is widely recognized that alterations in the diet strongly affect rumen functioning and performance of ruminants (e.g. roughage: concentrate ratio, or the fibre, starch, sugars and protein content of the feed). Increase the level of starch or rapidly fermentable carbohydrates, to enhance propionate production, reduces excess hydrogen and subsequent CH₄ production. Moreover, addition of readily fermentable carbohydrates (e.g., cereal grain) to diets fed at maintenance levels causes a proliferation in the ciliate population (Bonhomme, 1990). Ciliates are symbiotic with methanogens (Stumm, 1986; Finlay, 1994) and the increase in methane production when grain is fed at maintenance may be due to an increase in hydrogen transfer between these microorganisms (Krumholz, 1983).

Among important fermentation conditions are acidity (pH) of rumen fluid. The optimum pH for methane production is 7.0-7.2, but the gas production can occur in the pH range of 6.6-7.6. However, beyond this range, the activity of fibre degraders reduces (Arglyle, 1988; Dijkstra, 1992). Additionally, there is a different profile of volatile fatty acids and methane production with different carbohydrates fermented (Murphy, 1982; Friggens, 1988). Simple carbohydrates produce more methane (0.45 moles/mole of hexose) as compared to complex carbohydrates (0.3 moles/mole of hexose). Forage: concentrate ratio also influence the acetate: propionate ratio, and methane emission decreases drastically from 6-12% (forage-based diet) to 2-3% when a diet with concentrate (90%) predominates (Johnson, 1995).

Methane yield can thus be related to many and different categories of factors. However, these factors are often interrelated and so are their effects on CH₄ yield in the rumen. This complicates the use of such factors to predict the course of fermentation in the rumen, the extent of organic matter digestion and the productive response of the ruminant. Due to these interrelationships, the contribution of a single feed component or type of carbohydrate to CH₄ yield is not necessarily constant (Smink, 2003), but may vary with a change of the dietary characteristics and the fermentation conditions in the rumen (Mills, 2001; Bannink, 2005).

Diet composition:

The type of feed offered to a ruminant can have a major effect on methane production. It is well known that methane production is influenced by quality and quantity of feed stuffs. The forages to concentrate ratio of the ration has an impact on the rumen fermentation and hence to acetate: propionate (A/P) ratio (Moss, 2000; Mitsumori, 2008). Van Soest (1982) indicated that a high grain diet and/ or the addition of soluble carbohydrates shifted the fermentation pattern in the rumen which give rise to a more hostile environment for the methanogenic bacteria in which passage rates are increased, ruminal pH is lowered and certain population of protozoa, ruminal ciliates and methanogenic bacteria maybe eliminated or inhibited, which results in lower CH₄ production. Russel (1998) suggested that rumen bacteria that produce propionate are more sensitive to pH than some bacteria that produce acetate and H₂, because the A/P ratio was dramatically increased and a large amount of H₂ was detected when the final pH in his experiments was less than 5.3. Moreover, the final pH range of 5.3 to 6.5, CH₄ production was highly correlated with A/P ratio, which depended on the pH and substrate (CH₄ = 0.02 + 0.05 pH; r² = 0.80) (Russell, 1998).

Forage type and quality can also increase voluntary feed intake, which consequently reduces retention time in the rumen and partly shifts digestion from the rumen to lower gut (Eckard, 2010). The digestion of starch and protein in the small intestine is characterized to be more efficient compared to rumen fermentation, resulting in a reduced conversion of feed energy to methane (Blaxter, 1965). Several authors have shown that including tannin rich legumes (sainfoin, lotus, sulla) and shrubs in the diet contribute to a decrease in methanogenesis due to the presence of condensed tannins (Eckard, 2010; Blaxter, 1965; Waghorn, 2007). Robertson and Waghorn (2002) observed that CH₄ production from grazing dairy cows increased with forage maturity. A putative decrease in CH₄ with young fresh forages may be explained by a higher content of soluble sugars and linolenic acid. More generally, the correlation between forage quality and CH₄ emissions is low (Pinares-Patino,

2003). Methanogenesis tends to be lower when forages are ensiled than when they are dried, and when they are finely ground or pelleted than when coarsely chopped (Boadi, 2004; Beauchemin, 2008). Improving forage quality tends to increase voluntary intake and reduces retention time in the rumen, promoting energetically more efficient post-ruminal digestion and reducing the proportion of dietary energy converted to CH₄ (Blaxter, 1965).

