Enteric Methane Mitigation

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Introduction

The world is experiencing unprecedented extreme weather events due to climate change, caused by accumulation of greenhouse gases (GHG). Methane is a GHG with global warming potential of 28 times that of carbon dioxide over a 100-year period and more effective at trapping heat during the time it is in the atmosphere. The largest source of methane is from agriculture and waste and particularly ruminants such as cattle (NASEM, 2018). Globally, livestock are responsible for about 14.5% of GHG emissions ranging from 4% in the US to 43% in New Zealand (Gerber et al., 2013). According to the Food and Agriculture Organization of the United Nations (FAO), demand for animal products is increasing, especially in countries with growing populations and income. Due to increases in ruminant population to satisfy demand, methane emissions from livestock rose more than 50% in the last 60 years and are expected to continue rising (FAO, 2017). In the US, agricultural emissions in general are about 10% of total GHG emissions with animal agriculture contributing to about 4% of total direct GHG emissions (EPA, 2021).

Enteric methane represents about 2 billion tons of CO_2 equivalent per year or more than 4% of annual global GHG emissions. Enteric methane production contributes to 70% of GHG emissions from livestock in the US (EPA, 2021); therefore, it is key to mitigating such emissions. About a quarter of livestock related methane emissions from enteric fermentation is from dairy cattle. The majority (about 72%) comes from beef cattle. However, the majority of manure related methane is from dairy cattle followed by swine. About a third of nitrous oxide emissions is from dairy cattle while beef contribute close to half (EPA, 2021).

A number of strategies have been developed to reduce enteric methane emissions. The mitigation strategies can be classified into (1) feed manipulation, (2) rumen modifiers, and (3) increasing animal production through genetics and management (Knapp et al., 2014). The objective is to review the status of mitigation options that have high potential to reduce enteric methane emissions from livestock. The review is not intended to be exhaustive but rather highlighting potential mitigation options that can be deployed within the next 5 to 10 years.

Feed Manipulation

There are several ways to manipulate feed and diet to reduce enteric methane emissions. In this section we cover feed manipulation through addition of lipids, nitrate and improvement of forage quality.

Lipids

Added dietary lipids could decrease methanogenesis in several ways including (1) lowering the quantity of organic matter fermented in the rumen; (2) hindering the activity of rumen methanogens; and (3) through biohydrogenation of lipids rich in unsaturated fatty acids. Supplementation of dairy cow diets with lipids has been one of the most extensively experimented enteric methane mitigation strategies. A systematic review by Eugène et al. (2008) concluded that lipid supplemented diets containing, on average, 6.4% ether extract (EE) reduced methane production in lactating dairy cows by 9% (~30 g/cow per day) compared with diets containing 2.5% EE. Furthermore, they observed that this reduction was mainly a consequence of decreased DMI, although milk production and milk composition were not affected. A meta-analysis by Grainger and Beauchemin (2011) showed a persistent reduction in enteric methane per unit of DMI for dietary lipid supplementations. In another meta-analysis, Patra (2014) examined the impact of the composition of added lipids on enteric methane production and reported that fats with high concentrations of C12:0, C18:3, and polyunsaturated fatty acids had marked inhibitory effect on methane production independent of DMI in cattle. Odongo et al. (2007) fed Canadian dairy cows with myristic acid (C14:0) at 5% of dietary DM and observed methane intensity reduction of about 29% without altering DMI, milk yield, or milk fat percentage. Jayasundara et al. (2016) calculated that a one unit increase in EE from 3.0% of DM was associated with a 12.5 g/cow per day reduction in methane production, implying that total methane reduction associated with increased dietary EE from 3.0% to 6.0% of DM would be, on average, 37.5 g per cow per day.

