



## Invited review: Current enteric methane mitigation options

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### ABSTRACT

Ruminant livestock are an important source of anthropogenic methane (CH<sub>4</sub>). Decreasing the emissions of enteric CH<sub>4</sub> from ruminant production is strategic to limit the global temperature increase to 1.5°C by 2050. Research in the area of enteric CH<sub>4</sub> mitigation has grown exponentially in the last 2 decades, with various strategies for enteric CH<sub>4</sub> abatement being investigated: production intensification, dietary manipulation (including supplementation and processing of concentrates and lipids, and management of forage and pastures), rumen manipulation (supplementation of ionophores, 3-nitrooxypropanol, macroalgae, alternative electron acceptors, and phytochemicals), and selection of low-CH<sub>4</sub>-producing animals. Other enteric CH<sub>4</sub> mitigation strategies are at earlier stages of research but rapidly developing. Herein, we discuss and analyze the current status of available enteric CH<sub>4</sub> mitigation strategies with an emphasis on opportunities and barriers

to their implementation in confined and partial grazing production systems, and in extensive and fully grazing production systems. For each enteric CH<sub>4</sub> mitigation strategy, we discuss its effectiveness to decrease total CH<sub>4</sub> emissions and emissions on a per animal product basis, safety issues, impacts on the emissions of other greenhouse gases, as well as other economic, regulatory, and societal aspects that are key to implementation. Most research has been conducted with confined animals, and considerably more research is needed to develop, adapt, and evaluate antimethanogenic strategies for grazing systems. In general, few options are currently available for extensive production systems without feed supplementation. Continuous research and development are needed to develop enteric CH<sub>4</sub> mitigation strategies that are locally applicable. Information is needed to calculate carbon footprints of interventions on a regional basis to evaluate the impact of mitigation strategies on net greenhouse gas emissions. Economically affordable enteric CH<sub>4</sub> mitigation solutions are urgently needed. Successful implementation of safe and effective antimethanogenic strategies will also require delivery mechanisms and adequate technical support for producers, as well as consumer involvement and acceptance. The most appropriate metrics should be used in quantifying the overall climate outcomes associated with mitigation of enteric CH<sub>4</sub> emissions. A holistic ap-

Received March 21, 2022.

Accepted July 23, 2022.

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proach is required, and buy-in is needed at all levels of the supply chain.

**Key words:** antimethanogenic strategies, climate change, methane, mitigation, ruminants

## INTRODUCTION

Over 110 countries and supporters have signed the Global Methane Pledge ([www.globalmethanepledge.org](http://www.globalmethanepledge.org)) to decrease methane (CH<sub>4</sub>) emissions collectively by 30% from 2020 levels by 2030. Due to the relatively short life of CH<sub>4</sub> in the atmosphere and its high global warming potential, reducing CH<sub>4</sub> emissions is seen as a rapid way to help limit global warming to 1.5°C above preindustrial levels. Given that enteric CH<sub>4</sub> from ruminant livestock accounts for 30% of global anthropogenic CH<sub>4</sub> emissions (United Nations Environment Programme and Climate and Clean Air Coalition, 2021), there is increasing interest in its mitigation.

In recent years, tremendous advances have been made in understanding factors that affect CH<sub>4</sub> production in the rumen and development of mitigation practices. Detailed descriptions of the biochemistry (Ungerfeld, 2020) and microbiology (Morgavi et al., 2010; Huws et al., 2018) of fermentation and CH<sub>4</sub> production in ruminants have been published, along with thorough reviews of CH<sub>4</sub> mitigation (Beauchemin et al., 2008, 2020; Martin et al., 2010; Hristov et al., 2013a,b; Knapp et al., 2014; Arndt et al., 2022). This area of science is evolving at a rapid pace and, with increased pressure on ruminant sectors to decrease CH<sub>4</sub> emissions, there is a need for continual review to guide research, policy, and adoption.

This review stems from a comprehensive technical guidance document for the Food and Agriculture Organization of the United Nations (FAO) under the Livestock Environmental Assessment and Performance Partnership (FAO LEAP Partnership) program developed by an international group of scientists working on solutions for enteric CH<sub>4</sub> mitigation. The main intents of this review are to analyze the current possibilities for implementing strategies for mitigating enteric CH<sub>4</sub> emissions, establish research priorities for different production systems, and discuss biological, economical, regulatory, and societal barriers for adoption of each antimethanogenic strategy. We also discuss other antimethanogenic strategies being investigated that may have implications for future adoption. Whenever possible, we build on previous reviews and meta-analyses. Our review takes the approach of systematically discussing each mitigation option in terms of its mode of action, efficacy, potential for combining with other strategies, effects on other emissions of greenhouse

gases (**GHG**), impact on animal productivity, safety, adoption potential, and further research needs.

## METHANE MITIGATION METRICS

Several metrics must be considered when addressing the efficacy of a particular enteric CH<sub>4</sub> mitigation strategy. Antimethanogenic strategies may decrease total CH<sub>4</sub> production (absolute emissions, g/d), CH<sub>4</sub> yield (g/kg of DMI), or CH<sub>4</sub> intensity (g/kg of meat, milk, or wool produced). Methane mitigation can also be evaluated in terms of CH<sub>4</sub> energy loss as a proportion of gross energy intake (**GEI**), a variable known as **Ym**, and as grams of CH<sub>4</sub> produced per kilogram of digested OM. Methane yield, CH<sub>4</sub> produced per kilogram of digested OM, and Ym are variables important in research for helping to understand how emissions are mitigated by a certain strategy, and potential effects on the animal's energy utilization efficiency. Expressing CH<sub>4</sub> relative to DMI reveals how efficacious a mitigation strategy may be, independently of possible changes in feed intake, given that feed intake is the main factor driving CH<sub>4</sub> production. Methane production per kilogram of digested OM further adjusts CH<sub>4</sub> yield for the proportion of ingested feed actually digested and can reflect changes in the rumen fermentation profile. In turn, Ym provides a measure of how much extra ingested energy is potentially available for increasing animal production when methanogenesis is inhibited.

Importantly, in some cases, CH<sub>4</sub> may decrease when expressed as one metric but increase when expressed as another. Increasing feed intake by increasing forage digestibility or supplementing concentrates can decrease CH<sub>4</sub> yield, but absolute CH<sub>4</sub> emissions could remain unchanged or even increase if the animal's DMI increases. Strategies that improve animal performance and efficiency of production tend to decrease CH<sub>4</sub> intensity because they dilute the feed energy associated with the individual animal or herd maintenance, and thus represent a desirable improvement in the efficiency of GHG emissions relative to food supply. However, a decrease in CH<sub>4</sub> intensity may not decrease absolute emissions from a farm, sector, or area if individual feed intake or the number of animals, or both, increase to compensate for the decrease in CH<sub>4</sub> intensity.

## INCREASED ANIMAL PRODUCTIVITY

Intensification through improved feeding practices (quantity and quality of feed), animal management, improved animal health, breeding for greater productivity, and better reproductive performance results in greater individual animal production (Capper et al., 2009;

Beauchemin et al., 2020). Animals that produce more generally eat more, and digest and ferment more feed in their ruminants, thus producing more CH<sub>4</sub>. However, as the production of the individual animal increases, the emission of CH<sub>4</sub> on an animal product basis (i.e., CH<sub>4</sub> emissions intensity), decreases (Gerber et al., 2013). This phenomenon largely occurs through the dilution of maintenance effect; as nutrient intake increases, the proportion of nutrients ingested used for maintenance functions decreases, leaving a greater proportion of ingested nutrients for animal production (Capper et al., 2009). Mitigation potential through intensification of animal production is greater in low-producing than in high-producing animal systems (Gerber et al., 2013) and is attractive in low-income countries that need to produce greater quantities of nutritious foods (Tricarico et al., 2020).

Importantly, intensification usually increases upstream emissions of carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) resulting from the production of animal feed or even from pasture management, and also increases manure emissions. Therefore, changes in the emissions of all GHG must be taken into account in a life cycle assessment (LCA) approach (Beauchemin et al., 2020).

Intensification of animal production has resulted in large decreases in GHG emission intensity or carbon footprint [measured as kg of CO<sub>2</sub> equivalents (CO<sub>2</sub>e) per kg of product] of animal products. For example, in the United States, milk production increased by 59% between 1944 and 2007 through improved animal productivity, leading to a decrease in milk carbon footprint by almost two-thirds (Capper et al., 2009).

The relationship between carbon footprint and total GHG emissions is, however, variable. The change in total GHG emissions from ruminant production over time in a certain region, country, or globally depends on how the rate of decrease in carbon footprint relates to changes in individual animal production and numbers. Ungerfeld et al. (2022) examined the evolution of GHG emissions, expressed as CO<sub>2</sub>e, of the dairy, beef, and lamb production industries in 9 countries or regions, contrasting yearly rates of change of CO<sub>2</sub>e per animal product with yearly rates of change of total emissions CO<sub>2</sub>e for each industry in each country or region. Examination of that historical evidence showed that intensification and lowering the carbon footprint decreased total emissions of GHG in 4 of those case studies but increased GHG emissions in 5 of them. Although intensification and increases in animal productivity ameliorate total GHG emissions relative to a nonintensification scenario, intensification alone is insufficient to mitigate total emissions of GHG to the levels required to maintain global temperature increase

within 1.5°C by 2050, unless total emissions are capped (Ungerfeld et al., 2022).

Animal productivity can be increased without an increase in total GHG emissions if GHG intensity is reduced sufficiently to offset the increase in total animal product output. Additionally, selective breeding for improved feed efficiency without a decline in performance could result in animals utilizing less feed to produce the same amount of product (Løvendahl et al., 2018). Precision feeding, targeting nutrient requirements of each individual animal and thus decreasing feed input per unit of product, can be another tool to decrease CH<sub>4</sub> intensity, particularly in less-efficient animals (Fischer et al., 2020). It is important to continue investigating the improvement of feed efficiency through genetic selection and precision feeding, because greater production efficiency can help decrease total emissions of GHG with equal or greater animal production (Waghorn and Hegarty, 2011; Tricarico et al., 2022). The balance in total emissions of GHG ultimately depends on the relative proportional changes in feed efficiency and total animal production.

Improved animal productivity has been regarded as an attractive proposition for producers because it has the potential to increase economic margins of production (Gerber et al., 2013). However, the appeal to producers and success of intensification as a means of improving animal production profitability are highly dependent on the ratios of product prices to cost of production, risk aversion, access to high-producing breeds, access to credit, education and entrepreneurship, size of the production unit, and availability of technology applicable to local conditions, among other factors.