Dietary fat supplements:

Dietary fat seems a promising nutritional alternative to depress ruminal methanogenesis without affecting other ruminal parameters. Fatty acids, especially those of medium chain length (C8–C16) (e.g., coconut oil, canola oil, kernel oil etc.) have been found to decrease methane production (Machmuller, 1999; Dohme, 2000) and in study a 22% reduction was achieved by addition of sunflower oil to the diet (McGinn, 2004). Most of the oils and fatty acids that reduce methanogenesis reduce the ruminal level of protozoa that are known to be cosymbionts of methanogens. Therefore, a reduction in protozoan numbers is partly responsible for the decreased methane production induced by oils and fatty acids (Kobayashi, 2010). However, the effects of fat on methane production are not limited to those mediated via the rumen protozoa. Lipids have also been shown to inhibit methanogenesis even in the absence of rumen protozoa (Dohme, 1999), possibly due to the toxicity of long chain fatty acids to methanogenic bacteria (Henderson, 1973). Linolenic acid is toxic to cellulolytic bacteria (*F. succinogenes*, *R. albus* and *R. flavefaciens*) by disrupting their cell integrity, and to the cellulolytic fungus *Neocallimastix frontalis* grown *in vitro* [43]. In addition, biohydrogenation of polyunsaturated fatty acids results in an uptake of hydrogen. However, its influence on methanogenesis is low since the complete hydrogenation of 1 mol of linolenic acid spares 0.75 mol of CH₄ (Martin, 2010). A common effect for all lipid sources is that unlike other feed constituents such as forages and cereals they are not fermented in the rumen, and thus the decrease in fermented organic matter leads to a decrease in CH₄ production. Among common sources of lipids, coconut oil suffers from a possible negative effect of medium-chain fatty acids (FA) on human health, due to an increase in meristic acid in milk (Martin, 2010). In contrast, polyunsaturated FA are considered beneficial to human health. The inclusion of polyunsaturated FA in ruminant diets results in a limited increase of these FA's in milk and meat could thus be proposed as a way for CH₄ abatement provided that supplementation levels do not decrease feed efficiency or performance of animals (Martin, 2010).

Methane inhibitors:

A large number of halogenated methane analogues and related compounds have been studied for their efficacy in inhibiting CH₄ production. Some of them, including chloroform, amichloral and 2-bromoethanesulfonic acid, can be used directly to suppress methanogenesis, but they have an adverse effect on the animal (Dong, 1999). Chloroform reduced methanogenesis *in vitro* and *in vivo* (Bauchop, 1967; Clapperton, 1974), but is obviously not suitable for use in practice. Chloral hydrate, which is converted to chloroform in the rumen (Prins, 1965; Quaghebaur, 1971), inhibited methane production *in vivo* (Mathers, 1982) but lead to liver damage and death in sheep after prolonged feeding (Lanigan, 1978). In contrast, amichloral appeared to be safer and was found to increase live weight gain in sheep. Denman *et al* (2007) reported that bromochloromethane could decrease the number of methanogenic *Archaea* by 34% as assayed by qPCR and thus reduce methane production. But the anti-methanogenic activity of bromochloromethane has been reported to be transient (Sawyer, 1974). However, a combination of bromochloromethane with α -cyclodextrin was found to be more stable, and was shown to suppress methane emissions in sheep and cattle over a prolonged period (McCrabb, 1997). 2-bromoethanesulfonic acid (BES), a bromine analogue of coenzyme F involved in methyl group transfer during methanogenesis, is a potent methane inhibitor (Martin, 1985; Wolfe, 1982). BES is a specific inhibitor of methanogens and does not appear to inhibit the growth of other bacteria (Sauer, 1987; Sparling, 1987). However, when tested *in vivo* the inhibition in methanogenesis was transient suggesting that adaptation of the methanogenic population occurred (Van Nevel, 1995). Quaternary ammonium compounds inhibit methanogenesis at concentration of 25 mg/l or above but at prolonged periods of incubation the effect become transient (Tezel, 2006). Anthraquinone has also been shown to inhibit methanogenesis *in vitro* and in lambs by inhibiting the methyl-coenzyme M reductase (Garcia-Lopez, 1996; Kung, 1998).

