

Review: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation

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Meat and milk from ruminants provide an important source of protein and other nutrients for human consumption. Although ruminants have a unique advantage of being able to consume forages and graze lands not suitable for arable cropping, 2% to 12% of the gross energy consumed is converted to enteric CH₄ during ruminal digestion, which contributes approximately 6% of global anthropogenic greenhouse gas emissions. Thus, ruminant producers need to find cost-effective ways to reduce emissions while meeting consumer demand for food. This paper provides a critical review of the substantial amount of ruminant CH₄-related research published in past decades, highlighting hydrogen flow in the rumen, the microbiome associated with methanogenesis, current and future prospects for CH₄ mitigation and insights into future challenges for science, governments, farmers and associated industries. Methane emission intensity, measured as emissions per unit of meat and milk, has continuously declined over the past decades due to improvements in production efficiency and animal performance, and this trend is expected to continue. However, continued decline in emission intensity will likely be insufficient to offset the rising emissions from increasing demand for animal protein. Thus, decreases in both emission intensity (g CH₄/animal product) and absolute emissions (g CH₄/day) are needed if the ruminant industries continue to grow. Providing producers with cost-effective options for decreasing CH₄ emissions is therefore imperative, yet few cost-effective approaches are currently available. Future abatement may be achieved through animal genetics, vaccine development, early life programming, diet formulation, use of alternative hydrogen sinks, chemical inhibitors and fermentation modifiers. Individually, these strategies are expected to have moderate effects (<20% decrease), with the exception of the experimental inhibitor 3-nitrooxypropanol for which decreases in CH₄ have consistently been greater (20% to 40% decrease). Therefore, it will be necessary to combine strategies to attain the sizable reduction in CH₄ needed, but further research is required to determine whether combining anti-methanogenic strategies will have consistent additive effects. It is also not clear whether a decrease in CH₄ production leads to consistent improved animal performance, information that will be necessary for adoption by producers. Major constraints for decreasing global enteric CH₄ emissions from ruminants are continued expansion of the industry, the cost of mitigation, the difficulty of applying mitigation strategies to grazing ruminants, the inconsistent effects on animal performance and the paucity of information on animal health, reproduction, product quality, cost-benefit, safety and consumer acceptance.

Keywords: methane, mitigation, greenhouse gas emissions, rumen fermentation, ruminants

Implications

Enteric CH₄ from ruminants contributes approximately 6% of global anthropogenic greenhouse gas emissions. Thus, producers need cost-effective ways to lower emissions while meeting consumer demand for high-quality, safe and affordable food produced from healthy animals. Methane

emissions per unit of meat and milk have continuously declined over the past decades due to improvements in production efficiency and animal performance. However, decreases in both intensity and absolute emissions of CH₄ are needed to curb rising atmospheric greenhouse gas concentrations. The paper reviews future prospects for enteric CH₄ mitigation and provides a critical analysis of the knowledge gaps and future challenges for science, governments, producers and livestock industries.

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Introduction

Global demand for meat and milk is expected to increase by 73% and 58%, respectively, by 2050 compared with 2010 levels, due to continuous expansion of the world population, an emerging middle class, growing incomes and urbanization (Gerber *et al.*, 2013). Expansion of animal agriculture is a concern because it contributes to rising atmospheric concentrations of greenhouse gases (GHGs) and consequent climate change. Total global GHG emissions from livestock (animals, manure, feed production and expansion of lands into forested areas) are estimated to account for 14.5% of total anthropogenic emissions (Gerber *et al.*, 2013). Enteric CH₄ from ruminants contributes approximately 6% of global anthropogenic GHG emissions (40% of all livestock emissions; Gerber *et al.*, 2013). Methane has a much shorter lifetime (half-life; 8.6 years; Muller and Muller, 2017) than CO₂ in the atmosphere, which makes it an attractive amelioration target for short-term gains in global warming abatement.

Rising environmental awareness and vegetarianism, coupled with the emergence of synthetic milk and meat and limited resources, are challenging the ruminant livestock industries' social licence to operate. Thus, it is imperative that ruminant producers develop cost-effective ways to continue decreasing CH₄ emissions while meeting consumer demand for food. The GHG emissions per unit of meat and milk (also called carbon footprint and emission intensity) have declined over the past 50 years due to improvements in production efficiency and animal performance, and this trend is expected to continue, especially in countries with developing economies. However, the decline in emission intensity due to production efficiency gains is modest (<1%/year) and may be insufficient to offset the rising emissions from increasing demand for animal protein. Thus, a global concerted effort to reduce ruminant emissions is warranted.

Concern that enteric CH₄ from ruminants is contributing to anthropogenic GHG emissions has promoted a recent research focus on understanding the factors affecting methanogenesis in the rumen, as well as exploration of a broad range of potential mitigation strategies. Consequently, a plethora of CH₄-related papers has been published ranging from genome sequencing of rumen methanogens (Henderson *et al.*, 2015 and 2018; Seshadri *et al.*, 2018) to practices that can be adopted to mitigate emissions (Beauchemin *et al.*, 2009; Hristov *et al.*, 2013; Eckard and Clark, 2018). Thus, our intent is not to cite the vast numbers of individual papers published, but rather, present a critical analysis of the current knowledge gaps and prospects for CH₄ mitigation in view of the need for food security. We focus the discussion on knowledge learned, unresolved issues and future challenges for science, governments, farmers and ruminant livestock industries.

Historical perspective

A search of the literature related to rumen methanogenesis published between 1960 and 2018 (Scopus keywords:

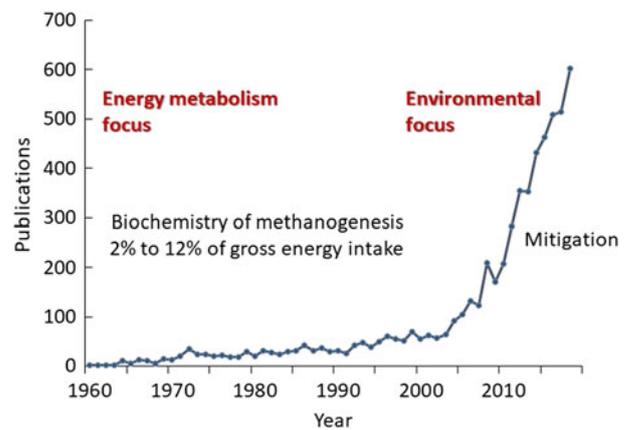


Figure 1 (colour online) Number of published papers related to enteric methanogenesis (Scopus search keywords: methane OR methanogenesis AND cow OR cattle OR sheep OR lamb OR rumen (total = 5845). A shift in research focus from energy metabolism to environment occurred in the early 2000s, indicating significant recent investment in CH₄ mitigation research.

methane OR methanogenesis AND cow OR cattle OR sheep OR lamb OR rumen) revealed almost 9000 papers (Figure 1). *In vitro* (e.g. Bauchop, 1967) and *in vivo* (e.g. Clapperton, 1974) research on inhibiting rumen methanogenesis started with the aim of improving energy utilization efficiency of rumen fermentation, with the ultimate goal of improving animal productivity. Some of the studies prior to the year 2000 also established many of the fundamentals of rumen methanogenesis, including rumen microbial ecology, carbohydrate fermentation and associated biochemical pathways, and modifiers of ruminal microbial activity and effects on methanogenesis (Hobson and Stewart, 1997). Early animal studies focused on energetics and reported CH₄ losses account for 2% to 12% of the gross energy consumed by ruminants (Johnson and Johnson, 1995). Many of the factors affecting energy losses as CH₄ were revealed including level of intake, carbohydrate type and lipid supplementation. Early animal studies were conducted in respiratory calorimetry chambers (whole animal chambers, head boxes and face masks), with the development of the sulphur hexafluoride tracer gas technique in the early 1990s being a significant innovation providing researchers with a low-cost means of measuring CH₄ production of individual animals without the need for restraint or enclosure (Johnson and Johnson, 1995). In the early 2000s, the number of CH₄-related publications increased rapidly, reflecting significant investment and shift in research focus towards mitigation in view of the increasing awareness of the environmental impact of CH₄ from ruminants (Figure 1).