## SELECTION OF LOW-METHANE-PRODUCING ANIMALS

Individual differences in CH<sub>4</sub> production exist among animals within the same herd and with the same feeding management (de Haas et al., 2017). Heritabilities of absolute CH<sub>4</sub> production in cattle and sheep were moderate, and were higher than the heritability of CH<sub>4</sub> yield in sheep (Rowe et al., 2019) but lower in dairy cows (Manzanilla-Pech et al., 2021). As with any selected trait, gains in lowering CH<sub>4</sub> production that are associated with host genetics are permanent and cumulative (de Haas et al., 2021; Manzanilla-Pech et al., 2021).

Possible associations with other traits of interest need to be considered. Selecting against total CH<sub>4</sub> production selects against DMI and production traits (Lassen and Løvendahl, 2016; de Haas et al., 2017; Breider et al., 2019; Manzanilla-Pech et al., 2021). Problems caused by using ratios of correlated variables as selection crite-

ria (i.e., CH<sub>4</sub> yield or intensity) as a means of overcoming positive correlations between CH<sub>4</sub> production and performance have been discussed (de Haas et al., 2017; Løvendahl et al., 2018; Breider et al., 2019; Manzanilla-Pech et al., 2021). However, a program selecting for ewes divergent in CH<sub>4</sub> yield, obtaining a 12% difference between divergent lines after 10 yr of selection, was also successful in selecting more productive animals: ewes with low CH<sub>4</sub> yield weaned heavier and leaner lambs that produced more wool (Rowe et al., 2019).

Another option for selection is residual CH<sub>4</sub> production, calculated as the residual of the regression of observed CH<sub>4</sub> production against variables such as DMI, daily body mass gain (ADG), milk production, and body mass. Working with 15,320 dairy cow records of CH<sub>4</sub> production and performance from 2,990 dairy cows from Canada, Australia, Switzerland, and Denmark, Manzanilla-Pech et al. (2021) found that residual CH<sub>4</sub> metrics were more suited for inclusion in selection indices than CH<sub>4</sub> production metrics. Inclusion of CH<sub>4</sub> production in a selection index will likely require establishment of a price on CH<sub>4</sub>. If the price of CH<sub>4</sub> is too low, the CH<sub>4</sub> production weight in the index will be equally low and it might not be worth implementing (Beauchemin et al., 2020; de Haas et al., 2021).

The association between enteric CH<sub>4</sub> emissions and feed efficiency needs to be clearly established. On the one hand, low-CH<sub>4</sub>-producing animals should theoretically have a better conversion of digestible to metabolizable energy; however, their lower rumen retention times may result in lower digestibility (Løvendahl et al., 2018). Studies comparing animals selected for low and high production efficiency have yielded differing results with respect to CH<sub>4</sub> production. Fitzsimons et al. (2013) observed that heifers with low residual feed intake (i.e., more efficient animals) emitted less total CH<sub>4</sub> and CH<sub>4</sub> per kilogram of metabolic body mass than those with high residual feed intake (i.e., less efficient animals). Freetly and Brown-Brandl (2013) reported that feed efficiency expressed either as the ADG:DMI ratio or residual feed intake was unrelated to CH<sub>4</sub> production in steers, whereas the ADG:DMI ratio was loosely but positively related to CH<sub>4</sub> production in heifers. Arndt et al. (2015) observed that highly efficient dairy cows lost less energy as CH<sub>4</sub> as a proportion of GEI compared with their low-efficiency counterparts. McDonnell et al. (2016) did not find associations between residual feed intake and total CH<sub>4</sub> production in heifers, but more efficient animals lost a greater proportion of GEI as CH<sub>4</sub>. Velazco et al. (2017) reported a negative association between residual feed intake and CH<sub>4</sub> production. Olijhoek et al. (2018), Flay et al. (2019), and Renand et al. (2019) did not find associations between residual feed intake and total CH<sub>4</sub> production or intensity, CH<sub>4</sub>

production per kilogram of body mass, or CH<sub>4</sub> as a proportion of GEI. Importantly, low residual feed intake animals have consistently demonstrated greater CH<sub>4</sub> yield because of their decreased DMI (Fitzsimons et al., 2013; McDonnell et al., 2016; Olijhoek et al., 2018; Flay et al., 2019), which indicates that residual feed intake is more influenced by DMI than by CH<sub>4</sub> production. The latter aspect is the result of feed efficiency being a complex trait influenced by numerous aspects of digestive and metabolic efficiency apart from energy losses as CH<sub>4</sub> (Herd et al., 2004; Richardson and Herd, 2004).

It should be noted that in the studies discussed above, animals were selected based on feed efficiency or residual feed intake, and CH<sub>4</sub> production was compared in high- and low-efficiency animals; more studies are needed comparing feed efficiency and animal performance of animal lines selected for high and low CH<sub>4</sub> production. Manzanilla-Pech et al. (2021) found positive correlations between residual CH<sub>4</sub> and residual feed intake in a multi-country data set of records from cows that had not been selected by either trait, implying that those animals that produced less CH<sub>4</sub> converted feed to milk more efficiently. As for any other trait subjected to genetic selection, the possible existence of genotype by environment interactions should be considered when anticipating how a genotype selected for low CH<sub>4</sub> production would perform in other countries or regions, or in a different production system, or with different diets and rumen microbiota.

One of the main challenges in selecting animals with low CH<sub>4</sub> production is measuring CH<sub>4</sub> of a large number of animals in commercial farms, which is not within the reach of most commercial breeders (de Haas et al., 2021). Sniffers to measure CH<sub>4</sub> concentration in the exhaled air at a feeder or during milking have been used with some success (Difford et al., 2019). Methane production needs to be measured for weeks at a time, and a genetic selection program requires thousands of measurements (de Haas et al., 2017; Løvendahl et al., 2018), although measurements of CH<sub>4</sub> production in sires can potentially accelerate the spread of genetic progress. Proxies of CH<sub>4</sub> production, such as feed intake and feeding behavior, rumen VFA concentration, composition of the microbial community, and membrane lipids of methanogens in feces, have all been investigated as alternatives to direct measurement (Beauchemin et al., 2020). Milk fatty acid composition estimated through mid-infrared spectroscopy initially showed good results at an experimental scale, but to a lesser extent under commercial conditions with more animals (Løvendahl et al., 2018). Development of biomarkers to estimate CH<sub>4</sub> production reliably and that are sufficiently practical to implement at the farm scale is an area of considerable interest (Beauchemin et al., 2020).

The use of genomic selection toward low CH<sub>4</sub> production has also been proposed (de Haas et al., 2017).

Animal breeding is one of the few antimethanogenic strategies that can be applied to extensive production systems where animals are not supplemented. An additional advantage of this approach is that no major effects on other upstream or downstream emissions of GHG are expected. The greatest challenges to the selection of low-CH<sub>4</sub>-producing animals are the possible existence of undesirable associations between CH<sub>4</sub> production and animal productivity and developing reliable and practical proxies for predicting CH<sub>4</sub> production applicable to large numbers of animals.

## DIET REFORMULATION

### *Levels of Feed and Concentrate Intake, Source, and Processing*

This section discusses dietary strategies based on their direct effects on CH<sub>4</sub> production through rumen digestion and fermentation; effects occurring through an increase in productivity were discussed in a previous section (“Increased Animal Productivity”). Increasing feed intake of ruminants decreases retention time of feed in the rumen due to faster passage rates. Shorter retention time limits microbial access to OM, thus reducing the extent of rumen fermentation and leading to a decline in CH<sub>4</sub> yield and Y<sub>m</sub>, although total CH<sub>4</sub> emissions increase as more feed is ingested and digested (Arndt et al., 2022). Increasing the proportion of concentrate in the diet increases dietary energy density, decreases the proportion of structural carbohydrates, increases rumen outflow rate, and lowers rumen pH, decreasing CH<sub>4</sub> production per unit DMI and of feed fermented (Janssen, 2010). Processing of grains and feeding concentrates with rapidly fermentable starch promotes starch fermentation in the rumen and increases propionate production, which serves as a sink of metabolic hydrogen alternative to methanogenesis (Janssen, 2010; Ungerfeld, 2015). Rapid fermentation rate of grains also lowers rumen pH and inhibits the growth of protozoa (Janssen, 2010), thereby reducing the role of protozoa in protecting methanogens from oxygen toxicity and decreasing the supply of H<sub>2</sub> for methanogenesis (Newbold et al., 2015).

The efficacy of increasing levels of concentrate is variable. Based on an intercontinental database for beef cattle, van Lingen et al. (2019) reported a CH<sub>4</sub> yield of 20.7 g/kg of DM (range: 6.29 to 35.1 g/kg of DM) and a Y<sub>m</sub> of 6.3% (1.9 to 10.4%) for high-forage (≥25%) diets compared with a CH<sub>4</sub> yield of 15.2 g/kg of DM (7.50 to 30.9 g/kg of DM) and Y<sub>m</sub> of 4.5% (2.3 to 8.7%) for low-forage (≤18%) diets. The meta-analysis

by Arndt et al. (2022) reported a decrease in total CH<sub>4</sub> as well as CH<sub>4</sub> yield and intensity, without an increase in total CH<sub>4</sub> production through decreasing the forage-to-concentrate ratio. In terms of grain sources, absolute CH<sub>4</sub> production and CH<sub>4</sub> yield appear to follow the order wheat and steam-flaked corn < corn < barley, with the ranking highly dependent on the composition and extent of processing of the grain (Beauchemin and McGinn, 2005; Moate et al., 2017, 2019). In their meta-analysis, Arndt et al. (2022) found greater decreases in total CH<sub>4</sub> with barley than with corn, with differing results for CH<sub>4</sub> intensity of growth and milk production. Grain processing methods (application of various combinations of heat, moisture, time, and mechanical actions) can modify the fermentation of starch and rumen pH. Compared with a dry-rolled corn-based diet, feeding a steam-flaked corn-based diet to steers reduced CH<sub>4</sub> yield by 17% (Hales et al., 2012).

