Inhibitors of methanogenesis. Review.

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Abstract

One way to reduce methane release during the digestion process, is to use feed additives and methanogenesis inhibitors. Methane inhibitors block, or inhibit methane formation by altering the structure or function of key drivers of methane formation.

The mode of action of these feed additives is by altering fermentation to promote alternative uptakes of H2, such as propionate production, or by inhibiting methanogenesis (McAllister and Newbold, 2008; Martin et al., 2010). Ruminal modifiers do not act directly on methanogens, but rather on the conditions that promote methanogenesis. These feed additives include lipids, plant secondary compounds, and essential oils (Honan et al., 2021).

The efficacy of lipids has been studied extensively, and although the addition of medium-chain and polyunsaturated fatty acids has been shown to significantly reduce enteric CH4 production, the results have been variable. Similarly, secondary plant compounds and essential oils have shown inconsistent results, ranging from a significant reduction to a moderate increase in CH4 emission in the gut.

Due to the continued interest in this area, research is expected to accelerate in the development of feed additives that may provide options to reduce CH4 emissions in the ruminant digestive tract.

Keywords: methane; inhibitors

Introduction

Many feed additives with different efficiencies have been tested to reduce CH4 emissions in the gut. The mode of action of these feed additives is by altering fermentation to promote alternative uptakes of H2, such as propionate production, or by inhibiting methanogenesis (McAllister and Newbold, 2008; Martin et al., 2010). Some examples of feed additives are ionophores (e.g., monensin), electron acceptors (e.g., nitrate, malate, fumarate, sulfate), plant bioactive compounds (e.g., tannins, saponins, essential oils), enzymes, and direct-feeding microbes (e.g. exogenous enzymes, yeasts), dietary lipids (e.g., vegetable oil, animal fats), and CH4 inhibitors (e.g., bromochloromethane, 2-bromoethane sulfonate, chloroform, cyclodextrin, algae, 3-NOP). Some compounds are effective in reducing methane emissions, but their use is limited due to toxicity, reduced feed intake, degradability of fibre in the ration and animal performance, or environmental interaction issues (Hristov et al., 2013a).

One way to reduce methane release during the digestion process is to use feed additives and methanogenesis inhibitors. Methane inhibitors block, or inhibit methane formation by altering the structure or function of key drivers of methane formation. Commonly known additives that fall under this denominator include 3-nitrooxypropanol (3NOP) and bromoform, occurring/existing naturally in the red seaweed Asparagopsis taxiformis (Carrazco, 2021). On the other hand, rumen fermentation modifiers lead to further beneficial changes in the rumen environment. In addition to reducing methane formation, rumen fermentation modifiers can also improve animal performance and health, for example through increased availability of dietary protein to animals. Essentially, a lot of protein and nitrogen is lost by the rumen microbes. Rumen modifiers help "protect" the protein from use by less desirable microbes, thereby increasing the availability of that protein to the animal itself. These modifiers can be both artificial such as ionophores, and natural compounds such as tannins and essential oils. Red seaweeds are of interest because of their enormous potential as a feed additive. A. Taxaformis reduces methane emissions more than any other additive tested without affecting feed digestibility. Numerous studies have been conducted with A. taxiformis as an additive and has been found to reduce the amount of methane (methane production/kg dry matter intake) up to 55% in dairy cattle and up to 98% in beef cattle. Although researchers are still unsure exactly how A. taxiformis helps reduce methane emissions, it has been found to naturally contain bromoform, which is a known methane inhibitor. As a natural product, A. taxiformis also shows additional beneficial properties that can improve production or animal health.

Feed additives reducing methane production and release

A series of studies by Zain et al. (2021), using feed additives (direct-fed microbes), and various methanogenesis inhibitors (plant bioactive compounds and lipids), were tested to determine their effect on nutrient digestibility and methane production in plantation waste-based feeds. In vitro and in vivo experiments were conducted on different ruminant species. Plant bioactive compounds, such as tannins have been shown to reduce methane production through their ability to defaunate the rumen. Tannins may also have a direct effect on methanogens and indirectly by reducing fiber digestion. In addition, feed additives from direct-fed microbes (DFM), such as Saccharomyces cerevisiae, Bacillus amyloliquifaciens and Aspergillus oryzae can be used in ruminants to enhance their performance. In addition, virgin coconut oil as a dietary lipid contains medium-chain fatty acids, mainly lauric acid, which can inhibit the development of protozoan ciliates and methanogenic bacteria that produce methane in the rumen.