Rumen fermentation and microbiome associated with methanogenesis

Carbohydrates are the main dietary source of energy for ruminants. In the rumen, polysaccharides (mainly cellulose, hemicellulose and starch) are hydrolysed to glucose and other

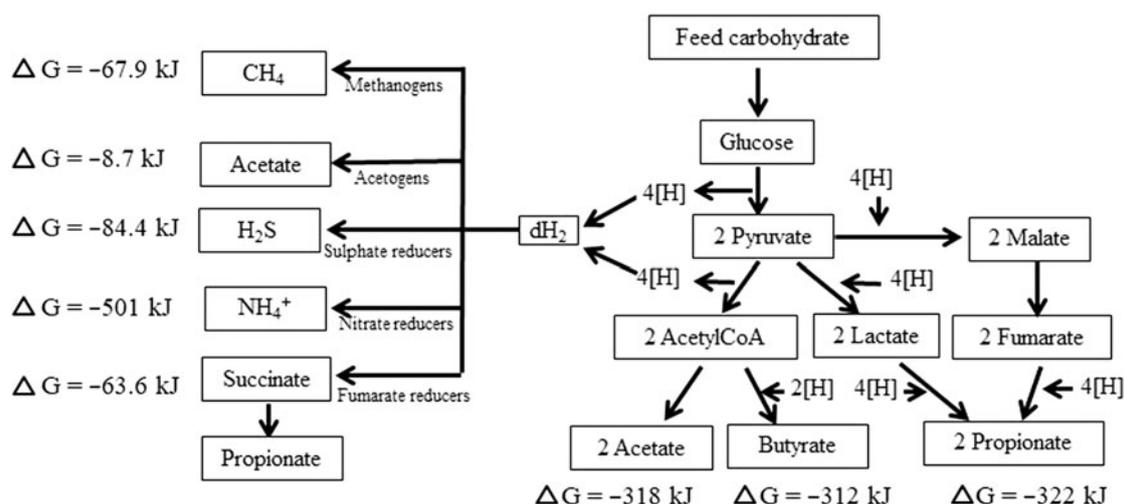


Figure 2 Scheme of the major pathways of rumen fermentation including generation and incorporation of metabolic hydrogen ($[H]$) and dihydrogen (H_2). Estimated Gibbs energy changes are based on Kohn and Boston (2000) and Ungerfeld and Kohn (2006) without considering ATP generation. Generation and incorporation of $[H]$ are estimated based on 1 mol of glucose fermentation according to the following reactions: $C_6H_{12}O_6$ (glucose) \rightarrow $2 C_3H_4O_3$ (pyruvate) + $2 [2H]$; $2 C_3H_4O_3 + 2 HSCoA$ (non-esterified coenzyme A) \rightarrow $2 C_2H_3OSCoA$ (acetyl coenzyme A) + $2 CO_2 + 4 [2H]$; $C_2H_3OSCoA + H_2O$ (water) \rightarrow $C_2H_4O_2$ (acetate) + $HSCoA$; $2 C_2H_3OSCoA + 2 [2H] \rightarrow C_4H_8O_2$ (butyrate) + $2 HSCoA$; $2 C_3H_4O_3 + 2 [2H] \rightarrow 2 C_3H_6O_3$ (lactate); $2 C_3H_6O_3 + 2 [2H] \rightarrow 2 C_3H_6O_2$ (propionate) + $2 H_2O$; $2 C_3H_4O_3 + 2 [2H] + 2 CO_2$ (carbon dioxide) \rightarrow $2 C_4H_6O_5$ (malate); $2 C_4H_6O_4$ (fumarate) + $2 [2H] \rightarrow 2 C_3H_6O_2 + 2 CO_2$.

hexoses and pentoses (Figure 2). Monosaccharides are further metabolized to volatile fatty acids (VFAs) and CO_2 . Metabolic hydrogen ($[H]$) is released in the metabolism of monosaccharides to VFA, reducing intracellular co-factors, and for fermentation to continue, co-factors must be re-oxidized. This happens to a large extent through hydrogenase activity and formation of dihydrogen (H_2 , i.e. molecular hydrogen). Ruminant H_2 exists in two forms, dissolved H_2 (dH_2) and gaseous H_2 (gH_2), with only dH_2 being available for microorganisms (Wang *et al.*, 2014). Dihydrogen does not accumulate in the rumen because it is transferred from the fermentative consortium of bacteria, protozoa and fungi to methanogenic archaea that use it to reduce CO_2 and other one-carbon compounds via the hydrogenotrophic pathway to CH_4 . Most methanogens can also utilize formate generated in acetyl-CoA formation from pyruvate as a $[H]$ donor for ruminal methanogenesis (Schauer and Ferry, 1980), with the unused formate being rapidly converted to H_2 and CO_2 . To a much lesser extent, CH_4 can also be produced in the rumen through the utilization of methyl groups (methylotrophic pathway) and less commonly from acetate (Huws *et al.*, 2018).

Thus, CH_4 represents the largest sink of $[H]$ in the rumen. Importantly, while most $[H]$ produced by the fermentative microbiota is transferred to methanogens as dH_2 and used in methanogenesis (Janssen, 2010), there are other important pathways of $[H]$ utilization such as propionate production, which can incorporate $[H]$ in reduced co-factors generated in intracellular reactions as well as dH_2 produced by other cells (Henderson, 1980; Figure 2). A strong positive relationship between the concentrations of dH_2 and propionate indicates that increased ruminal dH_2 can facilitate reactions incorporating $[H]$ into propionate production (Wang *et al.*, 2016).

When methanogenesis is inhibited *in vitro*, gH_2 can accumulate up to 100 times the control treatments with functional methanogenesis (Chalupa *et al.*, 1980). *In vivo* gH_2 emissions can increase by 60- (Hristov *et al.*, 2015) or even 600-fold (Vyas *et al.*, 2018) when CH_4 production is inhibited. Although the release of gH_2 represents an inefficiency of energy utilization, the loss of energy as H_2 represents on average only 2.7% of the energy potentially available from the decrease in CH_4 production (Ungerfeld, 2018).