Mevastatin and lavastatin, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, can inhibit growth and methane production of *Methanobrevibacter* strains isolated from the rumen (Miller, 2001). Since *Archaea* are the only bacteria known to possess biosynthetic HMG-CoA reductase, HMG-CoA reductase inhibitors would have the potential to specifically inhibit rumen methanogens without inhibiting other rumen bacteria [63]. Inhibitors directly toxic to methanogens are a powerful tool to stop methanogens from producing methane. However, as a result of these inhibitors, Hydrogen, which could suppress the activity of rumen fermentation, can be expected to accumulate in the rumen (Baker, 1995).

Ionophores:

Ionophores are lipid-soluble molecules. Some ionophores are used as antibiotics or as growth-enhancing feed additives for growing cattle. Adding ionophores to the diet of ruminants has been shown to reduce CH₄ production (Moss, 2000). It promotes the production of propionate at the expense of acetate and reduces feed intake (Ramin, 2013; Johnson, 1995; Baker, 1995; Hegarty, 1999). Ionophores are generally effective against Gram-positive bacteria but exhibit little or no activity against Gram-negative bacteria and methanogens in the rumen (Nagaraja, 1997). Furthermore, entodiniomorphs (*Entodinium*, *Diplodinium* and *Ophryoscolex*) in rumen ciliates are sensitive to ionophores (Dennis, 1986). It is assumed that ionophores are able to modify rumen fermentation based on their antimicrobial spectrum, which has been examined using culturable strains (Mitsumori, 2008).

Monensin is the most commonly used ionophore in ruminant nutrition and was originally developed as a coccidiostat in poultry (Chapman, 2010; Rodehutsord, 2013). Monensin is considered as a growth promoter due to its favorable effects on rumen fermentation including methane reduction, propionate enhancement and ammonia reduction, together with its preventive effects on coccidiosis, bloat and lactic acidosis. These effects are attributed to a selective antimicrobial action of monensin on rumen microbes. Review papers indicate that methane reduction by monensin ranges from 4 to 31% (Schelling, 1984; Rumpler, 1986). A recent report indicated that long term administration of monensin to dairy cattle stably reduced methane by 7% and that this reduction persisted for 6 months with no adverse effect on milk yield (Kobayashi, 2010; Odongo, 2007). Further investigations are needed to verify whether monensin is able to reduce methane production without adverse effects on rumen fermentation, and to clarify its effect on microbial protein synthesis on the basis of commonly used feedstuffs in ruminant production.

This family of additives is not permitted in many countries including the European Union. Research has focused on the use of other compounds that can be substituted for ionophores but have the same inhibitory effect. Plant extracts have shown inhibitory effects on CH₄ production. Using a rumen simulation technique, McAllister and Newbold (McAllister, 2008) reported that a commercial allicin product originating from garlic was effective in inhibiting CH₄ production at a dose of 20 µg/mL without any adverse effect on daily VFA production. Tannins have also been reported to reduce emissions of CH₄ from ruminants, either by reducing the methanogen population in the rumen directly or by reducing the protozoa population, which tends to increase the molar proportion of propionate in the rumen (Bhatta, 2009). Tavendale *et al* (Tavendale, 2005) reported a decrease in CH₄ production when legume forages containing condensed tannins were

incubated *in vitro*. Reductions in H₂ production or direct inhibitory effects on methanogens were the suggested mechanisms by which tannins reduced CH₄ production (Ramin, 2013).