Nitrate

Nitrate (NO_3^-) is an inorganic anion and acts as an alternative hydrogen sink in the rumen. Supplementing a diet with nitrate is regarded as an effective and promising methane mitigation strategy by competing with methanogens for available hydrogen through its reduction of ammonia in the rumen. Studies have shown major reductions in methane emissions with nitrate supplementation, but with large variation in response. In a meta-analysis, Lee and Beauchemin (2014) demonstrated that nitrate is a viable candidate for feed additive that could be used to mitigate enteric methane emissions in ruminants. Similarly, van Gastelen et al. (2019) demonstrated that methane production was indeed consistently decreased when feeding nitrate to different types of ruminants. Feng et al. (2020) quantified the amount of methane emissions expected to be reduced by nitrate supplementation through a meta-analysis. The authors reported that nitrate supplementation reduced methane emissions (production in g/d) by 20.4% in dairy and 10.1% in beef cattle on average. Similarly methane yield (in g/kg of DMI) was reduced by 15.5% in dairy and 8.95% in beef cattle in a dose-dependent manner. The mitigating effect of nitrate on methane production and yield was greater in dairy than in beef cattle. However, effect of type of cattle appears to be related to slow-release nitrate use in beef cattle. A greater nitrate dose enhanced the nitrate mitigating effect on methane production and yield, whereas an increased DMI reduces the mitigating effect of nitrate on methane production.

Forage quality

Forages constitute the major proportion of dairy cow diets; however, few studies have investigated the effect of forage type on enteric methane emissions. Corn silage usually contains greater amounts of starch (e.g., 30% DM; Maizex, 2015) than silages from other forages (e.g., 9.4% DM in barley silage; Oba and Swift, 2013). Feeding more starch without compromising rumen health (i.e., acidosis) and/or production (e.g., milk fat depression) has been shown to be associated with less methane losses (Mills et al., 2003) and improved milk yields (Khorasani et al., 1994). Therefore, increasing the proportion of corn silage at the expense of cereal or legume silage is considered a promising enteric methane mitigation strategy, provided that the desired maturity stage of corn corresponding to high starch contents is achieved. It may be possible to decrease enteric methane yield by up to 15% with forage quality improvement; however, possible trade-offs from increased methane emissions from manure and implications related to increased production of whole corn silage in place of other silage crops need to be evaluated at the whole farm scale (Jayasundara et al., 2016).

Feed Additives

Recent advances in understanding of the rumen and methanogenesis has led to development of feed additives that have the potential to reduce enteric methane emissions substantially. Due to continued interest in this area, research is expected to accelerate in developing feed additives that can provide options in mitigating enteric methane emissions. In this section we will discuss mitigation options that directly affect methanogenesis in the reticulo-rumen. These include inhibitors that target methanogens or other microbes associated with methane emissions and vaccines. Innovations that have the potential to reduce enteric emissions including 3-nitrooxypropanol (3NOP) macroalgae and plant secondary compounds are discussed below.

3-Nitrooxypropanol (3NOP)

The compound, 3NOP, is a highly specific inhibitor of methanogenesis in the reticulo-rumen. Several studies using 3NOP as an additive have reported reduction in methane emissions from beef and dairy cattle up to 60%. Dijkstra et al. (2018) conducted a meta-analysis of the effectiveness of 3NOP and reported an average of 32.5% reduction; however, there was differences in type of animals. In dairy cattle, at an average 3NOP supplementation of 81 mg/kg DM, there was a 39% reduction in enteric methane while in beef cattle the emission was reduced by 22.2% with average supplementation of 144 mg 3NOP/kg DM. The authors attributed the greater efficacy of 3NOP in decreasing methane emissions in dairy cattle compared with beef cattle to the higher feed intake level in dairy cattle. Higher feed intake levels increase rumen concentrations of fermentation products, including VFA and hydrogen. Larger feed intake levels in dairy cattle than in beef cattle may be associated with relatively (i.e., per unit of feed fermented) greater alternative hydrogen sinks for rumen methanogenesis, resulting in relatively lesser concentrations of enzyme catalyzing methane formation and elevated inhibitory potential of 3NOP. 3NOP is a compound consisting of a molecule of propylene glycol and nitrate

and resembles a key molecule in methane formation - methyl-coenzyme M reductase (MCR). 3NOP specifically targets MCR, which is a nickel enzyme and only active when its Ni ion is in the +1 oxidation state (Duin et al., 2016). MCR catalyzes the methane-forming step in the rumen fermentation. 3NOP preferably binds into the active site of MCR and effectively inactivates it. 3NOP is demonstrated to inhibit growth of methanogenic archaea at concentrations that do not affect the growth of nonmethanogenic bacteria in the rumen (Duin et al., 2016). According to Hristov et al. (2015), supplementation of 3NOP did not significantly affect feed intake or milk production.