Propionate, an alternative $[H]$ sink to CH_4 (Janssen, 2010), is the main glucose precursor for ruminants, and therefore desirable to enhance in animals with high demands for glucogenic precursors. Reductive acetogenesis, the formation of acetate from CO_2 and H_2 (Figure 2), is also a desirable $[H]$ incorporating process, as acetate is an energy source and building block in long chain fatty acid synthesis. However, reductive acetogenesis is thermodynamically outcompeted by methanogenesis in the normal rumen (Ungerfeld and Kohn, 2006) but would be a beneficial $[H]$ sink to enhance in a methanogenesis-inhibited rumen fermentation (Ungerfeld, 2013). Some inorganic electron acceptors like nitrate and sulphate (Figure 2) can thermodynamically outcompete methanogenesis in the rumen (Ungerfeld and Kohn, 2006), although the availability of these $[H]$ acceptors is low with most diets unless these electron-accepting compounds are supplemented.

In theory, redirecting $[H]$ away from methanogenesis to fermentation end-products that can be absorbed and utilized by the host animal, as well as to microbial biomass synthesis, helps to not only decrease CH_4 emissions but may potentially also benefit productivity of the host animal (see the 'Methane, animal productivity and incentives to lower emissions' section). So far, however, this potential has not been consistently realized (Ungerfeld, 2018).

The rumen microbiome associated with methane emissions

There have been tremendous advances made in characterizing archaea responsible for methanogenesis in the rumen, including the variability among animals and how the archaea are affected by diet and mitigation strategies (Henderson *et al.*, 2015; Tapio *et al.*, 2017; Huws *et al.*, 2018; Seshadri *et al.*, 2018). Henderson *et al.* (2015) conducted a comprehensive global census of the microbial community composition of rumen and foregut samples (from 379 cattle, 106 sheep, 59 deer, 52 goat and 72 others) from 35 countries. Although the samples were from a wide range of animals, locations, diets and conditions, the dominant archaeal groups were surprisingly similar. *Methanobrevibacter gottschalkii* and *Mbb. ruminantium* were found in almost all samples, accounting for, on average, 74% of all archaea. Together with a *Methanosphaera* sp. and two *Methanomassiliicoccaceae*-affiliated groups, the five dominant methanogen groups comprised almost 90% of the archaeal communities. About 78% of archaea were hydrogenotrophic, while 22% were methylotrophic using methyl groups from methanol or methylamines, whereas methanogens that used acetate were rare (Seshadri *et al.*, 2018). It appears that rumen archaea are much less diverse than rumen bacteria, which probably reflects the narrow range of substrates they use. This limited diversity provides an opportunity to develop CH₄ mitigation strategies that target these few dominant methanogens. Recent whole genome sequencing of rumen methanogens has provided insight into their metabolic processes (Seshadri *et al.*, 2018), which could lead to the development of microbiome-based mitigation approaches, such as small molecule inhibitors that target enzymes, vaccines and other approaches that affect the rumen archaea (Leahy *et al.*, 2013).

High-throughput sequencing has been used to study relationships between CH₄ production and the microbial community composition quantified in terms of 16S rRNA or 18S rRNA gene abundance, as well as functional gene abundance and expression. The reader is directed to several excellent reviews (e.g. Leahy *et al.*, 2013; Tapio *et al.*, 2017; Wallace *et al.*, 2017; Huws *et al.*, 2018). Most (Shi *et al.*, 2014; Danielsson *et al.*, 2017), but not all (Wallace *et al.*, 2015), studies report weak or no relationships between CH₄ production of individual animals and total abundance of archaea. Rather, it appears that the composition of the archaeal community and differential gene expression of methanogenesis pathways are more highly associated with CH₄ production (Shi *et al.*, 2014; Wallace *et al.*, 2015; Danielsson *et al.*, 2017). Studies have also shown an association between CH₄ production and the abundance of 16S rRNA genes of specific bacterial phyla and genera (Wallace *et al.*, 2015; Danielsson *et al.*, 2017). As well, a positive association was reported between CH₄ production and protozoa (Tapio *et al.*, 2017), but surprisingly no association was found between archaea and protozoa in the global rumen census (Henderson *et al.*, 2015).

A positive association between CH₄ production and the abundance of functional genes encoding for methanogenesis enzymes has been reported in some studies (Shabat *et al.*, 2016). Transcription of the entire methanogenesis pathway was augmented in high-emitting sheep (Shi *et al.*, 2014). It is tempting to speculate that a positive relationship between CH₄ production and the abundance of genes encoding for methanogenesis enzymes or their transcripts might indicate that the rate of CH₄ formation is kinetically controlled by the activity of one or more methanogenic enzymes. Yet, cause–effect relationships were not demonstrated in the studies discussed, thus the inverse might occur and methanogens may grow and regulate the expression of genes encoding for methanogenesis enzymes depending on other limitations to producing CH₄ (Browne and Cadillo-Quiroz, 2013). Methanogenesis might be enzyme limited after feeding, when the elevation of dH₂ concentration precedes the increase in CH₄ and methanogen 16S rRNA gene copies or methanogenesis mRNA transcripts (van Lingen *et al.*, 2017).

Increasing our understanding of the complexity of the rumen microbiome in relation to host and external factors (e.g. diet, mitigation) is key to decreasing CH₄ production from ruminants in the future, in addition to maximizing the efficient use of feed resources. Advances in enzyme and gene-based approaches may facilitate the future development of CH₄ mitigation compounds that specifically target methanogens and their enzymes (Henderson *et al.*, 2018), including ongoing efforts to sequence complete genomes of methanogens (e.g. Leahy *et al.*, 2013; Li *et al.*, 2016). Metagenomic and metatranscriptomic research to reveal the complexities and functionality of the rumen microbiome should continue to be an area of high-priority research to improve the environmental sustainability of ruminant production.

Strategies for mitigating methane emissions

While numerous strategies have been proposed for CH₄ mitigation (Hristov *et al.*, 2013; Knapp *et al.*, 2014), many are difficult to implement on-farm (e.g. protozoa defaunation), have low mitigation potential (e.g. yeast, bacterial direct-fed microbials, saponin, ionophores) or are at a very early stage of development (e.g. bacteriocins, phages). Accordingly, the following discussion focuses on mitigation strategies with potential for on-farm adoption in the short or medium term (Table 1).