Some experiments evaluating concentrate supplementation of grazing animals have shown a decrease in CH<sub>4</sub> yield and intensity (Jiao et al., 2014), although most reported no change in any CH<sub>4</sub> production metric (Arndt et al., 2022; Vargas et al., 2022). The discrepancies for pasture studies may be attributed to the substitution rate (concentrate vs. pasture), pasture quality, or differences in methodology to estimate DMI. Increasing concentrate intake can be easily combined with other mitigation strategies. Several studies have shown additive effects of concentrate and oil inclusion on mitigating total CH<sub>4</sub> emissions and emission intensity (e.g., Bayat et al., 2017). Methanogenesis inhibitors such as 3-nitrooxypropanol (**3-NOP**) show synergy with concentrates, whereby the mitigation potential of inhibitors is increased in high-concentrate diets (Schilde et al., 2021).

Increased feed intake and use of grain and grain processing are accompanied by increased emissions of CO<sub>2</sub> and N<sub>2</sub>O from the use of fossil fuels and nitrogen (N) fertilizers used during feed production and manufacture (Beauchemin et al., 2009). Furthermore, conversion of pastureland to cropland results in the loss of soil carbon. Changes in digestibility of nutrients can alter manure amount and composition (Beauchemin and McGinn, 2005; Hristov et al., 2013a,b) and CH<sub>4</sub>, ammonia, and N<sub>2</sub>O emissions from manure.

Greater concentrate intake increases feed costs and can cause clinical and subclinical acidosis (Hristov et al., 2022). Milk protein and fat concentrations might also decrease, particularly when feeding wheat- or oat-based diets compared with corn- or barley-based diets (Moate et al., 2019). A decrease in milk components could reduce the profitability for dairy producers. Increased feeding of concentrates is easily adoptable in production systems in which intensification is pos-

sible. However, substantial increases in concentrate use would be difficult, or even impossible, to implement in many areas of the world where cereal crops cannot be grown or are too expensive (Beauchemin et al., 2009). Such production practices would also be associated with substantial land use change. Furthermore, feeding ruminants grains that could be directly consumed by humans can be regarded as an inefficient practice that does not take advantage of the ability of ruminants to convert fibrous feeds unsuitable for humans into useful products (Beauchemin et al., 2020). More research is required to characterize how grain source and processing method influence enteric CH<sub>4</sub> emissions, and to identify the appropriate ration formulations with cereal-based diets that counteract the negative effect on milk fat while retaining a CH<sub>4</sub> mitigation effect. Further research should also focus on evaluating total GHG emissions using an LCA for individual farms and geographical regions (Beauchemin et al., 2008).

### Lipid Supplementation

Dietary lipids elicit their CH<sub>4</sub>-mitigating effect through several mechanisms, including toxicity against methanogens and protozoa; biohydrogenation of UFA serving as a minor sink of metabolic hydrogen; and shifting rumen fermentation to promote the production of propionate, resulting in lower CH<sub>4</sub> production (Newbold et al., 2015). Also, as they are largely unfermentable (except for the glycerol moiety), the replacement of carbohydrates with lipids contributes to decreased enteric CH<sub>4</sub> emissions.

Supplementation of dietary lipids is an effective CH<sub>4</sub> mitigation strategy, although efficacy depends on the form, source, and amount of supplemental fat; degree of saturation; fatty acid carbon chain lengths; and nutrient and fatty acid composition of the basal diet (Patra, 2013). Various meta-analyses have been conducted to elucidate the CH<sub>4</sub>-mitigating effect of dietary lipids in ruminants (Beauchemin et al., 2008; Patra, 2013; Arndt et al., 2022). The antimethanogenic effects of dietary lipids vary considerably over a broad range of conditions. The effects vary from a decrease in CH<sub>4</sub> yield of 5.6% (Beauchemin et al., 2008) to between 3.8 and 4.3% per 10 g/kg (DM) of supplemental fat depending on source (Patra, 2013, 2014). Medium-chain fatty acids such as myristic acid and PUFA in fish, sunflower, linseed, and canola oils are the most effective fatty acids for reducing CH<sub>4</sub> emissions (e.g., Grainger et al., 2010). Supplementation with lipids was more effective, although effectiveness was more variable, for sheep than for cattle in the meta-analysis by Grainger and Beauchemin (2011). However, it should be noted that different lipid sources were used in the sheep and cattle

studies, which could have influenced the responses of each species to lipid supplementation.

Breaking the hull of oilseeds through grinding, crushing, or rolling them before feeding ensures availability of the lipids in the rumen. Oils are typically more effective than crushed oilseeds (Beauchemin et al., 2008), although this comparison depends on the extent of processing of the oilseeds. In a meta-analysis, Arndt et al. (2022) showed that feeding oils or fats versus oilseeds had comparable mitigation effects on total CH<sub>4</sub> production (−20 and −20%), CH<sub>4</sub> yield (−15 and −14%), and CH<sub>4</sub> intensity for milk production (−12 and −12%). However, feeding oilseeds had no effect on CH<sub>4</sub> intensity for ADG, whereas supplemental oils and fats reduced CH<sub>4</sub> intensity of ADG by 22% (Arndt et al., 2022). Few studies have examined the long-term effects of dietary lipids on CH<sub>4</sub> emissions. Indications are that they have persistent antimethanogenic effects (Jordan et al., 2006; Grainger et al., 2010), although a recent study with grazing dairy cows found transient effects of oilseed supplementation (Muñoz et al., 2021). The inhibitory effect of dietary lipids on CH<sub>4</sub> emissions is greater with concentrate-based diets than with forage-based diets (Patra, 2013), possibly because of the lower rumen pH associated with concentrate-based diets, which enhances the inhibitory effect of fatty acids on methanogens (Zhou et al., 2015).

Combinations of dietary lipids with other mitigation strategies have been investigated. An additive effect of dietary lipids on CH<sub>4</sub> abatement was confirmed when canola oil was combined with 3-NOP (Zhang et al., 2021) and when linseed oil was combined with nitrate (Guyader et al., 2015). However, there was no additive effect when soybean oil was combined with extracts rich in tannins (Lima et al., 2019) or saponins (Mao et al., 2010).

Feeding a high concentration of lipids can decrease feed and fiber digestibility (Patra, 2013, 2014; Arndt et al., 2022), which might increase the excretion of OM and nutrients and emissions of CH<sub>4</sub> from manure (Hassanat and Benchaar, 2019), although this may not occur with levels of total dietary lipid supplementation <6% DM. Supplementing fats leads to an increase in feed emissions associated with the cultivation and transportation of refined oils or of whole or crushed oilseeds. Supplementing fats at 4 to 6% of dietary DM (total dietary fat of 6 to 8% maximum) can improve milk production but feeding higher concentrations of fats can have detrimental effects on rumen fermentation, feed digestion, and animal performance (Patra, 2013, 2014). The meta-analysis conducted by Arndt et al. (2022) showed that feeding oils and fats decreased DMI (by 6%) and fiber digestibility (by 4%) but had no effect on milk production or ADG. However, feed-

ing oilseeds did not affect DMI but decreased digestibility (by 8%) and ADG (by 13%), with no effect on milk production (Arndt et al., 2022). Supplementing dietary lipids rich in long-chain UFA can improve the nutritional quality of meat or milk by increasing the content of healthful fatty acids including PUFA, CLA, and vaccenic acid (Bayat et al., 2015). However, high dietary UFA may decrease milk fat production, especially when diets contain high concentrate or rumen pH is low (Bougouin et al., 2019; Sun et al., 2019).

Lipid supplementation is not known to pose a risk to the safety of animals and humans; it is readily available and can be easily implemented in intensive or confined feeding systems. Feeding refined oils can be costly, and they often do not fit into least-cost ration formulations. Alternatively, processed oilseeds can be less expensive and might stimulate the adoption of supplementing dietary lipids. Although limited options exist to apply this strategy in grazing systems, there have been promising efforts to breed grasses with high levels of fats rich in PUFA (Winichayakul et al., 2008) or providing supplemental fat through drinking water (Osborne et al., 2008).

Further research is needed to identify cost-effective and sustainable fat sources and their respective supplemental level that would reduce CH<sub>4</sub> emissions without impairing feed digestibility and animal production. Studies are also required to ascertain the long-term effect of supplemental fats in suppressing CH<sub>4</sub> emission. Considering the potential impact on feed emissions and nutrient excretion, the effectiveness of this mitigation strategy needs to be addressed using LCA.

## FORAGES

Pastures and forage crops comprise 26% of the land and 70% of agricultural area globally (FAO, 2022) and are the main component of ruminant livestock diets. The unique digestive system of ruminants allows them to produce high-quality protein in the form of meat and milk from forages, avoiding direct competition for grain that can be used as human food. However, intake of cellulosic material augments enteric CH<sub>4</sub> emissions, with substantial variation due to forage source, chemical composition, digestibility, forage preservation, grazing management, and other factors. This variation creates opportunities for CH<sub>4</sub> mitigation through forage management. Forage production systems are highly variable and dependent upon farm site conditions (e.g., soil type and fertility, water, climate) and management practices. These factors affect forage yield and nutritive value, carbon storage in soils, animal performance, manure excretion and, ultimately, GHG emissions. Therefore, in all cases, a change in forage management

to decrease enteric CH<sub>4</sub> emissions needs to be assessed using regionally specific farm-level LCA that account for changes in forage and animal productivity, as well as emissions and sinks from all components of the farming system, including soil carbon.

## Digestibility

Increasing forage digestibility usually increases DMI and improves animal performance, which decreases CH<sub>4</sub> yield and intensity. Digestibility of forages conserved as hay or silage can be maximized by harvesting at a vegetative stage; in pastoral systems, digestibility can be enhanced by optimizing grazing management to decrease forage maturity (e.g., adjusting stocking rates, ensuring pregrazing herbal mass is not excessive; Vargas et al., 2022). Although CH<sub>4</sub> intensity decreases, absolute CH<sub>4</sub> production due to increased forage digestibility usually remains constant or increases due to greater DMI and increased OM fermentation in the rumen (Arndt et al., 2022). For example, cows fed fresh herbage cut after a shorter regrowth period produced more milk and the same amount of CH<sub>4</sub>, thus CH<sub>4</sub> intensity was 12% less with a shorter grass regrowth period (Warner et al., 2015). Warner et al. (2016) compared grass ensiled at 3 stages of maturity and reported that ensiling less-mature grass resulted in greater DMI, digestibility, and milk and CH<sub>4</sub> production, with CH<sub>4</sub> intensity being 24% less for the least compared with the most mature silage. On the other hand, total CH<sub>4</sub> production was 6% greater compared with that of the most mature silage. Macome et al. (2018) evaluated grass ensiled at 4 stages of maturity and reported that CH<sub>4</sub> yield and intensity of dairy cows was 16 and 21% less, respectively, for the least compared with most mature grass. Total CH<sub>4</sub> production was not reported in that study.