Defaunation:

Protozoa provide a habitat for 10 to 20% of rumen methanogens and produce acetate and butyrate as substrates for methanogenesis (Rodehutsord, 2013; Stumm, 1982)]. The elimination of protozoa (defaunation) is reported to considerably decrease the methane production compared with the normal faunated animals (Williams, 1988; Santra, 2002). Defaunation can be done by lowering the pH in the rumen (e.g. feeding grain), if the pH fall below 5.0, the ciliate protozoa are to be completely eliminated. In addition, the inclusion of fat in ruminant diet depress protozoal numbers (Ikwuegbu, 1982; Czerkawski, 1975) and the use of lipids as a defaunating agent has been suggested (Moss, 2000; Newbold, 1988).

It is possible to get defaunated animals with a consequently lower methane production, when they are isolated as new-borns from other ruminants (Faichney, 1999). Hegarty (Hegarty, 1999) reviewed further possibilities to eliminate protozoa by the use of synthetic chemicals (e.g. copper sulphate or calcium peroxide). Plant secondary metabolites i.e., saponin-containing plants are reported to suppress or inhibit protozoa and certain bacteria in the rumen (Cheeke, 2000). Saponins are glycosides which interact with the cholesterol present in the membrane of protozoa and causes cell lysis (Hess, 2003).

Consequently, the elimination of the rumen protozoal population to mitigate methanogenesis appears interesting, but this option should be carefully evaluated in terms of livestock performances. The absence of protozoa from the rumen can have diverse effects on animals that can be either negative or positive depending on the diet and the type of production targeted. Up to now, however, practical defaunation techniques are not available (Martin, 2010).

Acetogenesis:

An alternative strategy to reduce ruminal methanogenesis would be to rechannel substrates for methane production into alternative products, in fact, potential microbes for CO₂-acetate fermentation exist in the rumen, but CO₂-CH₄ fermentation is always predominant in the rumen (Crutzen, 1995; Fievez, 1999). Acetogenic bacteria in the hindgut of mammals and termites produce acetic acid by the reduction of carbon dioxide with hydrogen and reductive acetogenesis acts as important hydrogen sink in hindgut fermentation (Lajoie, 1988; Demeyer, 1991). Adding acetogenic bacteria daily as a feed additive to the diet of ruminants can inhibit CH₄ production, as they compete with methanogens by using H₂ and producing acetic acid. Joblin (Joblin, 1999) reported that artificially increasing the

concentration of acetogens through exogenous inoculation could be useful for competing against methanogens as they have many-fold greater hydrogen threshold concentration than methanogens. Similarly, Lopez *et al* (1999) found that acetogens suppressed methane production when added to the rumen fluid *in vitro* but that they did not persist in the rumen. However, they concluded that it might be possible to achieve the same metabolic activity using acetogens provided as a daily-fed feed additive. Unfortunately, it has been reported that in the rumen conditions, acetogens are unable to compete with methanogenic *Archaea*. Acetogens are more active in the gut of termites and the human colon than in the rumen (Immig, 1996; Klieve, 1999), furthermore acetogens are more numerous in the hind-gut than in the rumen of the dairy cow (Fievez, 1999).

Vaccines and other factors:

Several biotechnological strategies are currently being explored. Vaccination is a novel strategy to reduce CH₄ production in ruminants. This approach has the benefit of using the animal's immune system to produce antibodies against specific methanogens, instead of chemicals, drugs, or antibiotics that may be potentially harmful to the animal or the environment. Baker (Baker, 1995) has proposed that it may be possible to immunize ruminants against their own methanogens with associated decreases in CH₄ output. Shu *et al* (1999) have shown that such an approach can successfully reduce the numbers of *Streptococci* and *Lactobacilli* in the rumen. In a further study by Wright *et al* (Wright, 2004), two vaccines were developed, named VF3 (based on three methanogen strains) and VF7 (based on seven methanogen strains), which produced a 7.7% methane reduction per dry matter intake, despite only approximately 20% of the methanogen population being targeted. The same research group also created a vaccine based on five methanogen strains that was administered in three vaccinations to sheep (Williams, 2009). An additional vaccine has recently been developed using subcellular fractions of *Methanobrevibacter ruminantium* M1. Twenty sheep were vaccinated and then revaccinated three weeks later and the antisera was found to cause agglutination of methanogens and decrease growth and methane production *in vitro* (Wedlock, 2010).