Increasing animal productivity

Improved animal performance through superior animal management, health, nutrition and genetics lowers CH₄ emission intensity (g CH₄/kg product) because fewer animals and consequently less total feed are used to produce a given amount of product (Capper *et al.*, 2009). However, absolute emissions (g/animal per day) may increase as animals consume additional feed to meet their energy requirements. There are many industry-wide examples that illustrate how

Table 1 Assessment of select strategies for enteric methane mitigation in the short or medium term based on the information provided in the text

Strategy	CH ₄ decrease potential			Feasibility of implementing on-farms	Limitations	Key references
	Amount (g/day)	Intensity (g/kg product)	Expected availability			
Management and breeding						
Increased animal productivity (through nutrition, genetics, health and management)	Uncertain (can increase)	Low	Immediate	Potential greatest for production systems that are not already optimized	Adoption limited by knowledge transfer, economics, perception, limitation of resources and others	Capper <i>et al.</i> (2009); Hristov <i>et al.</i> (2013); Legesse <i>et al.</i> (2016)
Animal breeding for low-CH ₄ production	Low	Low	Unknown, possibly within 10 years	Can be incorporated into multiple trait selection index	Need robust ways of measuring CH ₄ of large numbers of individual animals. Relationships between CH ₄ production and economically important traits are unknown. Need to know long-term persistency on different diets and effects on animal health	Pickering <i>et al.</i> (2015); Løvendahl <i>et al.</i> (2018)
Animal breeding for feed efficiency and residual feed intake	Low	Low	Immediate	Can be incorporated into multiple trait selection index	Existence of genotype × environment interactions needs to be determined. Relationship to productivity-related traits at pasture unknown. Lack of information on the biological regulation of the trait	Basarab <i>et al.</i> (2013); Kenny <i>et al.</i> (2018)
Nutrition						
Lipids	Medium	Medium	Immediate	Feasible for ruminants fed diets, but difficult to implement for grazing ruminants	Can be expensive. Potential negative effects on fibre digestibility. Need more information on fat × diet interactions. Effects on meat and milk quality need further study	Grainger and Beauchemin (2011); Patra (2013)
Concentrates	Low to medium	Low to medium	Immediate	Feasible, but limited scope for further increase in grain feeding	Decrease in enteric CH ₄ does not always reduce total greenhouse gas emissions. Can increase risk of acidosis. Concentrates can be fed to other livestock and consumed by people	Hristov <i>et al.</i> (2013)
Improved forage quality	Highly variable	Low	Immediate	Feasible, but highly dependent upon weather and other environmental factors	Adoption limited by knowledge transfer and potential trade-off between yield and quality. Absolute emissions may increase, but improved animal performance decreases intensity	Hristov <i>et al.</i> (2013)
Rumen microbiome and fermentation manipulation						
Vaccine	Unknown, possibly low to medium	Unknown, possibly low to medium	Unknown	Experimental. Limited published results. Would be particularly relevant for grazing ruminants	Effects on CH ₄ production, animal health and productivity will need to be established	Wedlock <i>et al.</i> (2010); https://www.nzagrc.org.nz/vaccine.html

Table 1 (Continued)

Strategy	CH ₄ decrease potential			Feasibility of implementing on-farms	Limitations	Key references
	Amount (g/day)	Intensity (g/kg product)	Expected availability			
Early life programming	Unknown	Unknown	Unknown	Experimental. Most feasible for intensive systems, difficult to implement for extensive pasture-based production systems	Research is in early stages. If effective, it may be economically attractive. Implications for animal health and performance unknown. Need to establish long-term persistency	Yáñez-Ruiz <i>et al.</i> (2015)
Chemical inhibitors (3-nitrooxypropanol)	High	High	Currently undergoing approval in some countries	Feasible for ruminants fed diets but difficult to implement for grazing ruminants	Need more information on animal performance, dose × diet effects and safety. Not approved by regulatory agencies. Unknown consumer acceptance	Dijkstra <i>et al.</i> (2018); Ungerfeld (2018)
Algae	Medium to high	Medium to high	Unknown, experimental	Few algae species contain active compounds. Need to dry material, which may require energy	Safety concerns related to bromoforms. Will need approval from regulatory authorities in most countries. Life cycle assessment needed to account for upstream emissions	Machado <i>et al.</i> (2014); Li <i>et al.</i> (2018)
Nitrate	Low to medium	Low to medium	Available, but requires approval by regulatory officials in some countries	Useful in low-protein diets. Can be used in place of urea, maximum of 2% of dietary DM. Can be used in blocks for grazing ruminants	Risk of toxicity to non-adapted animals. Potential increase in N excretion if N requirements of animals are exceeded. Not approved in some countries	van Zijderveld <i>et al.</i> (2011); Lee and Beauchemin (2014)
Tannins	Low to medium	Low to medium	Immediate	Extracts can be added to diets. Tannin-containing forages can be incorporated into pastures	Much of the research has been conducted in vitro. Need more information of effectiveness of different source and types of tannins for CH ₄ reduction. Need information on whether digestibility and performance is negatively affected	Jayanegara <i>et al.</i> (2012); Cobellis <i>et al.</i> (2016)

improvements in animal performance over time have decreased CH₄ and total GHG emissions intensity (e.g. Capper *et al.*, 2009; Legesse *et al.*, 2016). However, CH₄ intensity decreases in a curvilinear manner with increased animal productivity; thus, increasing the productivity of lower-producing animals has a relatively large impact, whereas a further increase in the productivity of high-producing animals has a relatively small impact. A life cycle assessment that accounts for all changes in GHG associated with the changes in practices to enhance animal productivity is necessary before recommending this approach for CH₄ mitigation. Ultimately, for reduction in emissions intensity to translate into absolute reductions in emissions, a net decrease in animal numbers will be required.

Animal breeding

Heritabilities of CH₄ production on an absolute emission basis (g CH₄/day) are moderate and estimated at 0.29 and 0.40 in sheep and cattle, respectively, but much lower at 0.13 and 0.19, respectively, on a yield basis (g CH₄/kg dry matter intake (DMI)) (Pickering *et al.*, 2015). Incorporating CH₄ production in a genetic selection programme represents a major challenge because of the difficulty of measuring CH₄ in a manner that reflects the long-term CH₄ phenotype of the animal (Løvendahl *et al.*, 2018). Methane production is mainly driven by DMI and fermentability of the feed, so emissions fluctuate over the long term depending upon the status of the animal and the diet, and diurnally depending upon the timing of feeding. Obtaining accurate and low-cost estimates of CH₄ production for a large group of animals under commercial conditions is challenging. Some animal breeding programs use a 'sniffer' technique to measure breath CH₄ concentration at a feeder or during milking. Although this technique has many sources of error (source-sampling distance, air turbulence, cow's head movement; Wu *et al.*, 2018), it has been shown to be correlated ($r=0.75$) to flux methods when used by skilled researchers (Difford *et al.*, 2019). Additionally, the development of proxies (see the 'Biomarkers to estimate methane emissions' section) that are highly correlated with CH₄ production would be beneficial for identifying low-CH₄ animals in a genetic selection program (Negussie *et al.*, 2017).

Relationships between CH₄ production and economically important traits are largely unknown (Basarab *et al.*, 2013), although Breider *et al.* (2019) recently showed genetic correlations of 0.49 to 0.54 between CH₄ production and milk yield indicating that genetically selecting for lower CH₄ production may decrease productivity. Thus, it is not clear whether genetic selection for CH₄ traits would provide any further advantage over selection for production traits, such as growth and milk production, which lower CH₄ emission intensity (Capper *et al.*, 2009; Legesse *et al.*, 2016). Another major limitation is that economic indexes developed for commercial sire selection are based on multiple traits weighted for their economic value. With the low economic value of CH₄ mitigation, this trait would have minor weighting in a multi-trait index. Furthermore, it is not clear whether

selecting low-emitting animals may result in reduced feed efficiency (Løvendahl *et al.*, 2018), especially of high-forage diets, given the finding of Pinares-Patiño *et al.* (2011) that low-CH₄ sheep have lower feed digestibility than high-CH₄ emitting sheep.