Tannins are known for their ability to bind and denature proteins. When included in the ration of cattle, this protein binding results in reduced protein degradation in the rumen by microbes, resulting in more protein degraded in the abomasum (it is the acid compartment of the stomach where proteins are broken down). In addition to this, some classes of tannins have also shown the potential to reduce intestinal methane emissions by 13-16% in dairy cattle. But when added too much to feed, tannins can make it more difficult for cattle to digest feed and even cause them to take in less feed, which can ultimately lead to lower animal performance.

Like tannins, essential oils are secondary plant metabolites that vary greatly in structure and function. Best known for their antioxidant and immunological properties, key essential oils also have the ability to reduce methane emissions and improve animal health and performance in various aspects. Although, some essential oils appear to have beneficial effects, when applied alone, essential oils appear to have enhanced effects, when offered as a blend of several components. Two essential oil blends of interest are Agolin and Mootral. Agolin is a commercially available blend of coriander seed oil and extracts of common nutmeg and wild carrot. When included in cattle rations, Agolin has demonstrated the ability to improve animal performance, while reducing intestinal methane emissions. A recent study at UC Davis and the CLEAR Center found an 11% reduction in methane intensity (methane/cow production; methane production/kg energy-adjusted milk), when dairy cows were fed 1g/head/day of Agolin. Other trials where Agolin was fed found a 6% reduction in methane production and up to 20% reduction in methane intensity and a marked increase in animal performance, including milk yield. Mootral is another essential oil blend composed of garlic and citrus extracts. Although, more research is needed, the results are favorable, demonstrating a reduced amount of methane 12 weeks after starting the supplement.

Scientists from the Netherlands, Australia, Brazil, the United Kingdom and the United States are evaluating both the effectiveness and health effects of several methane-reducing feed additives. These include red seaweed, ozone, the enzyme inhibitor 3-nitrooxypropanol (3-NOP) and the essential oil agoline, as well as blends of garlic and citrus, oregano and green tea /https://www.darigold.com/6-feed-additives-reduce-cows-methane-emissions/.

Eubiotics are innovative feed additives that play an essential role in maintaining animal performance and welfare by supporting gut health. Good gut health is a prerequisite for efficient and environmentally friendly farm animals in modern farming systems. A proper balance of microflora in the intestinal tract (known as eubiosis) is essential for optimal gut function /https://www.dsm.com/anh/products-andservices/products/eubiotics.html/. Types of eubiotics, Eubiotics fall into five categories:

- Organic acids
- Probiotics
- Prebiotics
- Phytogenic or essential oils
- Enzymes for gut health

All five types of supplements contribute positively to modulating the microbiome, leading to improved gut health.

Bovaer® is a feed additive that allows farmers to achieve a significant and immediate reduction in the environmental footprint of meat, milk and dairy products. On average, it reduces intestinal methane emissions by 30% from dairy cows and 45% from beef cattle / https://www.dsm.com/. In the rumen of cows, microbes help break down food, releasing hydrogen and carbon dioxide. An enzyme combines these gases to form methane. Bovaer® is a feed additive that inhibits the action of this enzyme so less methane is generated. While it is acting, Bovaer® breaks down safely into compounds already naturally present in the rumen.

Martin (1998) points out that the addition of malate and fumarate, as direct metabolic precursors to propionate, reduce methane production when given in high malate feeds. Methane emissions are reduced by directing hydrogen into succinate rather than methane.

Dong et al. (1997) compared the effect of adding canola oil and coconut oil and indicated that coconut oil was a more effective methane inhibitor. Kongmuna et al. (2011) reported that addition of coconut with garlic powder improved in vitro fermentation in terms of VFA profile and resulted in reduced methane emission. Inclusion of sunflower oil in cattle rations resulted in a 22% reduction in methane emissions (McGinn et al. 2004).

In vitro studies show that garlic oil reduces CH_4 emissions. The active diallyldisulfide and allylmercaptan are "responsible" for most of its effects (Ankri and Mirelman, 1999). According to the authors, its antimicrobial activity is due to organosulfur compounds, especially allicin. The antimethanogenic effect of garlic oil is due to the direct inhibition of Archaea microorganisms in the rumen. Archaea have unique membrane lipids that contain glycerol bound to long-chain isoprenoid alcohols, which are essential for cell membrane stability.