Improved forage digestibility is easy to combine with other CH<sub>4</sub> mitigation strategies at the farm level. Forage management to enhance digestibility affects many other aspects of the farming system, highlighting the need to consider impacts on net GHG emissions. The other aspects of farming that need consideration include animal productivity, amount and composition of manure, forage biomass yields, carbon sequestration during forage growth, and forage crop inputs. Immature forages have greater N content, which can increase N voided to the environment if not balanced. Implementation of increased forage digestibility at the farm can be hindered by lack of agronomic information and technical support, as well as additional costs. Furthermore, some ruminant production systems (e.g., nonlactating beef cows, animals at maintenance energy intake) fill the unique niche of consuming high-fiber,

low-digestible feeds and crop residues and co-products not suitable for highly productive animals.

### Perennial Legumes

At the same physiological stage of maturity, legume forages contain less NDF than grasses. Although fiber in legumes is more lignified, the decline in fiber digestibility with advancing maturity is much greater for grasses than for legumes, especially in tropical locations. In addition, some legumes can contain secondary compounds that decrease  $\text{CH}_4$  production (refer to section “Tannins and Saponins”). Rate of passage from the rumen, and consequently DMI, can be greater for legumes than grasses, which should theoretically decrease  $\text{CH}_4$  yield. Animal performance is often increased with inclusion of legumes in ruminant diets, which decreases  $\text{CH}_4$  intensity. For example, Johansen et al. (2018) conducted a meta-analysis of temperate forages in dairy cow diets and concluded that, overall, legume-based diets resulted in higher DMI and milk yield than grass-based diets, although not all legumes were equally effective. Arndt et al. (2022) and Vargas et al. (2022), on the other hand, indicated variable and mostly no effects, respectively, of the inclusion of non-tannin-containing legumes in pastures, on different  $\text{CH}_4$  production metrics.

It is difficult to quantify the mitigation effect due to dietary inclusion of legumes because it depends on the quality of the forages being compared, as differences in feed intake and digestibility due to phenological stages can confound results. For temperate forages, a meta-analysis showed no difference in  $\text{CH}_4$  yield between legumes and C3 grasses, whereas in warmer environments, legumes produced 19% less  $\text{CH}_4$  than C3 or C4 grasses (Archimède et al., 2011). Similarly, working with sheep, Hammond et al. (2014) did not find differences between ryegrass and white clover in DMI, digestibility, or any  $\text{CH}_4$  production metric; in their experiment, rumen liquid and solid outflow rates were greater or tended to be greater for ryegrass than for white clover. Inconsistent results for  $\text{CH}_4$  production in experiments comparing ryegrass with white clover were reported in other studies (Hammond et al., 2011, 2013). Kennedy and Charmley (2012) also reported similar Ym values for cattle fed tropical grasses (5.4–7.2%) compared with tropical grass-legume mixtures (5.4–6.5%). However, there was one exception; the legume *Leucaena leucocephala* decreased Ym by 11% when its inclusion rate was doubled (Kennedy and Charmley, 2012). Although increased use of legumes may not consistently decrease  $\text{CH}_4$  production or yield,  $\text{CH}_4$  intensity can decrease if the nutritive value of the diet is improved with increased animal performance.

Perennial legume forages biologically fix N, which reduces the amount of N fertilizer used and associated emissions (Schultze-Kraft et al., 2018). The N fixed by legume forages is still subject to losses and thus contributes to  $\text{N}_2\text{O}$  emissions when their residues decay (Guyader et al., 2016). Perennial legumes can increase soil carbon storage (Little et al., 2017) and help prevent erosion and rehabilitate degraded soils, especially in tropical areas (Schultze-Kraft et al., 2018). Methane emissions from dairy manure slurry were less for alfalfa than for corn silage (Massé et al., 2016), although ammonia and  $\text{N}_2\text{O}$  emissions can be greater if CP intake of animals is increased by feeding legumes (Rotz et al., 2010). The high CP concentration of legumes can decrease the use of purchased supplements and associated emissions (Schultze-Kraft et al., 2018). Therefore, the net effect of increased use of perennial legumes is complex and regionally specific, and LCA need to be conducted before recommendations can be made. Further research is needed to assess impacts on animal and forage productivity under different management systems to identify optimum legume inclusion levels that minimize emission intensity in different regions.

### High-Starch Forages

Use of high-starch forages such as corn silage and small-grain cereals (e.g., barley, oat, triticale, and wheat in temperate locations; sorghum in semi-arid, warmer climates) can increase starch and decrease fiber concentration of diets. The resulting rumen fermentation promotes propionate production, which competes with methanogenesis for metabolic hydrogen and can also lower rumen pH and inhibit methanogens. With some diets, incorporating high-starch forages increases digestible energy intake of animals and enhances animal performance, thereby decreasing  $\text{CH}_4$  intensity. A meta-analysis for corn silage diets indicated that milk yield per tonne of DM was positively correlated with starch concentration ( $r = 0.65$ ) and NDF digestibility ( $r = 0.49$ ) and negatively correlated with NDF concentration ( $r = -0.72$ ; García-Chávez et al., 2020). In addition to decreased  $\text{CH}_4$  intensity,  $\text{CH}_4$  yield decreased up to 15% for diets containing corn silage compared with other forages (Hassanat et al., 2013; Gislón et al., 2020).

Rotz et al. (2010) reported that increasing the ratio of corn silage to alfalfa silage in dairy cow diets resulted in N being used more efficiently, which resulted in a small decrease in excreted N and in emissions of  $\text{N}_2\text{O}$ . Uddin et al. (2021) reported a 2.5% decrease in  $\text{CO}_2\text{e}$  per kilogram of fat- and protein-corrected milk for corn silage compared with alfalfa silage in the diet of lactating dairy cows. Little et al. (2017) showed that,



although replacing alfalfa silage with corn silage in the diet of lactating dairy cows lowered enteric CH<sub>4</sub> yield by 10%, differences in CO<sub>2</sub>e emission intensity between the 2 forage systems were minimal when soil carbon was accounted for. Thus, feeding high-starch forages to reduce enteric CH<sub>4</sub> emissions is not recommended unless substantiated by an LCA that includes soil carbon changes, an area of knowledge that is currently evolving. The greatest potential for high-starch forages to reduce total GHG emissions may take place when replacing another annual forage crop.

### High-Sugar Grasses

High-sugar cultivars of perennial ryegrass (*Lolium perenne* L.) have elevated water-soluble carbohydrate (WSC) concentrations (250 to 350 g/kg of DM; Rivero et al., 2020), mainly at the expense of CP and, in some cases, NDF concentration. The greater concentration of readily available carbohydrates decreases the acetate-to-propionate ratio in the rumen (Rivero et al., 2020). In vitro studies report less CH<sub>4</sub> production for high-versus low-sugar grasses (Lovett et al., 2006). Using modeling approaches, Ellis et al. (2012) estimated that an increase in WSC concentration of 40 g/kg of DM or more may be required to decrease in vivo CH<sub>4</sub> yield, and the mitigation potential also depends on concomitant changes in CP and NDF concentration and digestibility. Zhao et al. (2016) fed fresh perennial ryegrass to sheep and reported moderate inverse correlations ( $r = -0.44$  to  $-0.54$ ) between WSC concentration and various expressions of CH<sub>4</sub> production. A meta-analysis of 27 in vivo experiments found that for every 10 g/kg (DM) increase in WSC content, CH<sub>4</sub> yield decreased by 0.311 g/kg of DMI (Vera and Ungerfeld, 2022). The CH<sub>4</sub> mitigation potential of high-sugar grasses appears to be reduced when the forage is conserved as hay or silage (Staerfl et al., 2012).

An inverse relationship was reported between CP and WSC content across 195 samples of perennial ryegrass genotypes including conventional and high-WSC cultivars from 49 studies (N. Vera, Instituto de Investigaciones Agropecuarias, Vilcún, Chile; personal communication). A lower ratio of CP to WSC in high-WSC grasses improves rumen microbial protein synthesis, with less ammonia-N absorbed and excreted as urea in urine (Foskolos and Moorby, 2017), potentially resulting in lower ammonia and N<sub>2</sub>O emissions. Importantly, even if CP and WSC are genetically negatively related across conventional and high-WSC perennial ryegrass cultivars, lush swards generally have elevated contents of both CP and WSC because of their vegetative phenological stage, compared with more mature swards of the same cultivar. An LCA of milk production indicated

that CO<sub>2</sub>e per kilogram of milk was 3% less when dairy cows were fed high-sugar versus conventional ryegrass pastures (Soteriades et al., 2018).

A mechanistic model developed by Ellis et al. (2012) predicted a 3.3% average increase in DMI with increased WSC concentration (+39 g/kg of DM) of grass, leading to increased milk yield. However, a meta-analysis indicated that feeding dairy cattle high-sugar grasses did not increase milk production although urinary N excretion was decreased by 26% (Foskolos and Moorby, 2017). An aspect to be considered is that the lower CP concentration of high-sugar grasses may negatively affect productivity of high-producing ruminants if protein requirements are not met (Staerfl et al., 2012), and would require balancing for dietary protein. An LCA of diet changes may be required, also considering the forage yield of the different cultivars. Most of the research on high-sugar grass cultivars has been limited to the United Kingdom, Netherlands, and New Zealand. In vivo studies are needed to quantify the effects of high-sugar grasses on CH<sub>4</sub> production, forage crop yields, and animal performance in various production systems. The effect of selection for the high-sugar trait on fungal diseases and insect attack also requires further assessment.

### Pastures and Grazing Management

Grazing systems vary with climate, plant species, soil types, and livestock, and include season-long continuous grazing, rest-rotation grazing, deferred rotational grazing, and intensively managed grazing. These systems manage pastures to provide forage resources for animals, attempting to balance livestock nutritional requirements with herbage availability and quality while promoting rapid pasture regrowth and long-term pasture resilience. Grazing management can enhance herbage quantity and quality, leading to increased animal production per hectare (Congio et al., 2018; Savian et al., 2018), with increased soil carbon stocks and decreased CH<sub>4</sub> intensity (Guyader et al., 2016). Some pasture species also contain phytochemicals such as condensed and hydrolyzable tannins and saponins that may reduce enteric CH<sub>4</sub> production (MacAdam and Villalba, 2015; Kozłowska et al., 2020). In addition to traditional pasture-based systems, silvopastoral systems that incorporate trees and shrubs in pastures increase the amount of biomass per unit of area and provide other ecosystem services. Silvopastoral systems promote sustainable intensification of land, potentially increasing biodiversity, water use efficiency and biomass production, while promoting animal welfare by providing shade to alleviate heat stress (Mauricio et al., 2019).