Another approach for lowering CH₄ emissions through animal genetics is the selection of more efficient animals based on a measure of feed conversion efficiency such as residual feed intake (which compares the actual feed intake of animals to expected intake for maintenance and production; Kenny *et al.*, 2018). This trait is moderately heritable (0.26 to 0.43) and moderately repeatable across diets (0.33 to 0.67) (Basarab *et al.*, 2013), although there is evidence that animal re-ranking may occur with different types of diets (Kenny *et al.*, 2018). One limitation for research is that the measurement of residual feed intake requires accurate measurement of DMI of individual animals, which is challenging for grazing animals. Incorporating measures of improved feed efficiency into multi-trait selection-based breeding programs has the potential to reduce the amount of feed used for meat and milk production (lower DMI with minimal change in digestibility). Consequently, a decrease in absolute emissions of enteric CH₄ can be expected with improved feed efficiency, but this needs confirmation in additional research using a range of diets to ensure no genotype \times environment interaction exists.

Nutrition

Dietary manipulation can be a highly effective CH₄ mitigation approach, and many thorough reviews are available (Beauchemin *et al.*, 2009; Hristov *et al.*, 2013; Knapp *et al.*, 2014). The efficiency of a particular dietary CH₄ mitigation strategy depends on its effects on ruminal H₂ flow and concentration, the microbial community, fermentation pathways, residence time of feed in the rumen and interactions among these factors.

Lipids. Numerous studies have shown that low levels of lipid supplementation of diets (<4% of dietary DMI) can decrease CH₄ production (by up to 20%, although results are variable) while increasing energy density of diets and benefiting animal productivity in some cases. The results from meta-analysis studies indicate a 1% to 5% decrease in CH₄ (g/day) per 10 g/kg DM dietary fat (Grainger and Beauchemin, 2011; Patra, 2013) with medium chain (C12:0, C14) and polyunsaturated fatty acids being most potent (Patra, 2013). Lipids inhibit methanogenesis by replacing rumen fermentable organic matter in the diet, decreasing the numbers of ruminal methanogens and protozoa, and through biohydrogenation of unsaturated fatty acids (Patra, 2013). Biohydrogenation can provide an alternative [H] sink in the rumen to compete with methanogenesis, but this is quantitatively small (1% to 2% of [H] used for this reaction; Nagaraja *et al.*, 1997), albeit potentially greater when methanogenesis is inhibited. However, lipid supplementation is often costly and can decrease fibre digestibility and DMI, inhibit rumen fermentation, depress milk fat

synthesis and alter the fatty acid composition of products (Grainger and Beauchemin, 2011; Patra, 2013). Although lipid supplementation can be implemented immediately on commercial farms, overall it has low to moderate scope for CH₄ mitigation due to cost and potential negative effects on animal production and product quality.

Concentrates. Compared to forage-based diets, concentrate-based diets are associated with lower CH₄ yield (g/kg DMI; Johnson and Johnson, 1995) because fermentation of starch in concentrate results in more propionate and butyrate than cellulose in forage and thus competes with methanogenesis for [H]. Starch has a faster rate of digestion and fermentation than cellulose, resulting in elevated dH₂ (Wang *et al.*, 2014). Additionally, high-starch intake can decrease ruminal pH, which inhibits the growth of methanogens, but it can also reduce fibre digestibility and increase the risk of acidosis. While increased feeding of starch-based diets may improve animal performance and decrease CH₄ yield, its potential as a CH₄ mitigation strategy is low as the global capacity to increase concentrate feeding of ruminants is limited. Furthermore, grain-based diets ignore the importance of ruminants in converting fibrous feeds, unsuitable for human consumption, to high-quality protein sources (i.e. milk and meat). Changes in the emissions of GHG resulting from producing additional concentrate and land use change also need to be considered using a life cycle assessment approach.

Forages. Strategies to mitigate CH₄ production from ruminants consuming forage diets are needed given that grazing ruminants produce 75% of global ruminant CH₄ emissions (Food and Agriculture Organization of the United Nations, 1999). Some of the CH₄ emissions from grazing ruminants can be offset by enhancing soil carbon reserves, thereby removing CO₂ from the atmosphere (Guyader *et al.*, 2016b). Additionally, well-managed grazing systems can reduce the use of synthetic fertilizer by more effective use of manure and nitrogen-fixing plants, which decreases N₂O emissions. Forage-based ruminant systems also provide many other ecological benefits, such as conserving biodiversity, improving soil health, enhancing water quality and providing wildlife habitat (Guyader *et al.*, 2016b). Mitigation of CH₄ from forage-based diets can be achieved to some extent by improving forage quality and availability through grazing management, timing of harvest, use of forage species with superior digestibility, use of condensed tannin-containing plants (see the 'Phytochemicals' section) and storage of forages to conserve digestible nutrient content. However, differences in forage quality may not always alter absolute CH₄ emissions (g/day) (Beauchemin *et al.*, 2009). On the one hand, high-quality forage has a greater ratio of non-fibre carbohydrates to NDF and less lignified NDF, which promotes organic matter degradation in the rumen. Therefore, more [H] is available for methanogenesis and absolute CH₄ production is increased due to greater DM ingested and digested in the rumen. On the other hand, high-quality forage promotes greater DMI in animals, which is associated with greater rate

of passage from the rumen, and decreased CH₄ per gram of DMI. Also, animals fed high-quality forages are more productive and thus have lower CH₄ emission intensity. As a result, the net effects of forage quality on daily CH₄ emissions can be variable, but improved forage quality typically lowers emissions intensity as a result of enhanced animal productivity.

Rumen fermentation and microbiome manipulation

Vaccines. Vaccination against rumen methanogens has been promoted as a means of decreasing CH₄ emissions and would be particularly useful for pasture-based systems for which many other mitigation approaches cannot be easily implemented. The concept is based on the vaccine inducing the animal's immune system to produce antibodies in saliva, which upon entry into the rumen would suppress the growth of methanogens (Subharat *et al.*, 2016). Current vaccine development by AgResearch (Hamilton, New Zealand) targets cell surface proteins that are conserved among rumen methanogens (<https://www.nzagrc.org.nz/vaccine.html>). So far, research has demonstrated production of antibodies *in vivo* in response to a vaccine (Wedlock *et al.*, 2010; Zhang *et al.*, 2015); however, changes in rumen methanogen population or CH₄ emissions have been nominal (Eckard and Clark, 2018). Although a challenging undertaking, a vaccine, if successful, could make substantial contribution to CH₄ mitigation, although effects on animal health and productivity will need to be established.

Early life programming. Recent studies have focused on decreasing CH₄ formation through programming the rumen microbial community early in the animal's life, as reviewed by Yáñez-Ruiz *et al.* (2015). The central idea is that the developing microbial community of the newborn ruminant is more malleable so that its manipulation is more likely to have long-lasting effects compared to the established microbiome of the adult animal. There are reports of colonization of the rumen by methanogens as early as birth (Guzman *et al.*, 2015). Research with gnotobiotic animals also provides important insights about the potential of rumen manipulation in early life and the potential alternative [H] sinks to CH₄ that could be enhanced in the developing rumen.