Dietary oils such as coconut oil, sunflower oil, mustard oil, horseradish oil help to reduce the production of methane in the rumen (Roonal Pritam Kataria,2015).

Ionophores are antibiotics produced by bacteria. Ionophores increase the proportion of gram positive bacteria in the rumen resulting in a change in fermentation from acetate and butyrate to propionate, and therefore reduces methane production (Schelling, 1984). Monensin is the most studied ionophore and is routinely used in beef production etc. and recently in dairy cattle feeding in many countries. There have been a number of experiments with monensin as a rumen modifier in various production systems. The effect of the ionophore on methane production appears to be intermittent, transient and short-lived, indicating that microbial adaptation is occurring.

In a combined in vivo and in vitro trial (Klop, 2016) in cows fed a mixture of essential oils or lauric acid (C12:0), there was a transient effect of essential oils on CH_4 production, which may indicate microbial adaptation, while the CH_4 reduction effect of lauric acid (C12:0), persisted.

Lipids from oilseeds, vegetable oils, and protected ruminal fats from vegetable oils are commonly used as energy sources for dairy cattle (Chilliard et al., 2001). Oilseeds can be one of the effective ways to reduce enteric CH_4 production to mitigate CH_4 emissions from ruminants. Vegetable oils can mitigate CH_4 by directly inhibiting rumen protozoa and methanogens and increasing biohydrogenation of PUFA to act as a sink for hydrogen produced by rumen microbes (Wang et al., 2017). Fat supplements are also known to reduce CH_4 production (Beauchemin et al., 2009; Chung et al., 2011; Moate at al., 2011).

Influence of feed type and processing

In vitro substrate degradability and methane production from pea and groundnut forages were evaluated in a study (Ansah et al., 2021). Samples from three replicate batches (n = 3) of three peanut cultivars (Samnut 22, Chinese and Samnut 23) and two pea cultivars (Padi Tuya and Songotra) were incubated in buffered rumen fluid. The crude protein (CP) concentration of Songotra and Padi Tuya varieties ranged from 112 to 154 g kg-1 dry matter (DM), respectively. Both neutral detergent fibre (NDF) and acid detergent fibre (ADF) values were found to be higher in Samnut 22, with other varieties having values below 400 g kg-1 DM. The highest ($P<0.05$) DM and organic matter (OM) degradability was observed in Padi Tuya cultivar. Methane gas production, expressed as ml g - 1 DM incubated and ml g - 1 DM degraded, was higher ($P<0.05$) in Padi Tuya, Songotra and Chinese peanut cultivars. Pearson correlation showed a significant positive relationship between CP and metabolic energy (ME) and a negative relationship between CP and methane. The relationship between NDF, ADF and methane production was found to be negative. It can be concluded from the study that pea varieties have better and efficient degradability as compared to groundnut varieties.

Grain barley varieties adapted to grow under different climatic conditions in Iran (16 varieties named Bahman, Makoeei, CB-79-10, Sahand, Reyhan03, Reyhan45, Fajer, Nosrat, Valfajer, Kavir, MB-82-12, AB-23-14 , Nimrooz, Jenob, Dasht and Sahra) were analyzed for chemical composition, organic matter (OM), crude protein (CP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF) and soluble sugars. In vitro gas production technique was used to investigate the effect of evaluated varieties on gas production parameters (Ghezeljeh et al., 2011). The 24-h gas production data were also used to estimate the digestibility of organic matter and metabolic energy. The concentrations of crude protein, soluble sugar, EE, ash, NDF and ADF in the barley samples studied averaged 108, 35, 30, 24, 238.4 and 72 g/kg, respectively; with a significant difference ($P<0.001$) among the varieties Dasht had the highest crude protein (CP) and lowest NDF; while Makooei and Sahra showed the highest NDF, respectively. The digestibility of organic matter of the evaluated samples ranged from 75 to 81% (mean = 78%) and there were significant differences ($P<0.01$) among the varieties.