Grazing management for CH<sub>4</sub> mitigation considers pregrazing and postgrazing sward height and biomass to maximize herbage nutritional quality (Muñoz et al., 2016; Congio et al., 2018; Savian et al., 2018). Grazing management can lower enteric CH<sub>4</sub> intensity, but total CH<sub>4</sub> production has not changed in most studies (Arndt et al., 2022; Vargas et al., 2022), and it may increase if DMI is increased. In turn, changes in CH<sub>4</sub> production per unit of grazing area depend on changes in CH<sub>4</sub> production per animal and stocking rate. The extent to which grazing management lowers CH<sub>4</sub> intensity is extremely variable depending upon the production system and local conditions. For example, rotational grazing based on sward pre- and postgrazing heights increased digestible OM intake of sheep grazing Italian ryegrass, reducing CH<sub>4</sub> intensity by 63% and CH<sub>4</sub> production per hectare by 39%, although CH<sub>4</sub> production per animal was increased by 12% (Savian et al., 2018). For dairy cattle, optimizing grazing management improved milk production efficiency by 51%, while decreasing CH<sub>4</sub> intensity by 20% and CH<sub>4</sub> yield by 18%, although CH<sub>4</sub> emissions per hectare increased by 29% (Congio et al., 2018). Dairy cows grazing swards differing in pregrazing herbal mass had similar total CH<sub>4</sub> production, but the increase in DMI and milk yield with low herbage mass (lower in NDF concentration) resulted in 10% less CH<sub>4</sub> yield (Muñoz et al., 2016). For beef cattle, CH<sub>4</sub> production was greater for light versus heavy continuous grazing because plants were at a more advanced stage of maturity, but the additional CH<sub>4</sub> was more than offset by greater soil carbon sequestration (Alemu et al., 2017). Therefore, optimum grazing management needs to consider the productivity of animals as well as pastures and soil, and LCA is needed to account for all GHG emissions and removals (changes in soil carbon), and other ecosystem services provided by grassland ecosystems also need to be considered.

Implementation of improved pasture management by farmers can be hindered by additional costs (e.g., fences, water troughs, moving cattle, tree plantation) and lack of long-term, regionally relevant research. Extension services supported by public policies (e.g., payment for environmental services) may be needed to encourage adoption.

### Forage Preservation and Processing

Ensiling forage causes losses in DM and changes in nutritive value, but good management practices can be used to ensure excellent quality silage. Thus, the effect of ensiling forage on CH<sub>4</sub> production is expected to be highly variable depending upon the resulting forage quality and ensiling practices. Few *in vivo* studies have examined the direct effects of forage preservation

method on CH<sub>4</sub> production (Knapp et al., 2014). The impact of preservation methods on CH<sub>4</sub> emissions is mainly due to effects on animal performance, which affects CH<sub>4</sub> intensity (Evans, 2018).

Processing of forage by grinding and pelleting reduces particle size, which increases ruminal passage rate, decreases OM degradation in the rumen, and shifts fermentation toward propionate production with less CH<sub>4</sub> production. Johnson et al. (1996) reported a 20 to 40% decrease in CH<sub>4</sub> yield when forage was ground or pelleted compared with feeding long forage. However, total CH<sub>4</sub> production is likely not decreased or is even increased (Arndt et al., 2022), by forage processing in animals fed *ad libitum* due to increased DMI, especially for low-quality forages (Hironaka et al., 1996). A faster ruminal passage rate can also result in a reduction in forage digestibility if structural carbohydrates are not digested in the lower tract.

Forage preservation and processing increase the use of fuel for machinery and associated emissions compared with grazing fresh herbage. Moreover, reduced NDF digestibility due to processing can lead to increased manure emissions of CH<sub>4</sub> (Knapp et al., 2014), depending on how the manure is managed. Before recommending a change in forage preservation or processing for CH<sub>4</sub> mitigation, additional inputs required, effects on animal productivity, and whole-farm GHG emissions need to be considered.

## ACTION ON RUMEN FERMENTATION

### Ionophores

Ionophores are polyether compounds that increase permeability of cell membranes to ions in gram-positive bacteria and protozoa, resulting in retarded growth and death. Most targeted microorganisms produce H<sub>2</sub> and, in this way, ionophores decrease the availability of H<sub>2</sub> for methanogenesis, and shift fermentation from acetate to propionate (Duffield et al., 2008a). Reports on microbial adaptation to ionophores are conflicting (Appuhamy et al., 2013).

Monensin is routinely used in feedlot cattle production in many countries, but its effects on CH<sub>4</sub> production are generally small. The meta-analysis by Appuhamy et al. (2013) reported average decreases in total CH<sub>4</sub> production of between 3.6 and 10.7% in dairy cows and beef steers, respectively. Additionally, monensin improves feed conversion efficiency (Duffield et al., 2008b), which decreases GHG emissions from feed production needed to sustain animal production.

Monensin inclusion in manure at concentrations resulting from recommended inclusion in dairy cow diets did not affect manure CH<sub>4</sub> production (Arikan et al.,

2018). Monensin decreases the concentration of rumen ammonium, but there are contradictory results about its effects on N metabolism and release to the environment (Duffield et al., 2008a). Increases in emissions associated with manufacturing and transport of ionophores are small because they are typically included in the diet at concentrations of 50 mg/kg of DM or less. Use of monensin in beef and dairy animals is permitted in some countries and prohibited in others. Adoption is favored by intensive systems where animals are fed or supplemented daily, but slow-release forms, suitable for use in grazing cattle, are commercially available. It has been questioned whether widespread usage of monensin can contribute to antibiotic resistance, but these antimicrobials are presently not used in human medicine. Overall, ionophores can help achieve minor mitigation of enteric CH<sub>4</sub> production and intensity of ruminant products, and they have favorable effects on animal productivity. The use of ionophores in ruminant diets is already approved in many regions of the world, but with growing concerns over antimicrobial resistance, their use may become more limited in the future.

### 3-Nitrooxypropanol

3-Nitrooxypropanol is a molecule that when included in small doses (60 to 200 mg/kg of DMI) in ruminant feeds inhibits CH<sub>4</sub> production in the rumen. Chemical inhibitors of methanogens have been studied in vitro and in vivo since the 1960s. Research on some compounds was abandoned because of risks of toxicity, passage to animal products, volatility, or transient in vivo effects. 3-Nitrooxypropanol for CH<sub>4</sub> mitigation was patented in 2012 (Duval and Kindermann, 2012) and has been comprehensively investigated in silico, in pure enzyme-substrate systems, in in vitro pure and mixed cultures (Duin et al., 2016), and in vivo (e.g., Yu et al., 2021). 3-Nitrooxypropanol targets methyl-coenzyme M reductase, which catalyzes the last step of methanogenesis in methanogenic archaea. Its mechanism of action is established, as are the products resulting from its metabolism in the rumen (Duin et al., 2016).

On average and at typical inclusion levels in beef ( $144 \pm 82.3$  mg/kg of DM; mean  $\pm$  SD) and dairy ( $81 \pm 41.2$  mg/kg of DM; mean  $\pm$  SD) diets, 3-NOP decreases CH<sub>4</sub> production by 30% (Dijkstra et al., 2018; Kim et al., 2020), although decreases of 80% or greater have been obtained in some studies with high-concentrate diets (Yu et al., 2021). The effect of 3-NOP on CH<sub>4</sub> production is related to its level of inclusion in the diet (Yu et al., 2021) and is negatively affected by dietary concentration of NDF (Dijkstra et al., 2018; Yu et al., 2021).

Long-term in vivo inhibition of enteric CH<sub>4</sub> production by 3-NOP was initially reported by Hristov et al. (2015) and has since been confirmed in various studies (Yu et al., 2021). Although in most long-term studies, 3-NOP effectiveness has remained constant, a couple of studies have shown that 3-NOP effectiveness declined slightly over time, which might be related to the low dose used (Yu et al., 2021).

In the meta-analysis by Jayanegara et al. (2018), 3-NOP did not affect DMI of dairy and beef cattle, whereas the later meta-analysis by Kim et al. (2020) reported a decrease in DMI with 3-NOP in beef but not dairy animals. Both meta-analyses found increases or tendencies to increase milk fat and protein percentages with 3-NOP supplementation, although Kim et al. (2020) reported a decrease in milk yield with 3-NOP. Feed conversion efficiency has been shown to be improved in beef cattle supplemented with 3-NOP (Jayanegara et al., 2018). Digestibility of various dietary fractions was unaffected (Jayanegara et al., 2018) or improved (Kim et al., 2020), the latter of which might be due to decreased DMI (Illius and Allen, 1994). Heterogeneity among studies or the interactions between the experiment effect and the effect of 3-NOP supplementation were not reported by Jayanegara et al. (2018) or Kim et al. (2020), so it was not determined whether the observed effects were consistent across studies.

Because the recommended dietary concentration of 3-NOP is very low, CO<sub>2</sub> emissions associated with its manufacture and transport are also very low. Based on CO<sub>2</sub> emissions from 3-NOP production (Feng and Kebreab, 2020) and the average DMI, CH<sub>4</sub> production, and 3-NOP dose, it is estimated that the additional emissions of GHG associated with manufacturing and transporting 3-NOP would represent between 1.8 and 5.3% of the decrease in CH<sub>4</sub> emissions that it would elicit (calculations not shown). No effects on manure emissions of GHG as a consequence of feeding 3-NOP were observed by Nkemka et al. (2019) and Owens et al. (2020), although Weber et al. (2021) found soil-dependent effects. Hence, the effects of 3-NOP on manure emissions need further examination.

Chemical inhibitors can be easily combined with other mitigation strategies. Their adoption requires them to pass safety tests for animals, consumers, and the environment. In the animal, 3-NOP carbon is largely metabolized to CO<sub>2</sub>, carbohydrates, fatty acids, and amino acids, with less than 5% of the original compound excreted in urine (Thiel et al., 2019a). Mutagenic and genotoxic potential were not found (Thiel et al., 2019b). Chemical inhibitors of methanogenesis need approval by government agencies, which has been recently granted for 3-NOP in Brazil, Chile, and the

European Union, and is under consideration in other countries (Yu et al., 2021).