Abecia *et al.* (2013) observed that treating both does and their kids with the methanogenesis inhibitor bromochloromethane (BCM) resulted in less CH₄ emissions and greater rumen propionate concentration in kids when measured 3 months after BCM treatment of does and kids had ceased. The archaeal community composition also differed between treatments, but only if the mothers had also been treated with BCM, indicating that treating the mothers may be an additional means of influencing the early development of the rumen microbial community (Abecia *et al.*, 2018). Other studies showed no long-lasting effects on CH₄ production when anti-methanogenic (garlic essential oil and linseed oil) treatments were given to neonatal lambs (Saro *et al.*, 2018). It remains to be determined how the persistence of early life methanogenesis intervention is affected by the treatment applied, the animal and the diet, among other

variables. Although some early life studies have reported decreased methanogenesis in the short-term post-treatment, it will be necessary to assess whether mitigation is maintained throughout adulthood, the mechanisms involved and implications for animal health and performance. As the research is at an early stage, the feasibility of implementation on commercial farms where animals are in constant contact with each other is unknown.

Chemical inhibitors. The search for compounds that decrease CH₄ production when fed to ruminants is an important area of research, although challenging. In addition to CH₄ abatement, research in this area may theoretically lead to improved production efficiency through redirection of [H] from CH₄ towards compounds such as propionate or acetate formed through reductive acetogenesis that can be used by the animal (Janssen, 2010; Ungerfeld, 2013). The most common approach has been to use compounds that directly inhibit methanogenesis. Such compounds need to persistently lower emissions without toxic effects for animals, humans and the environment, and in order to be adopted by producers, they may need to be low cost and increase productivity and profitability. Furthermore, such compounds need to undergo thorough and costly regulatory processes before being commercially available. While the enormous cost and complexity of developing new compounds that are not already deemed safe for feeding to animals is daunting, this area of research should be a high priority.

Use of chemically synthesized inhibitors is one of the most promising strategies to decrease CH₄ emissions from ruminants (Liu *et al.*, 2011; Veneman *et al.*, 2016; Henderson *et al.*, 2018). Most inhibitors evaluated can be classified as analogues of CH₄ or analogues of methyl-coenzyme M, a co-factor involved in methyl transfer during methanogenesis. Some inhibitors that have been evaluated *in vivo* are trichloroacetamide, hemiacetal of chloral and starch, BCM, chloral hydrate, 9, 10-anthraquinone, nitroethane, 3-nitrooxypropanol (3-NOP) and chloroform (Ungerfeld, 2018). Some inhibitors are toxic, cause undesirable side effects or decrease methanogenesis only transiently, yet their study has generated useful proof of concept knowledge about the consequences of inhibiting rumen methanogenesis.

3-Nitrooxypropanol is a promising experimental CH₄ inhibitor currently under evaluation in large-scale dairy and beef cattle studies to support licencing by government authorities. Consistent CH₄ yield decreases of 20% to 40% have been reported depending upon animal, diet composition, dose and method of supplementing 3-NOP (Hristov *et al.*, 2015; Dijkstra *et al.*, 2018; Vyas *et al.*, 2018). No negative effects on diet digestibility (Romero-Perez *et al.*, 2014) and sustained decrease of CH₄ production over several months have been reported for lactating dairy cows (25% to 32%, 12-week study; Hristov *et al.*, 2015) and growing beef cattle (high-forage diet for 105 days, 37% decrease; high-grain diet for 105 days, 42% decrease; Vyas *et al.*, 2018). However, a study by McGinn *et al.* (2019) suggests that there

may be an adaptation to inhibitors over time, which is an area of research that needs to be pursued.

3-Nitrooxypropanol is a small molecule with a molecular shape similar to that of methyl-coenzyme M (Duin *et al.*, 2016). The methylated forms of coenzymes M and B are utilized as substrates by the nickel enzyme methyl-coenzyme M reductase in the last step of methanogenesis. 3-Nitrooxypropanol preferably binds into the active site of the reductase and then inactivates the reductase by oxidation of its active site Ni(I) (i.e. the nickel containing co-factor F430 of the reductase has to be in Ni(I) state for it to be active; Duin *et al.*, 2016). The effective dose of 3-NOP is relatively low (1–2 g/day), has high specificity towards methanogens, is degraded in the rumen to very low concentrations of nitrate, nitrite and 1,3-propanediol, residues in milk and meat are minute or non-existent and the safety risks of 3-NOP are reportedly low (Thiel *et al.*, 2019a and 2019b), although it waits to be seen whether the compound is approved by the regulatory authorities. Thus, 3-NOP has tremendous potential for CH₄ mitigation if commercially available, but product cost and consumer acceptance will factor into the acceptance of such a compound.

Algae. Algae can be classified by size (micro or macro) with macroalgae (seaweed) further classified based on pigmentation (green, red or brown) and habitat (freshwater, marine). Some types of algae concentrate phlorotannin and bromoforms, halogenated compounds that inhibit cobamide-dependent coenzyme M during methanogenesis. Machado *et al.* (2014) screened 20 species of tropical marine macroalgae *in vitro* and concluded that *Dictyota* (brown) and *Asparagopsis* (red) had the most potential for CH₄ production decrease. Kinley *et al.* (2016) further showed *in vitro* that *Asparagopsis taxiformis* supplemented at 20 g/kg of forage almost eliminated CH₄ production without negative effects on forage digestibility. Recently, Li *et al.* (2018) reported that feeding diets supplemented with up to 3% *A. taxiformis* to sheep decreased CH₄ production in a dose-dependent manner over a 72-day period, with 80% mitigation at the greatest dose and no changes in body mass gain. For algae to be adopted by farmers, a decrease in methanogenesis without negative side effects would have to be persistent. The safety of feeding bromoform-containing macroalgae to livestock will also need to be investigated, as bromoform can be toxic to the environment (i.e. ozone depletion) and can impair human health. Furthermore, a life cycle assessment will need to examine the CO₂ emissions from producing, harvesting, drying and transporting algae, which may offset potential decreases in CH₄ emissions from ruminants.