The effects of ruminal contents from Bunaji cattle, WAD sheep and WAD goats on in vitro gas production and forage degradability (DM) of Moringa oleifera, Millettia griffoniana, Enterolobium cyclocarpum and Gmelina arborea were compared in an in vitro study using incubation periods ranging from 0 - 48 hours (Aderinboye et al., 2016). Oven-dried leaf samples were incubated in three replicates with each inoculum source and incubations were conducted in duplicate to make six replicate treatments to estimate gas production kinetics using a nonlinear equation. The residue samples were analyzed for crude protein (CP), lignin (ADL), acidic (ADF) and neutral (NDF) detergent fibers. Gas production in cattle, sheep, and goats were highly correlated ($r = 0.98$; $P < 0.001$). The kinetics of gas production differed ($P < 0.05$) in the different -inoculum from cattle, showing shorter ($P < 0.05$) lag times and higher ($P < 0.05$) fermentation rates. Gas production also varied ($P < 0.05$) among the species considered, with M. oleifera recorded the highest volume of production. M. oleifera and E. cyclocarpum was higher ($P<0.05$) in dry matter degradation than M. griffoniana and G. arborea, irrespective of inoculum source. The results showed that in vitro gas production and dry matter degradation of forages varied among species. Therefore, ruminal fluid from cattle, sheep and goats can serve as a source of inoculum for ruminant feed screening.

Many experiments, both in vitro and in vivo studies, have been conducted to investigate the potential of phytochemicals on ruminal fermentation to enhance feed digestibility and reduce methanogenesis (Patra et al., 2012; Jayanegara et al., 2014; Marrez et al., 2017). Many of these have shown promising results, but the applicability in terms of efficient animal husbandry is questionable. Therefore, efforts are still underway to find a suitable feed additive to mitigate rumen CH_4 production while improving livestock production by reducing greenhouse gas emissions on the environment. In order to accomplish this challenging task, a thorough understanding of rumen development, microbial colonization, rumen microbiome interaction with the host and ration is required.

Concentrate rations have been shown to lower rumen pH, increase VFA concentrations, and induce metabolic disturbances (DeVries et al., 2014). In addition, feeding high starch diets significantly increases the activity of lactic acid consuming and producing bacteria in the rumen, as these microbes are not susceptible to lower pH and therefore utilize greater substrate availability (Nagaraja et al., 2007). Conversely, feeding a significant amount of roughages may limit feed consumption, energy efficiency and microbial protein synthesis in ruminants (Yang et al., 2006). Therefore, increasing the amount of starch in the ration is considered a promising strategy to reduce methanogenesis per unit dry matter intake by shifting ruminal fermentation to propionogenesis (Doreau et al., 2011). Increased ration starch levels indicate a relatively lower proportion of cellulolytic bacteria R. albus and R. flavefaciens, due to their higher sensitivity to low pH. However, in an acidic ruminal environment F. succinogenes remain stable due to their gram-negative nature and different cell membranes than R. albus and R. flavefaciens (Granja-Salcedo et al., 2016).

Feeding silage from different sources leads to the development of different types of microbes in the rumen. Feeding alfalfa silage increases the relative abundance of F. succinogenes and R. flavefaciens while decreasing CH_4 production in the rumen of cows compared to sweet sorghum silage. However, Ruminococcus albus and Ruminobacter amylophilus populations showed no change (Chen et al., 2019). Conversely, sheep fed an alfalfa hay ration had higher levels of Fibrobacter succinogenes in the rumen compared to Ruminococcus (Michalet-Doreau et al., 2001). According to Guo et al. (2019), fermented corn mash showed a positive effect on rumen bacterial diversity, favoring four bacterial species: Bacteroidetes, Lentisphaerae, Firmicutes, and Fibrobacteres, which accounted for 77% of the total bacterial abundance. Furthermore, feeding fermented corn mash shifted rumen fermentation kinetics in cows by increasing the relative abundance of Prevotella and stabilizing the rumen microbial ecosystem. However, a 10-25% reduction in CH_4 production was observed when green matter and pods from trees and shrubs were included in the cattle ration (Ku-Vera et al., 2020).

The effects of barley sprouts on rumen fermentation were analyzed in lambs at 3 months of age. The animals were fed different diets:Eragrostis curvula hay as control (1), grass hay plus 25% barley sprouts (2) and grass hay plus 50% barley sprouts (3). Animals were fed the ration for 61 days, including 10 days of adaptation. Four animals were used to collect rumen fluid. Methane emissions were analyzed for nine consecutive days, from day 52 to 60, using a handheld laser detector. Rumen contents were collected on day 61 using an esophageal gastric probe for volatile fatty acids and DNA sequencing. Sprout supplementation had significant ($p < 0.05$) effects on methane emissions and ruminal fermentation. Significant effects on rumen fermentation were observed for ammonia-nitrogen (NH3-N), acetic acid and a trend ($p < 0.0536$) for an increase in propionic acid. Barley sprouts reduced methane gas emissions, ammonia-nitrogen and increased animal body weight. Bacteroidota and Firmicutes bacteria were predominant among the species identified. The analysis showed a clear difference in the microbiome between animals in different groups fed different diets. The addition of sprouts improves the efficiency of feed utilization by the animals and can be strategically used as a climate-smart feed resource for ruminants.