Macroalgae

Some seaweed species, particularly *Asparagopsis*, contain bromoform and bromochlormethane as active ingredients that has been shown to be effective in vitro (Machado et al., 2016). Bromochloromethane in its pure form cannot be used as it is a banned substance under the Montreal Protocol. The first in vivo trial using *Asparagopsis armata* in cattle (Roque et al., 2019a) reported up to 67% reduction in methane production in dairy cattle with inclusion of 1% of organic matter (OM). The authors reported a decline in feed intake, particularly at the high level of inclusion, which might have compromised milk production. Kinley et al. (2020) reported that methane emissions in Brangus cattle declined 98% with inclusion of only 0.02% (OM basis) of *Asparagopsis taxiformis*. Additionally, they reported no reduction in feed intake or loss of productivity. Roque et al. (2021) conducted a longer-term study to investigate the potential for adaptation by microbes and interaction with feed quality. The authors reported that there was no evidence of microbial adaptation when steers were fed for up to 5 months. The efficacy was dependent on fiber concentration and ranged from about 50% in high NDF diets to 80% under feedlot conditions. Analysis of meat quality in supplemented groups showed that there was no interactive effect between treatments and time on the shelf life of steaks (Bolkenov et al., 2021). The efficacy of methane reduction appears to correlate with the concentration of bromoform compounds, which appear to be the main active ingredients although other yet to be identified substances may contribute to methane reduction as well (Vijin et al., 2020). Analysis of the meat from seaweed supplemented animals did not show any bromoform residue. The main barriers for adoption of macroalgae as a mitigation option are 1. Regulatory approval and 2. Scaling up production enough to feed cattle around the world. Further research and removal of barriers is required before widespread adoption.

Plant Secondary Compounds

Tannins

Tannins are soluble, phenolic compounds that accumulate within plant tissues likely due to ongoing metabolic processes and contribute to the plant defense system (Swanson, 2003). The methane mitigation mechanisms of tannins are not well understood but may be due to a combination of various factors. Several mechanisms have been proposed for the anti-methanogenic activity of tannins including direct inhibition of methanogens and the protozoa population associated with methanogens; decreasing

hydrogen production through inhibition of fibrolytic bacteria and fiber digestibility, and acting as an alternative hydrogen sink to methanogenesis (Aboagye and Beauchemin, 2019). Jayanegara et al. (2012) conducted a meta-analysis describing the relationship between rumen methane formation and the level of dietary tannin (hydrolysed or condensed) inclusion between in vivo and in vitro models. These authors reported that low levels of inclusions of tannins in animal experiments often yielded inconsistent results on methane production, but that variability seemed to diminish at higher doses, leading to setting the threshold for detecting treatment differences in animals to be >20 g/kg DM of tanniferous inhibitors. Furthermore, reduction in methane production was often followed by a suppression in OM and fiber digestibility. Care should be taken when supplementing tannins as several studies have shown that tannins bind and interact with dietary proteins in the gastrointestinal tract, which reduces nitrogen availability to the animal (Waghorn, 2008).

Essential oils and blends

Essential oils are naturally occurring chemical ompounds extracted from plants and used in fragrances and cosmetics and, to a lesser extent, pharmaceutical products for humans and animals (Honan et al., 2021). Volatile in nature, they contribute to the phenotypic expression of the plant including color and scent (Benchaar et al., 2008). Consumption of essential oils affects rumen microbial communities and fermentation patterns in a varying manner, depending on the source (Benchaar and Greathead, 2011). Many essential oils hold a high affinity for lipid and bacterial membranes, leading to disruption, but the broad antimicrobial effect is likely to be due to a combination of mechanisms (Helander et al., 1998). Numerous plants such as cinnamon, lemongrass, ginger, garlic, juniper berries, eucalyptus, thyme, citrus, oregano, mint, rosemary and coriander have been screened in vitro (Becnhaar et al., 2008; Nanon et al., 2015). However, only few have been studied in vivo.