Alternative [H] sinks. Nitrate is a competitive [H] acceptor in the rumen that uses [H] at the expense of methanogenesis during its reduction to nitrite and subsequently ammonia. Additionally, nitrate can exert direct toxic effects on methanogens through its reduction intermediate nitrite (Lee and Beauchemin, 2014). Stoichiometrically, reduction of 1 mol (62 g) of nitrate to ammonia in the rumen should lower

CH₄ production by 1 mol (16 g). However, in feeding studies this potential is never reached because both nitrate and nitrite can be absorbed from or passed out of the rumen, increasing the risk of toxicity, and nitrite may be undesirably metabolized to other end-products such as N₂O, another potent GHG. A number of feeding studies have examined the short- and long-term effects of nitrate supplementation of diets (mainly as calcium nitrate). In studies lasting several months, dietary inclusion of nitrate (about 20 g/kg DM) persistently lowered CH₄ by up to 12% in beef cattle (Lee *et al.*, 2017) and 16% in dairy cows (van Zijderveld *et al.*, 2011). Nitrate is a source of non-protein N and can help supply the N requirements of the rumen microorganisms, which can be beneficial for low-protein diets, but the addition of nitrate to a diet already sufficient in N would result in increased N voided to the environment. Feeding nitrate to animals slightly increases nitrate residues in tissues (Doreau *et al.*, 2018) and milk (Guyader *et al.*, 2016a), but the levels are very low and not considered harmful to humans. However, increased nitrate concentrations in the rumen cause nitrate and methemoglobin levels to increase potentially causing nitrate poisoning of animals. Although risk of poisoning can be reduced by gradual adaptation of animals (Lee and Beauchemin, 2014), nitrate supplements are not approved for livestock in many countries (USA, Canada). Overall, the cost of calcium nitrate relative to urea (more than double) and the potential safety risks to animals fed nitrate are major impediments to using nitrate for CH₄ mitigation.

Phytocompounds. Secondary plant compounds such as essential oils, tannins, saponins, flavonoids and organosulphur compounds have been investigated for their potential anti-methanogenic properties. One of the most comprehensive studies of this kind is the EU project 'Rumen-up' that evaluated 500 plants and plant extracts for their effects on *in vitro* fermentation and identified at least 25 as having potential value as feed additives (<https://www.abdn.ac.uk>). Numerous essential oils (e.g. derived from garlic, thyme, eucalyptus, oregano, cinnamon and rhubarb) have been shown to decrease CH₄ production *in vitro*, but very few compounds have been shown to have long-term anti-methanogenic effects *in vivo* (Cobellis *et al.*, 2016). Garlic oil, which contains the organosulphur compounds alliin, diallylsulphides and allicin, appears to be one of the most effective phytocompounds for CH₄ decrease *in vitro*; thus, this effect needs to be evaluated in future animal studies.

Condensed and hydrolysable tannins also offer promise for CH₄ mitigation. Tannins are polyphenolic compounds found in various plants with complex and diverse chemical structure that have an affinity to bind to proteins and other compounds. The CH₄ response to feeding tannins is highly variable depending upon the source, type and molecular weight of the tannins, and the methanogenic community present in the animal. A meta-analysis of 30 *in vitro* and *in vivo* experiments showed that increasing levels of tannins decreased CH₄ production expressed relative to digestible

organic matter (Jayanegara *et al.*, 2012). Furthermore, the *in vitro* batch culture studies appeared to predict the *in vivo* responses reasonably well up to a level of 100 g tannin/kg DM. Meta-analysis of the *in vivo* studies indicated a decrease of 0.109 L CH₄/kg DMI per g tannin/kg DMI ($r^2 = 0.47$). However, a major limitation with tannins is that at low concentrations (<20 g/kg DMI), typical of many forages and feed supplements, CH₄ responses are highly variable. Furthermore, part of the CH₄ decrease due to tannins can be caused by a concomitant decline in DMI and nutrient digestibility. Nevertheless, the use of tannins as a potential CH₄ mitigation strategy warrants further investigation to identify the types and doses of tannins that reduce CH₄ without adverse effects on animal performance. Use of tannin-containing forages is particularly relevant for grazing ruminants as many forage legumes contain tannins, condensed tannins have been shown to aid in the control of gastrointestinal parasites (Min and Hart, 2003), and tannins can improve N utilization (Jayanegara *et al.*, 2012).

Reducing net emissions v. emissions intensity

In discussing CH₄ mitigation, it is important to consider the implications of the different metrics used (Eckard and Clark, 2018). Agricultural supply chain markets increasingly require certification of the carbon footprint of products, an emission intensity metric based on GHG emissions per unit of product produced. However, under the UN Framework Convention on Climate Change (UNFCCC, 2015), signatories have made commitments towards absolute reductions in their national GHG emissions. Fifty-four countries specifically mention a goal of decreasing livestock emissions (Richards *et al.*, 2015). A net reduction in GHG emissions will require net reductions in CH₄ emissions, yet cost-effective options for decreasing livestock CH₄ are limited at present.

A focus on emissions intensity still allows the livestock industries to grow with increasing efficiency of production, while decreasing CH₄ emissions relative to a 'business as usual' scenario. However, if the rate of growth of meat and milk production as a result of increasing demand for animal products is greater than the decrease in CH₄ emissions intensity, absolute CH₄ emissions from the livestock sector will continue to increase in the future. Thus, an approach targeting increases in animal productivity and decreases in absolute CH₄ emissions of individual animals, possibly combined with a decrease in consumption of livestock products, is needed (Garnett, 2009). Providing livestock producers with cost-effective options for decreasing CH₄ emissions is therefore imperative to lower carbon footprint of livestock products while also meeting international targets for net reductions in livestock emissions. There are few examples where both emissions intensity and net decreases in CH₄ emissions can be achieved, and this is an area requiring further research.

For increased milk and meat production to fill the increasing demands for food from animal origin to occur, along with

a decrease of absolute CH₄ emissions from the livestock industry, the decrease in GHG emissions intensity (i.e. including N₂O and CO₂ emissions) of the livestock industry would have to be proportionally greater than the increase in production. We can envision three possible, non-excluding avenues to achieve substantial decreases in absolute CH₄ production. A first possibility is to increase individual animal productivity and efficiency, thus requiring fewer animals and thus generating less total CH₄, for the same total production from the industry (Capper *et al.*, 2009; Legesse *et al.*, 2016). Decreasing CH₄ intensity through improvements in animal productivity involves intensification and consequences on the emissions of GHG other than CH₄ would also need to be considered. While this option is attractive in that it allows the industry to continue growing, net GHG emissions will continue to increase if not accompanied by measures to curb the growth of the industry (e.g. reduced consumption of animal products) or decrease GHG emissions per animal. Furthermore, intensification can lead to greater vulnerability under a changing climate (Henry *et al.*, 2018). Thus, improving individual animal productivity as a GHG mitigation approach will likely be insufficient if not accompanied by measures to decrease CH₄ emissions per animal. A second possibility is to explore the combination of various anti-methanogenic strategies (Table 1) that when applied together have substantial additive effects (e.g. linseed oil + nitrate; Guyader *et al.*, 2015). A third possibility is the development and use of specific chemical inhibitors of methanogenesis, such as 3-NOP. However, regulatory approval and consumer acceptance will be required, and improvements in animal performance or government incentives may be necessary to offset the added cost to producers. It is not clear whether greater decrease of CH₄ production may be achieved by combining inhibitors with other anti-methanogenic strategies, an area that needs further examination.