Hydroponic forage sprouts contain bioactive catalysts (enzymes) that can aid in forage digestion (Salo, 2019). In addition, the liquid fraction of the sprouts is a potential source of nutrients for ruminal microbes as the sprouts contain over 80% moisture content. Hydroponic forage sprouts for ruminants improve the digestibility of nutrients and increase the activity of ruminal enzymes (Farghaly et al., 2019). There is a hypothesis,that the addition of barley sprouts would affect enteric methane emissions, ruminal fermentation and microbiota.

Organic acids and other additives

Two experiments were conducted in dairy cows to evaluate the effect of different additives on enteric methane production, ruminal fermentation, feed digestibility and energy balance. In both experiments animals were fed identical rations containing: lauric acid, myristic acid, linseed oil and calcium fumarate. These additives were included at 0.4, 1.2, 1.5 and 0.7% of the dry matter of the ration, respectively. Animals in experiment 1 /n = 20/ and experiment 2 /n = 12/ were fed limited amounts of feed to determine the effect of dry matter intake on methane production (Zijderveld, et al., 2011). In experiment 1, methane production and energy balance were investigated using open-loop indirect calorimetry. In experiment 2, 10 animals with a fistula in the rumen were used to measure rumen fermentation characteristics. Inclusion of dietary supplements reduced methane emissions (g/d) by 10%. Urea content of milk was lower in experiment 1 and tended to be lower in experiment 2. The apparent total digestibility of fat in the digestive tract, but not that of starch or neutral detergent fiber, was higher. The reduction in methane production (g/d) was not significant when methane emissions were expressed per kilogram of milk produced. In Experiment 2, cows had reduced numbers of protozoa in ruminal supernatant compared to control cows.

Nitrates are naturally occurring compounds found in a variety of sources including soil, plants and water. Nitrate supplementation has been found to reduce methane emissions by up to 16% in dairy and 12% in beef cattle. The downside to this is that nitrate can be converted to nitrite, which can be toxic to ruminants if levels are too high or feeding high doses of nitrate is prolonged. Therefore, the amount of nitrate in the diet should be carefully monitored to prevent the risk of toxicity.

A recently discovered substance that is being investigated is 3-nitrooxypropanol (3-NOP) (Duval and Kindermann, 2012). It has been shown to be very effective in reducing enteric CH4 emissions in dairy cows by 30% (Hristov et al.,2015) with no negative effects on intake and lactation efficiency (Haisan et al., 2014, 2017; Hristov et al., 2015; Van Wesemael et al., 2019). The mode of action of 3-NOP has been shown to inhibit methanogenesis by targeting methyl-coenzyme M reductase in the final stage of CH4 formation from archaea in the rumen (Duin et al, 2016) and appears to inhibit only methanogens (Haisan et al., 2014; Lopes et al., 2016) without compromising animal health (Hristov et al., 2015) and thus be a promising option for its application as a feed additive (Duin et al., 2016)

In dairy cattle fed 3-NOP at 60 mg/kg feed DM, Lopes et al. (2016) reported an average 34% reduction in intestinal CH_4 and an undetectable to 1.33 g/d increase in H_2 emissions. Similarly, Hristov et al. (2015a) by feeding 3-NOP at 40, 60 and 80 mg/kg DM feed to dairy cows reported a 30% average reduction in intestinal CH_4 emissions.

Conclusion

Ruminal modifiers do not act directly on methanogens, but rather on the conditions that promote methanogenesis. These feed additives include lipids, plant secondary compounds, and essential oils (Honan et al., 2021).

The efficacy of lipids has been studied extensively, and although the addition of medium-chain and polyunsaturated fatty acids has been shown to significantly reduce enteric CH_4 production, the results have been variable. Similarly, secondary plant compounds and essential oils have shown inconsistent results, ranging from a significant reduction to a moderate increase in CH4 emission in the gut.

Due to the continued interest in this area, research is expected to accelerate in the development of feed additives that may provide options to reduce CH_4 emissions in the ruminant digestive tract.

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