Research on the discovery of new chemical inhibitors is ongoing (Henderson et al., 2018). The greatest hurdles for the widespread adoption of 3-NOP or other chemical inhibitors that may be discovered in the future are the additional feeding cost from their inclusion in animal diets, if no consistent benefits in productivity are obtained, and the difficulty of delivering the required dose to grazing ruminants in extensive production systems in a format that works over extended periods (Hegarty et al., 2021; Ungerfeld et al., 2022).

### Macroalgae

Macroalgae (seaweeds) have highly variable chemical composition, depending upon species, time of collection, and growth environment, and they can contain bioactive components that inhibit methanogenesis. Red seaweeds such as *Asparagopsis taxiformis* and *Asparagopsis armata* accumulate halogenated compounds, of which bromoform is the most abundant (Machado et al., 2016). Methane halogenated analogs react with vitamin B<sub>12</sub> to block the last step of methanogenesis in methanogenic archaea (Wood et al., 1968). Other seaweeds contain polysaccharides, proteins, peptides, bacteriocins (produced by surface-associated bacteria), lipids, phlorotannins, saponins, and alkaloids that are known to decrease CH<sub>4</sub> production by suppressing archaea and protozoa, and in some cases cause an undesirable decrease in nutrient degradability (Abbott et al., 2020).

In vivo studies with sheep, steers, and dairy cows reported dose- and diet-dependent decreases between 9 and 98% of CH<sub>4</sub> production by supplementing *Asparagopsis* to the diet (Li et al., 2018; Roque et al., 2019a, 2021; Kinley et al., 2020; Stefenoni et al., 2021). A substantial decrease in CH<sub>4</sub> yield for cattle was confirmed in a meta-analysis (Lean et al., 2021). Efficacy of *Asparagopsis* for CH<sub>4</sub> mitigation depends on its concentration of bromoform, which ranges from 3.0 to 51.0 mg/kg of DMI (Kinley et al., 2020; Roque et al., 2019a, 2021; Stefenoni et al., 2021). Additionally, *Asparagopsis* may be more effective at decreasing CH<sub>4</sub> production with high-concentrate than with high-forage diets (Roque et al., 2021). There are preliminary concerns about *Asparagopsis* losing effect in the long term (Hristov et al., 2022). Studies on the efficacy of other seaweeds on CH<sub>4</sub> production are mostly limited to in vitro conditions (Abbott et al., 2020), although interest is growing.

Dietary supplementation with *Asparagopsis* reduced feed intake in a dose-dependent manner in most but not all experiments. A meta-analysis of experiments

supplementing *Asparagopsis* or brown algae reported no effects on DMI or ADG and, depending on the estimation method, a significant or numerical decrease in the feed to body mass gain ratio and increase in milk yield (Lean et al., 2021). *Asparagopsis* supplementation was reported to increase feed efficiency in some small-scale beef studies (Kinley et al., 2020; Roque et al., 2021). There were no effects of *Asparagopsis* inclusion in the diet on carcass quality, meat quality, or taste (Kinley et al., 2020; Roque et al., 2021), although a possible decrease in beef shelf life was reported at a high dose of *Asparagopsis* inclusion (Bolkenov et al., 2021). The effect of *Asparagopsis* inclusion on manure emissions is unknown.

Long-term oral exposure of animals to high concentrations of bromoform can cause liver and intestinal tumors; hence, the EPA (2000) classified the compound in Group B2: probable human carcinogen. Within the dietary concentrations used (<0.5% of seaweed/DMI), bromoform residues were not detected in milk, meat, fat, organs, or feces from sheep and beef or dairy cattle fed *Asparagopsis* (Li et al., 2018; Kinley et al., 2020; Roque et al., 2019a, 2021). In contrast, Muizelaar et al. (2021), with no control animals in their study, reported passage of bromoform to milk in nonadapted dairy cows. However, bromoform was not detected in milk after 10 d of continuously feeding *Asparagopsis* at any level of supplementation, raising the possibility that microbial adaptation may play a role in reducing the flow of bromoform into milk. In an in vitro study, bromoform was degraded within 12 h of incubation, with dibromomethane as the main degradation product (Romero et al., 2022). Accumulation of iodine and bromide in milk (Stefenoni et al., 2021) and iodine in meat (Roque et al., 2021) has been reported in animals fed *Asparagopsis*. Assuming a milk iodine concentration of 3 mg/L, as reported by Stefenoni et al. (2021) for cows supplemented *Asparagopsis*, Lean et al. (2021) estimated an iodine consumption 15-fold greater than the maximum tolerable for children younger than 3 yr drinking 1 L/d of milk. Further residue and safety studies are needed, including effects on organ histology of treated animals (Glasson et al., 2022). For other seaweeds, potential toxicity and residues in meat and milk will depend on the content of toxic minerals and the level of inclusion of seaweeds in the diet.

The GHG emissions of growing, harvesting, processing (drying), storing, and transporting seaweeds on a large scale need to be considered in an LCA to determine the net effect on GHG intensity of meat and milk production. There is also potential to purify or extract seaweed bioactives, which would decrease emissions related to drying and transportation. The potential global depletion of stratospheric ozone was

estimated to be relatively small for Australian *Asparagopsis* growth conditions (Jia et al., 2022), but impacts on aquatic biodiversity would need to be considered if *Asparagopsis* were harvested directly from the ocean. On the other hand, seaweed cultivation can result in net CO<sub>2</sub> fixation and export part of the carbon to the deep sea, where it can be buried in sediments (Duarte et al., 2017). Ridoutt et al. (2022) estimated that inclusion of *Asparagopsis* in Australian feedlot diets could substantially contribute to decrease net emissions of GHG from the feedlot sector in that economy.

Consequently, adoption of *Asparagopsis* depends on the ability to sustainably grow the algae in aquaculture or marine systems with consistent concentration of the active compounds, which need to be maintained throughout transporting, handling, storage, and animal feeding. Concentrations of minerals such as iodine need to be controlled so that transfer to animal products does not exceed safe limits. In addition, the feeding of *Asparagopsis* may need to be approved by regulatory bodies before widespread adoption. Inclusion of other seaweeds in ruminant diets may be acceptable to consumers if there is no risk of toxicity and no off-flavors in meat or milk. More in vivo research is needed to determine CH<sub>4</sub> mitigation and productivity changes under different diet and management conditions for both bromoform-containing and other seaweeds (Lean et al., 2021). Use of macroalgae as an antimethanogenic strategy may be feasible in confined and mixed systems, but it is likely to be challenging to implement in extensive systems. Animal delivery mechanisms that do not reduce the efficacy of the bioactive compounds of macroalgae need to be designed for supplementing animals in extensive systems.

### Alternative Electron Acceptors

Alternative electron acceptors are organic (e.g., fumarate, malate) and inorganic (e.g., nitrate, sulfate) compounds that draw electrons away from methanogenesis and incorporate them into alternative pathways. Organic electron acceptors are rumen fermentation intermediates that are metabolized to VFA (mainly propionate), which can be absorbed and used by the ruminant host (Carro and Ungerfeld, 2015). When completely reduced to ammonium, nitrate is incorporated into microbial protein, and also absorbed through the rumen wall and converted into urea in the liver and kidneys (Yang et al., 2016). Sulfate is reduced to hydrogen sulfide (dissimilatory reduction) and expelled, and is also incorporated into the synthesis of microbial sulfur amino acids (assimilatory reduction; Drewnoski et al., 2014).

In general, in vivo effects of malate and fumarate on enteric CH<sub>4</sub> production range from no effects in some studies to mild or moderate effects (10 to 23%) in others (Carro and Ungerfeld, 2015). The average decrease in CH<sub>4</sub> production in 56 treatment means from 24 studies in which nitrate was supplemented was estimated to be 13.9% at the mean nitrate dose (16.7 g/kg of DM), with greater efficacy in dairy than in beef cattle, in which the difference might be caused by the encapsulated nitrate used in most beef studies (Feng et al., 2020). The meta-analysis by Arndt et al. (2022) found that the inclusion of fumarate and nitrate as organic and inorganic electron acceptors, respectively, decreased total CH<sub>4</sub> production by 16 and 17%, respectively. Nitrate supplementation decreased CH<sub>4</sub> intensity for growth and milk production by 12 and 15%, respectively, as well as causing a slight decrease in DMI of 3% without affecting animal production. Fumarate supplementation did not affect CH<sub>4</sub> intensity of milk production, and there was no information on its effect on CH<sub>4</sub> intensity of body mass gain.

Nitrate can be partially converted to N<sub>2</sub>O in the rumen and expelled (Petersen et al., 2015). Nitrous oxide is a very potent GHG; hence, supplementation with nitrate to mitigate CH<sub>4</sub> emissions can have effects on the emissions of other GHG. Furthermore, if nitrate is supplemented to an N-sufficient diet, the extra N will be voided to the environment and increase N<sub>2</sub>O emissions to the atmosphere and contaminate ground water with nitrate. Nitrate supplementation does not benefit animal productivity unless added to an N-deficient diet (Yang et al., 2016), as is often the case in tropical and subtropical regions. In that regard, Nguyen et al. (2016) reported an improvement with nitrate supplementation in DMI and ADG of lambs fed N-deficient chaff.

Nitrite is an intermediate in the reduction of nitrate that can be absorbed through the rumen wall and react with hemoglobin to form methemoglobin, which cannot transport oxygen. This condition can be fatal, although it is possible to gradually adapt the rumen to nitrate supplementation (Lee and Beauchemin, 2014; Yang et al., 2016). Traces of nitrate can pass to milk (Guyader et al., 2016) and tissues (Doreau et al., 2018) but are considered safe for consumers. Nitrate feeding to animals is not approved in North America (Beauchemin et al., 2020), but carbon credits can be obtained by feeding nitrate to beef in Australia (<https://www.legislation.gov.au/Details/F2015C00580>). Due to the risks of acute toxicity, nitrate supplementation can only be recommended in production systems where feed intake is closely managed.

The use of nitrate (e.g., calcium nitrate) as a source of nonprotein N is usually more expensive than urea

(Callaghan et al., 2014). At present, the adoption of nitrate as an antimethanogenic strategy might be feasible in some cases but is mostly dependent on carbon market pricing, mitigation of ammonia and N<sub>2</sub>O emissions from manure, and the availability of safe in-feed delivery procedures.

There are few studies of nitrate supplementation to grazing animals, with most being in mixed systems with nitrate offered mixed with substantial amounts of concentrates (van Wyngaard et al., 2018, 2019; Granja-Salcedo et al., 2019). Supplementing nitrate in a molasses lick block to grazing beef cows resulted in lower and more variable intake of the nitrate N supplement than urea blocks, resulting in lower free conceptus mass and BCS (Callaghan et al., 2021). High-nitrate-containing forages have mostly been investigated from the perspective of them causing toxicity, but there may also be merit in exploring their ability to lower enteric CH<sub>4</sub> emissions in temperate climates.