Unresolved issues and future direction

Mitigating emissions from extensive ruminant systems

A major constraint for decreasing global enteric CH₄ emissions from ruminants is the difficulty of applying mitigation strategies to grazing ruminants. It is estimated that about 60% of global agricultural land is grazed, supporting 360 million cattle and more than 600 million sheep and goats (FAO, 1999). Low-CH₄ diet formulations and feed additives are limited to animals fed total or partial mixed rations and very few mitigation approaches are available for pasture-based systems. Hence, most mitigation strategies for pastured ruminants focus on reducing emission intensity through animal breeding, supplementation to improve animal performance, grazing management and forage species selection (i.e. legumes, tannin-containing forages). There is an urgent need to develop additional mitigation strategies for grazing ruminants. Inhibitors (e.g. 3-NOP) offer great potential for CH₄ reduction but the challenge is administering

these compounds to grazing ruminants. It may be possible to develop a slow release formulation or delivery mechanism such as a pasture mineral block, liquid feed or water containing dissolved inhibitors or other mitigation agent to provide the required daily dose. The potential of self-regulated intake of such compounds needs further investigation, with attention to possible effects on animal health and residues in meat and milk.

Methane, animal productivity and incentives to lower emissions

Some CH₄ mitigation approaches increase the cost of feeding, thus a production benefit from decreasing methanogenesis may be needed to encourage widespread adoption by farmers. Theoretically, inhibiting CH₄ production increases the efficiency of conversion of digestible energy to metabolizable energy (ME) by decreasing energy losses in gases (Johnson and Johnson, 1995). In diets with 70% energy digestibility, energy losses as CH₄ range between 3% and 17% of digestible energy consumed. Thus, a moderate decrease in CH₄ production (e.g. 25%) may only increase ME by 0.75% to 4.25%, and given the efficiency of conversion of ME to net energy for production (i.e. <65%), it might be difficult to quantify a change in productivity. More severe inhibition of CH₄ production (e.g. >50%), without a decrease in DMI or digestibility, may be necessary to cause noticeable increases in animal productivity. Furthermore, the increase in ME resulting from a decrease in CH₄ might be smaller than estimated because unusable [H] sinks can accumulate in the methanogenesis-inhibited rumen (e.g. formate; Ungerfeld, 2015). Also, the additional ME may not contribute to greater animal productivity if increased flow of absorbed VFA does not match the animal's requirements or there are limitations in the supply of other nutrients. Thus, the decrease in CH₄ production may have to be substantial to obtain noticeable gains in production.

A second equally important consideration is how much CH₄ production can be effectively decreased in a production setting. A meta-analysis on the use of chemical inhibitors of methanogenesis reported average maximal decreases in CH₄ *in vivo* for individual experiments of 28% and 48% for milk and growth studies, respectively (Ungerfeld, 2018). The question of why methanogenesis inhibition by chemical compounds has been incomplete and variable is an important one. Compounds like CH₄ halogenated analogues, BES and 3-NOP universally target methanogens by inhibiting the last step of methanogenesis (Duin *et al.*, 2016). It is possible that some rumen methanogens less affected by chemical inhibitors occupy niches left by the most inhibited methanogens, resulting in incomplete inhibition. Metabolism of such compounds also occurs (Duin *et al.*, 2016) and may partly explain incomplete inhibition. Areas of interest for future research are the differences in the archaeal community and gene expression of methanogenesis pathways induced by anti-methanogenic compounds, the metabolism of these compounds in the rumen and long-term effects of combinations

and rotations of chemical inhibitors and other additives and dietary ingredients.

It is often said that decreasing CH₄ production is a win–win situation for the environment and livestock producers. However, increased animal performance due to CH₄ decrease is still highly speculative, as few studies have been conducted where a substantial decrease in CH₄ production was achieved over a long enough period to monitor effects on animal production (Ungerfeld, 2018). In the dairy study by Hristov *et al.* (2015) where 32% reduction in CH₄ was achieved by supplementing diets with 3-NOP, no improvement in milk production occurred although BW gain tended to increase possibly indicating enhanced body reserves. In the beef study by Vyas *et al.* (2018) where approximately 40% decrease in CH₄ was achieved using 3-NOP, a 3% to 5% improvement in gain : feed was reported. While there can be potentially promising improvements in animal performance coupled with decreases in CH₄ production, further long-term studies are needed.

Biomarkers to estimate methane emissions

While significant progress has been made in methods to measure CH₄ production (Hammond *et al.*, 2016), these techniques are expensive and technically challenging, and thus limited to research. The development of accurate, inexpensive and easy to use proxies for CH₄ production of individual animals may enable implementation of low-CH₄ management and breeding systems on farms. As reviewed by Negussie *et al.* (2017), a range of proxies have been explored including feed intake and behaviour, rumen fermentation metabolites, rumen microbiome, milk composition, membrane lipids (archaeol) of methanogens in faeces and lasers/sniffers that measure CH₄ concentration. For dairy cows, use of milk mid-IR spectroscopy to detect fatty acid composition and predict CH₄ emissions is based on the principle that the precursors for CH₄ and *de novo* synthesis of milk fatty acids both arise in the rumen. Mid-IR spectroscopy analysis of milk fatty acids has good potential to predict CH₄ production of individual cows on commercial dairy farms (van Gastelen and Dijkstra, 2016; Vanlierde *et al.*, 2018), especially when combined with additional information such as feed intake, nutrient composition of the feed, parity and lactation stage. This approach could allow CH₄ production to be incorporated in dairy cow breeding programs. Auffret *et al.* (2018) investigated the microbial communities and genetic markers associated with high/low CH₄ emitting cattle varying in breed and diet. They found the methanotrophic *Methylomonas* genus to be negatively correlated with CH₄ production. However, rumen microbiome profiling appears to be a poor to moderately accurate predictor of CH₄, and it is also costly and difficult for routine on-farm implementation. Nevertheless, the development of biomarkers for CH₄ production is at a relatively early stage and should be a priority area for future research.

Conclusions

A tremendous amount of research has been published in the past 50 years that has improved the understanding of the

complex processes of rumen fermentation and methanogenesis in ruminants, and the means by which enteric CH₄ production can be mitigated. While significant scientific advances have been made, arguably few cost-effective mitigation strategies are currently available to producers. Individually, most mitigation strategies are likely to have low to moderate (<20%) impact on decreasing CH₄ emissions, with the exception of chemical inhibitors, where long-term reductions of 20% to 40% may be feasible in commercial feeding operations. Thus, combining strategies may be necessary to achieve the substantial decreases in CH₄ production needed by the ruminant livestock industries and should receive research priority in the near future. Furthermore, there is a paucity of information on the mechanisms and effects of these mitigation strategies on animal performance, animal health and whether they will be accepted by consumers. Continued investment in research is critical because breakthrough technologies based on an understanding of rumen fermentation, microbiome and host animal are needed to achieve the decrease in CH₄ required. While substantial decreases in emission intensity (g CH₄/animal product) have been attained over the past decades due to production efficiency gains, increasing demand for animal protein, coupled with demands for lower emissions food, will require decreases in both emission intensity and absolute emissions (g CH₄/animal per day). Studies are needed to develop strategies to achieve both CH₄ mitigation and improvements in animal performance to benefit society and livestock producers. A major constraint for decreasing global enteric CH₄ emissions is the difficulty of applying mitigation strategies to grazing ruminants, a challenge that needs further investigation.

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Declaration of interest

These authors are unaware of any potential conflict of interest.

Ethics statement

Conducting this review involved no animal handling or procedures.

Software and data repository resources

No data were deposited in an official repository.

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