Some studies have used an essential oil 'blend' or 'complex' containing extracts from multiple plants. For example, Mootral is synthesized from natural products including garlic- and flavonoid-containing citrus extract and has demonstrated anti-methanogenic properties (Eger et al. 2018; Vrancken et al., 2019). The garlic component in Mootral targets methanogenic archaea populations and protozoal communities in the rumen and has led to nearly complete inhibition of methane production in vitro at a dosage of 2 g experimental mixture/day, without compromising bacterial population (Eger et al., 2018). A 23.2% decrease in methane yield (26.8% expressed in methane production) was observed in Angus x Hereford crosses after 12 weeks of treatment by supplementing Mootral at 1.58 g/kg DM (Roque et al., 2019b). Adverse effects on DMI, ADG and feed efficiency were not detected over the 12-week trial. Lactating cattle offered Mootral incorporated in pellets at a rate of 0.64 g/kg DM for Holstein-Friesian and 1.21 g/kg DM for Jersey herd experienced suppression of methane of 20.7% and 38.3%, respectively (Vrancken et al., 2019). Additionally, 3–5% increase in milk yield across breeds was observed with increased feed efficiency in the Jersey cattle.

Improved Efficiency

Animal efficiency has been a goal for decades for both beef and dairy cattle to produce more with less input. Increasing animal production efficiency is accompanied by decreases in methane emission intensity (methane emitted per unit of milk produced, often measured as CH_4 g/kg of milk) (Hristov et al., 2013). Factors affecting methane intensity include forage-to-concentrate ratio, forage quality, forage type, grazing management, and breeding strategies.

Forage-To-Concentrate Ratio

Decreasing the forage-to-concentrate ratio has been shown to reduce methane emissions and intensity (Tyrell and Moe, 1972). Aguerre et al. (2011) conducted an *in vivo* study to investigate the effects of different forage-to-concentrate ratios (47:53, 54:46, 61:39, 68:32) on emissions and production responses in dairy cattle. The lowest forage-to-concentrate ratio resulted in a 17% decrease in methane emissions (g/d) compared to the highest ratio. The authors also found that methane emissions per kilogram DMI, milk, and 3.5% ECM had a positive, linear relationship with forage level in the diet. Starch fermentation shifts VFA production away from butyrate and acetate and towards propionate (Ungerfeld, 2020). Butyrate and acetate are associated with hydrogen production, leading to more hydrogen available for methanogenesis. Conversely, propionate uses hydrogen and is a competitive sink against methanogenesis. (Benchaar et al., 2001; Ungerfeld, 2020). In addition, starch decreases the pH of the rumen environment, inhibiting methanogens and decreasing hydrogen availability, thus decreasing the digestibility of fiber in the diet and the production of methane (Van Kessel and Russell, 1996). Although concentrates reduce methanogenesis, excessive starch in the diet leads to subacute ruminal acidosis, laminitis, and decreased milk fat, compromising animal health and productivity.

Forage Quality

Improving forage quality is a potential strategy in reducing methane emissions and intensity (Hristov et al., 2013). When DMI increases with highly digestible feeds, methane produced per unit of feed consumed decreases (Hristov et al., 2013). Forage quality is determined by maturity, climate, and plant species (Eugène et al., 2021). As a plant matures, its fiber and lignin content increase, resulting in lower digestibility and higher enteric methane when fed to cattle (Jung and Allen, 1995). Lower fiber content, and thus higher digestible feeds, leads to faster fermentation and increased propionate production (van Gastelen et al., 2019; Ungerfeld, 2020). The impact of forage quality on methane emission and intensity varies between studies and animals (Hristov et al., 2013). van Gastelen et al. (2019) used 19 studies to assess the effects of increased digestibility (expressed as OM digestibility) of grass silage on dairy and beef cattle. In dairy cattle, the authors found that an average of 25% OM digestibility improvement resulted in an average decrease of 10% for methane yield (g/kg DMI) and 19% for methane intensity (g/kg milk). However, beef cattle saw no effects of improved digestibility on yield or intensity. The authors attribute this difference in animals to differences in feed intake

relative to body weight, as well as the composition of the diets. Most of the dairy studies incorporated concentrates in the diet, which contributed to the reduction in methane emissions.