In general, the effects of fumarate and malate on animal productivity have been inconsistent. Malate stimulates *Selenomonas ruminantium* and can help prevent lactate acidosis by promoting lactate metabolism (Carro and Ungerfeld, 2015). Fumarate and malate are natural intermediates of rumen fermentation regarded as safe and registered as feed ingredients in the European Union and the United States (Carro and Ungerfeld, 2015). Feeding fumarate and malate to ruminants is largely limited by cost because of the relatively high levels of inclusion needed and the relatively small effects on CH<sub>4</sub>.

### Essential Oils

Essential oils are complex mixtures of volatile lipophilic secondary metabolites that are responsible for a plant's characteristic flavor and fragrance (Benchaar and Greathead, 2011). When extracted and concentrated, or chemically synthesized, essential oils may exert antimicrobial activities against bacteria and fungi (Chao et al., 2000). Chemically, essential oils are variable mixtures of principally terpenoids, and a variety of low-molecular-weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, and, exceptionally, N- and S-containing compounds, coumarins, and homologs of phenylpropanoids (Dorman and Deans, 2000).

Most essential oils exert their antimicrobial activities by interacting with processes associated with the bacterial cell membrane, including electron transport, ion gradients, protein translocation, phosphorylation, and other enzyme-dependent reactions (Dorman and Deans, 2000). Gram-positive bacteria appear to be more susceptible to the antibacterial properties of essential oils

than gram-negative bacteria. However, phenolic compounds [e.g., thymol and carvacrol contained in some essential oils (e.g., thyme and oregano)] can inhibit the growth of gram-negative bacteria by disrupting the outer cell membrane (Helander et al., 1998). Rumen gram-positive bacteria are involved in fermentation processes coupled with the production of CH<sub>4</sub> through the release of H<sub>2</sub> (Owens and Goetsch, 1988).

Essential oils such as oregano, thyme, garlic oil, and its derivatives have been shown to decrease CH<sub>4</sub> production in vitro (Cobellis et al., 2016) but results from in vivo studies have been far less conclusive (Benchaar and Greathead, 2011; Hristov et al., 2022). Commercial products containing various essential oils have been shown to decrease CH<sub>4</sub> production in limited in vivo studies. For instance, a commercial product of oregano oil (Orego Stim, Meriden Animal Health Ltd.) and a green tea extract, and their mixture, fed to lactating dairy cows did not affect total CH<sub>4</sub> production or CH<sub>4</sub> intensity but reduced CH<sub>4</sub> yield by between 16 and 22% (Kolling et al., 2018). Feeding 15 g/d of a commercial product containing citrus extract and allicin from garlic (Mootral GmbH) to feedlot steers reduced enteric CH<sub>4</sub> yield by 23% only after 12 wk of supplementation, but the persistency of the decrease thereafter was not determined (Roque et al., 2019b). A 9% decrease in absolute CH<sub>4</sub> production in long-term studies was reported in a meta-analysis for a mixture of coriander, eugenol, geranyl acetate, and geraniol (Agolin Ruminant; Agolin SA) when fed to dairy cows at the rate of 1 g/d (Belanche et al., 2020).

Some essential oils and their compounds have been reported to reduce dietary protein degradation in vitro but in vivo studies have been inconsistent (Cobellis et al., 2016). If this decrease is accompanied by a reduction in urinary N excretion, potential reductions in N<sub>2</sub>O and ammonia may occur. In general, feeding most essential oils to ruminants does not negatively affect animal productivity or product (milk, meat) quality (Benchaar et al., 2009); in some cases, improvements have been reported (Belanche et al., 2020). However, adverse effects of some essential oils on feed digestion have also been reported (Benchaar et al., 2009; Cobellis et al., 2016). There is potential for transfer of essential oils to animal products, which could affect the organoleptic properties of meat and milk. If feed digestion is depressed, the amount and composition of manure excreted and associated emissions could also increase.

At doses generally recommended, the probability of essential oils being toxic to animals is low. However, caution should be taken especially if essential oils are fed at high doses. Some essential oil compounds such as estragole and methyl eugenol have exhibited genotoxic properties (Burt, 2004) and may be potentially irritat-

ing and cause allergic dermatitis during handling. The sulfur-containing compounds in garlic have been shown to be responsible for hemotoxic effects in beef cattle (Rae, 1999). Organo-sulfur compounds from garlic are known to cause cell damage (Amagase, 2006).

Essential oils have a wide spectrum of antimicrobial activity, which makes it difficult to target specific microbial groups so as to avoid adverse effects on feed digestion in the rumen. The challenge remains to identify essential oils with a fairly consistent composition that selectively inhibit rumen methanogenesis, with lasting effects and without depressing feed digestion and animal productivity. Because essential oils are highly volatile, most commercial products are coated and formulated to control the release of the active ingredient once added to the animal's diet. However, some essential oils are unstable and their efficacy may also be affected by improper storage or exposure to heat during feed manufacturing processes such as extrusion or pelleting.

There is a need to conduct more in vivo studies to determine the efficacy of essential oils. The range of essential oils available is extensive (>3,000) and more work is needed to identify the most effective ones to reduce enteric CH<sub>4</sub> production. Many of the concentrations that have shown effects in vitro are too high for in vivo applications; thus, more research is warranted to determine optimal doses under various dietary conditions. Furthermore, the favorable effects obtained in vitro are not as marked as those in vivo, potentially due to microbial adaptation. Consequently, further long-term animal studies are needed to investigate the extent to which microbes adapt to these substances. Also, more work is required to assess the transfer of essential oils into animal products and the potential effect on meat and milk quality. Opportunities exist to combine with other mitigation strategies with different or similar mechanisms of action.

### Tannins and Saponins

Several secondary plant compounds including tannins and saponins have been evaluated for their potential to decrease CH<sub>4</sub> production from ruminants (Jayanegara et al., 2012; Kozłowska et al., 2020). Tannins are polyphenolic plant compounds with affinity to bind to proteins and other compounds. They are classified as either condensed or hydrolyzable, and both types of tannins have been shown to exert antimethanogenic effects directly by inhibiting some methanogens and indirectly by decreasing numbers of protozoa, which symbiotically host methanogens (Aboagye and Beauchemin, 2019). A portion of the decrease in CH<sub>4</sub> can also be due to

a decline in DMI and nutrient digestibility, which can undesirably affect animal production.

There is a growing body of literature indicating that tannins decrease CH<sub>4</sub> production, albeit with highly variable responses depending upon the source, type, and molecular weight of the tannins and the methanogenic community present in the animal (Aboagye and Beauchemin, 2019). A meta-analysis of in vivo studies indicated a linear decrease in CH<sub>4</sub> yield of 3.53% ( $R^2 = 0.47$ ) with each 10 g/kg DM addition. However, the decrease in CH<sub>4</sub> yield was accompanied by a decrease in OM digestibility of 2.6% per 10 g/kg DM addition. Even though part of the CH<sub>4</sub> decrease effect by tannins could be ascribed to less OM being digested, CH<sub>4</sub> production on a digestible OM basis also declined (Jayanegara et al., 2012). Low-molecular-weight hydrolyzable tannins (and their subunit gallic acid) appear to be more effective than high-molecular-weight condensed tannins at decreasing CH<sub>4</sub> emissions without adversely affecting digestibility (Aboagye and Beauchemin, 2019). However, hydrolyzable tannins are susceptible to microbial hydrolysis in the gut, producing metabolites that may elicit toxic effects to the animal after absorption (McSweeney et al., 2001). Thus, care must be exercised when feeding high (i.e., >30 g/kg of DM) concentrations of tannins, especially hydrolyzable tannins.

Most tannin-containing legumes grown in temperate locations [e.g., sainfoin (*Onobrychis viciifolia*), birds-foot trefoil (*Lotus corniculatus*), and cicer milk vetch (*Astragalus cicer*)] contain relatively low concentrations (<20 to 50 g/kg of DM) of condensed tannins, thus CH<sub>4</sub> reductions are relatively small in contrast to tropical legumes and shrubs that contain greater concentrations of tannins. Consequently, there is interest in supplementing the diets of nongrazing animals with concentrated extracts from tannin-containing shrubs and trees [e.g., *Acacia mearnsii*, chestnut (*Castanea* spp.) and quebracho (*Schinopsis balansae*); Aboagye and Beauchemin, 2019].

Replacing grass hay (*Cynodon* spp.) with tropical nonconventional shrubs (*Orbignya phalerata*, *Combretum leprosum*) in the diet of sheep reduced the relative abundance of archaea methanogens when the condensed tannin content of the diet increased from 0.1 to 28.5 g/kg of DM (Abdalla Filho et al., 2017). Supplementing sheep with *Acacia mearnsii* bark (condensed tannin content: 279 g/kg of DM) did not affect CH<sub>4</sub> emission (Lima et al., 2019). Replacing 75% corn grain with low-tannin sorghum (*Sorghum bicolor*; condensed tannin: 6.1 g/kg of DM) maximized ruminal microbial biomass production and lamb growth and reduced CH<sub>4</sub> emission intensity (316 vs. 209 L/kg of ADG; Soltan et al., 2021).

Another important environmental benefit of dietary tannins is their ability to improve N utilization in ruminants (Jayanegara et al., 2012). Tannins bind dietary proteins in the gastrointestinal tract, which can improve N utilization, decrease urinary N losses, and decrease ammonia and N<sub>2</sub>O emissions from excreta (Hristov et al., 2022). Consequently, an LCA approach needs to be used when evaluating tannins as a GHG mitigation strategy. It should also be considered that dietary tannins can decrease nutrient digestibility, especially of proteins. This can be particularly important in grazing animals in the tropics and subtropics, where pastures are often deficient in N (Waghorn, 2008).

Although there is substantial evidence that tannins reduce enteric CH<sub>4</sub> production, more animal feeding studies are required to identify the optimum concentrations of different sources of condensed and hydrolyzable tannins. Furthermore, effects on DMI, digestibility, animal performance and health (e.g., preventing bloat and controlling parasites), and products (e.g., effects on rumen biohydrogenation of fatty acids; Waghorn, 2008; Patra and Saxena, 2011; Tedeschi et al., 2014; Aboagye and Beauchemin, 2019) also need to be considered. There is a paucity of information available on whether combining tannins with other mitigation strategies produces additive effects on mitigation.