Biological strategies to control methanogens are one approach influencing CH₄ production. Bacteriophages against rumen bacteria, not rumen *Archaea*, have been detected in the rumen (Swain, 1996; Klieve, 2004). The presence of phages against archaea in the rumen has also been suggested (Newbold, 1996). Some bacteriocins are known to reduce CH₄ production *in vitro* (Lee, 2002; Callaway, 1997). Nisin is thought to act indirectly, affecting hydrogen-producing microbes in a similar way to that of the ionophore anti-biotic, monensin (Callaway, 1997). There is a single *in vivo* result

reporting a significant 10% decrease of CH₄ emissions in sheep with this bacteriocin (Santoso, 2004). In contrast, the expected effect of nisin on the improvement of nitrogen metabolism was not observed in other *in vivo* reports (Santoso, 2004; Russell, 2002) implying that the same may happen if CH₄ was measured. A bacteriocin obtained from a rumen bacterium, bovicin HC5, decreased CH₄ production *in vitro* up to 50% without inducing methanogens' adaptation (Lee, 2002). The reported inhibitory effect on methanogenesis of spent culture from *Lactobacillus plantarum* 80 is also probably induced by a bacteriocin or a similar compound. The compound(s) in question reduced numbers of methanogens, but, like many other inhibitors that are efficient *in vitro*, the effect was lost in sheep after continuous administration for a few days (Nollet, 1998).

Plants and their extracts:

Many compounds extracted from plants have been screened and utilized for their ability to reduce rumen methanogens, because of the increased awareness of the hazards associated the use of antibiotics and chemical feed additives (Wallace, 1996). One such compound is saponin. These are glycosides found in many plants that have a direct effect on rumen microbes. Saponins decrease protein degradation and favor at the same time microbial protein and biomass synthesis (Makkar, 1996), two processes that result in reduced availability of hydrogen for CH₄ production (Dijkstra, 2007). However, the mode of action of saponins seems to be mostly related to their anti-protozoal effect (Newbold, 2006). For tannin-rich plants, the antimethanogenic activity has been attributed mainly to the group of condensed tannins. Hydrolysable tannins, although they also affect methanogens (Field, 1989), are usually considered more toxic to the animal (McSweeney, 2001) and have not been extensively tested. The condensed tannins appear to decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins act more through inhibition of the growth and/or activity of methanogens- and/or hydrogen-producing microbes (direct effect). Most essential oils and active components tested at high doses have inhibited rumen microbial fermentation, confirming their antimicrobial activity. Garlic oil, cinnamaldehyde, eugenol, anethol, and capsaicin are potential options for application as additives for ruminants. However, even though *in vitro* research is still needed for screening new products and determining mechanisms of action, there is an urgent need to conduct *in vivo* studies to determine the optimal dose in units of the active component, the potential adaptation of rumen microflora to the action of these additives, the fate of these products in the animal and the presence of residues in meat or milk, and the effects on animal performance (Calsamiglia, 2007).

Conclusions:

There is growing worldwide interest in reducing methane emissions from domestic ruminants. Methane is a potent greenhouse gas and its release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. Methane mitigation options from ruminants focus on increasing production per animal, modifying diet, decreasing numbers of methanogens and methanogen activity and by reducing livestock numbers. Moreover, stimulation of propionate production could be the best alternative hydrogen sink to methanogenesis in the rumen. Therefore, a strategy for abatement of methane production should be considered concurrently with a strategy to enhance propionate production. A universal feed additive that works under different conditions may not exist because effects have been diet and ruminal pH dependent. Although various feed additive candidates are now available to achieve this aim, the choice of additive must depend on the potency, safety, and expense of the candidate additive. Models predicting the amounts of CH₄ produced by ruminants are more of an interest for inventories such as IPCC and for developing mitigation strategies. Emphasis should be placed on developing models predicting CH₄ production with minimum requirements needed as input variables, with the goal of achieving higher accuracy. For evaluating mitigation strategies, models that are able to predict feed intake with reasonable accuracy and that rely on input data available at the time of predictions should be developed. It would also be useful to develop ration formulation models, in which CH₄ production per unit product can be used as a constraint.

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