Forage Type

Methane emissions also have the potential to be reduced when corn silage replaces grass silage (Hristov et al., 2013). van Gastelen et al. (2019) compared the effects of using corn silage in the place of grass silage in beef or dairy cattle across 23 studies, finding an average of 8% decrease in methane intensity from replacing grass silage with corn silage. The authors found a smaller effect of this dietary strategy in beef cattle compared to dairy, attributing this difference to the different responses in DMI and apparent total-tract digestibility. Dairy cattle fed corn silage increased their DMI, leading to increased fermentation and more propionate production (van Gastelen et al., 2019). Although replacing grass silage with corn silage in the diet shows promise in reducing enteric emissions, manure methane could increase from corn silage, and carbon dioxide emissions from the soil are greater for corn compared to grass silage (Eugène et al., 2021). Therefore, more research is needed on the whole-system effects of corn silage on the environment.

Legume forages have also been shown to reduce methane emissions (Hristov et al., 2013). When considering dietary energy, the replacement of timothy hay with alfalfa showed a 21% reduction in methane emissions within a modelling approach (Benchaar et al., 2001). The lower fiber content of legumes and faster passage rate allows for decreased methane production. Some legumes also contain tannins (van Gastelen et al., 2019; Eugène et al., 2021), which were discussed previously as a plant secondary compound capable of reducing enteric methane emissions. Legume silages also have the added benefit of lower nitrogen input from fertilizer and more nitrogen provided to the animal, increasing animal productivity while decreasing ammonia emissions (Eugène et al., 2021). However, excessive intake of legumes with high concentrations of protein (such as red or white clover) increases the risk of bloat.

Grazing Management

Grazing contributes to 45 and 57% of total beef and milk production throughout the world (Zubieta et al., 2021), so its management is an important consideration in methane mitigation. Rotational grazing allows for more efficient use of forage, therefore, it is a proposed strategy for reducing methane intensity in dairy and beef cattle (Hristov et al., 2013). DeRamus et al. (2003) found that annual methane emissions under rotational grazing decrease by 22% compared to continuous grazing. However, Zubieta et al. (2021) emphasized that the impact of rotational or continuous grazing on methane intensity is based on stocking rate, herbage allowance, and herbage mass. Although limited studies assess the impact of rotational grazing on methane intensity, its additional environmental benefits include a preservation of biodiversity and reduction of soil erosion (Thomspon and Rowntree, 2020). Other grazing strategies include concepts discussed previously: graze at ideal maturity/quality of forages, include leguminous forages (Archimede et al.,

2011), supplement with concentrates (Zubieta et al., 2021), and incorporate plant secondary compounds (Thompson and Rowntree, 2020).

Breeding Strategies

In addition to management and nutritional approaches, breeding for more efficient and low methane emitting animals has the potential to reduce methane emissions and intensity. Genetic traits that contribute to more efficient dairy cattle, and thus lower methane emission intensity, include milk protein, milk fat, survival, and calving interval. In addition, methane production is considered a heritable trait, with a heritability ranging from 0.21 to 0.35 (De Haas et al., 2011; Lassen and Løvendahl, 2016). By actively selecting for lower methane emitting cows, methane production and intensity can be reduced. De Haas et al. (2021) quantified the impact of adding methane emissions in the Dutch breeding strategy, finding that selective breeding has the potential to reduce methane intensity between 13 and 25% by 2050. Limitations to breeding for low methane emitters include the lack of knowledge on the full biological implications and impractical methane measurement techniques on the animal level, hindering direct selection and collection of data (De Haas et al., 2011). Once these limitations are overcome, breeding for low emitting animals could provide cost-effective, cumulative, and permanent effects on methane reduction (De Haas et al., 2011; 2021).

Summary

Enteric methane production contributes to most of the GHG emissions from livestock; therefore, it is key to mitigating such emissions. A number of strategies have been developed to reduce enteric methane emissions. These vary from those that directly target methanogenesis to indirectly reducing emissions by improving feed efficiency. Recent advances in understanding of the rumen and methanogenesis has led to development of feed additives that have the potential to reduce enteric methane emissions substantially. Overall, more research is needed on feed additives to understand methane reduction, rumen adaptation, and cattle health implications in the long-term. Due to continued interest in this area, research is expected to accelerate in developing feed additives that can provide options in mitigating enteric methane emissions. Increased animal production efficiency or improved reproduction would also indirectly reduce methane emissions as it reduces methane intensity (methane produced per unit of product). Improved efficiency could be achieved through better forages (such as high sugar/starch or low fiber) or better management particularly in grazing systems. Breeding for low methane emissions has also shown a promise in selecting breeds for reduced enteric methane emissions.

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