Saponins are structurally diverse molecules that are divided into 2 groups: triterpene and steroid glycosides, with the major sources of saponins included in ruminant diets being from *Yucca schidigera*, *Quillaja saponaria*, *Camellia sinensis*, and *Medicago sativa* (Jayanegara et al., 2014). The antimethanogenic effect of saponins is mainly due to an inhibition of rumen protozoa and associated methanogens, with the resulting promotion of propionate production (Adegbeye et al., 2019). The CH<sub>4</sub>-mitigating effect of saponins is highly variable depending on the source, chemical structure, dose of saponins, diet composition, and likelihood of the rumen microbes adapting to degrade saponins. A meta-analysis of in vitro studies indicated that CH<sub>4</sub> (mL/g of DM) was decreased in a curvilinear manner with increasing dose of saponins (Jayanegara et al., 2014). Many in vitro studies have evaluated relatively high dose rates, but high concentrations of saponins (>5% DM) and steroidal saponins from some plants can be toxic to animals. Extrapolating the in vitro results from Jayanegara et al. (2014) indicates that a 1.32% decrease in CH<sub>4</sub> yield would be expected per 10 g/kg dietary DM addition, but with considerable variation in CH<sub>4</sub> reduction at the low concentrations of saponins that are typically included in ruminant diets. Relatively few in vivo studies have examined the use of saponins for CH<sub>4</sub> mitigation, and the results have been variable. Saponins can exert a small or moderate

antimethanogenic effect at practical feeding rates, but more animal feeding studies are required to identify the optimum concentrations of different saponin sources. Additionally, saponins can improve N utilization efficiency and consequently exert environmental benefits on reducing ammonia and N<sub>2</sub>O emissions from manure (Yurtseven et al., 2018).

### Direct-Fed Microbials

Direct-fed microbials are live microorganisms that when ingested can modify rumen fermentation. They are usually added to diets to improve fiber digestion or enhance lactate utilization to stabilize rumen pH, but have also been explored for their ability to mitigate CH<sub>4</sub>. Direct-fed microbials may redirect metabolic hydrogen into alternative pathways to methanogenesis; for example, lactate, succinate, or propionate production, reductive acetogenesis, or anaerobic respiration (Jeyanathan et al., 2014). Another approach is the use of bacteria that inhibit the growth of methanogens (Jeyanathan et al., 2014). Yeasts and the filamentous fungus *Aspergillus oryzae* have also been studied for their effects on CH<sub>4</sub> production (Jeyanathan et al., 2014).

Effects of yeasts, *A. oryzae*, and lactic acid bacteria on CH<sub>4</sub> production are variable, which might be expected as they have not been selected for their ability to mitigate CH<sub>4</sub> production (Jeyanathan et al., 2014). However, they can promote milk yield (Hristov et al., 2013a) and thus decrease CH<sub>4</sub> intensity. In in vitro cultures, addition of some strains of propionibacteria have caused small decreases in CH<sub>4</sub> production (Alazzeh et al., 2012), whereas some fumarate reducers have resulted in stronger decreases in CH<sub>4</sub> (Kim et al., 2016). Reductive acetogens had minimal or no effects on in vitro rumen fermentation, unless methanogenesis was simultaneously inhibited (Nollet et al., 1997).

Although some coculture and mixed culture experiments have generated proof-of-concept results that direct-fed microbials can reduce CH<sub>4</sub> emissions, these results have seldom been confirmed in vivo. This may be explained by the high doses of direct-fed microbials that can be studied in vitro but are not applicable in vivo. In vivo experiments with propionibacteria fed to steers found no effects (Vyas et al., 2014b, 2016) or a slight decrease in CH<sub>4</sub> yield (Vyas et al., 2014a).

Direct-fed microbials can potentially act synergistically with other strategies such as chemical inhibitors of methanogenesis, as accumulated H<sub>2</sub> resulting from the inhibition of methanogenesis could be incorporated into beneficial pathways, such as reductive acetogenesis (Nollet et al., 1997). Nitrate- and nitrite-reducing bacteria have been added to rumen in vitro cultures



along with nitrate to inhibit CH<sub>4</sub> production and simultaneously enhance nitrate reduction to ammonium, avoiding nitrite accumulation (Jeyanathan et al., 2014). However, this approach only resulted in a numerical decrease in plasma nitrite concentration in sheep (de Raphélis-Soissan et al., 2014).

There is a lack of information about the effects of direct-fed microbials on animal performance in experiments where a decrease in CH<sub>4</sub> production was observed. In the study by Vyas et al. (2014a) in which supplementation of propionibacteria caused a small decrease in CH<sub>4</sub> yield, no effects on animal productivity were observed. Effects on upstream emissions of other GHG associated with growing, storing, and transporting direct-fed microbials are presumably low. Effects on digestibility and manure composition, and emissions of CH<sub>4</sub> and N<sub>2</sub>O from manure are unknown and would have to be studied for each direct-fed microbial.

Many direct-fed microbials are approved for animal feeding and commercially available. Direct-fed microbials have potential for adoption in production systems where animals are supplemented daily, as most of them are unlikely to persist in the rumen, but they may be less promising for extensive production systems. Preparations of direct-fed microbials should maintain viability and be easy and practical to use, store, and transport. Any direct-fed microbial shown to be effective *in vivo* would also require applied research to establish its optimum dose as well as frequency and mode of administration.

## EARLY-STAGE MITIGATION STRATEGIES

The global effort to curb CH<sub>4</sub> emissions is driving significant investment and innovation by the private and public sectors. Recent advances in characterizing the rumen microbiome, genome sequencing of rumen methanogens, and an in-depth analysis of the enzymatic pathways involved in methanogenesis are leading to new CH<sub>4</sub> mitigation approaches (Huws et al., 2018). Some early-stage technologies are discussed below, although the list is incomplete and likely to grow in future years. Most of the research to date has focused on mitigation of CH<sub>4</sub> from ruminants in confinement systems, but technologies to reduce emissions from grazing animals would have the largest effect on reducing emissions from global ruminant livestock.

### *Immunization Against Methanogens*

Development of an antimethanogenic vaccine that stimulates the immune system of animals to produce antibodies against methanogens was initiated over 2 decades ago. However, this approach of mitigating CH<sub>4</sub>

emissions has proven to be challenging. Vaccination against methanogens has been shown to induce the presence of antibodies in serum and saliva and their delivery to the rumen (Subharat et al., 2015; Zhang et al., 2015). Furthermore, the antibodies have been shown to be stable in rumen fluid for a few hours (Subharat et al., 2015, 2016) and to agglutinate methanogens *in vitro* (Wedlock et al., 2010). Although individual steps in the development of vaccines against methanogens have been successful, effects on *in vivo* CH<sub>4</sub> production have so far been small or nonexistent (Wright et al., 2004; Williams et al., 2009; Zhang et al., 2015; for a systematic review, see Baca-González et al., 2020). Results in *in vitro* mixed cultures have been variable and time-dependent (Cook et al., 2008). The lack of effects on CH<sub>4</sub> production and the observed increase in methanogens diversity may be due to a lack of broad-spectrum effectiveness of the vaccine on the rumen methanogenic community (Williams et al., 2009). Furthermore, the between-animal variability and interregional differences in the rumen microbiome make it challenging to develop a widely applicable vaccine. Work in this area has stimulated programs such as the Hungate 1000 and the Global Rumen Census, which have made a considerable contribution to our knowledge of rumen ecology. Despite these technical challenges, an immunization strategy against methanogens would be a very desirable approach to controlling CH<sub>4</sub> emissions, especially from extensive and pastoral ruminant production systems.

### *Early-Life Intervention*

In contrast to adult ruminants, preruminants undergo various stages of microbial colonization; thus, early-life interventions to modify the microbiota in a manner that decreases CH<sub>4</sub> emissions later in life have been explored (Yáñez-Ruiz et al., 2015). The concept of early-life intervention is very attractive in that the cost of applying long-lasting modifications for a short period to animals with a small body size would be greatly diminished compared with that in adult animals, in which most interventions have to be applied continuously. Furthermore, this strategy may be advantageous for grazing ruminants where supplementation of feed ingredients at the appropriate dose is challenging. Research on early-life interventions is at an early stage with few and contradictory results. Goat kids that had received bromochloromethane for 3 mo after birth had 20% lower CH<sub>4</sub> yield than control kids 3 mo after bromochloromethane was discontinued; the effect was greatest when both kids and their mothers were treated (Abecia et al., 2013). Meale et al. (2021) administered 3-NOP to heifers until 14 wk of age, finding that those animals still produced 17.5% less CH<sub>4</sub> than the control

animals between 57 and 60 wk of age. On the other hand, Debruyne et al. (2018) supplementing coconut oil to goat kids, Saro et al. (2018) working with a mixture of linseed and garlic oil administered to lambs, and Cristobal-Carballo et al. (2021) with a mixture of chloroform and 9,10-anthraquinone administered to calves did not obtain long-lasting inhibition of rumen methanogenesis after the methanogenesis-inhibition treatments were stopped. Efficacy may depend on the CH<sub>4</sub> mitigant used, dose, mode and duration of administration, and the animal species, among other factors. Understanding the mechanisms behind the differing results and early-life rumen microbial ecology would be important to evaluate the future possibilities of this CH<sub>4</sub> mitigation strategy.

### Phage and Lytic Enzymes with Activity Against Methanogens

Archaeal phage produce lytic enzymes that break down pseudomurein, the principal cell wall component of rumen methanogens. Preliminary research has shown that their disruptive activity could reduce the production of CH<sub>4</sub> in the rumen. A novel archaeal lytic enzyme (PeiR) displayed on bionanoparticles was shown to reduce CH<sub>4</sub> production in specific pure methanogen cultures by up to 97% over a period of 5 d (Altermann et al., 2018). Efficacy of the lytic enzyme was lower against methanogens that were more phylogenetically distant from *Methanobrevibacter ruminantium* M1, the original host of the provirus. No published in vivo or in vitro mixed culture studies have investigated the ability of phage or their lytic enzymes to reduce ruminal CH<sub>4</sub> emissions. Although this research is at a limited proof-of-concept stage, the approach is presumed to be low risk as phage are already used in therapeutic applications in medicine and food safety.

### Defaunation of the Rumen

Elimination of protozoa causes the loss of their methanogenic symbionts, resulting in a decrease in CH<sub>4</sub> production in the rumen. Meta-analyses estimate that removal of protozoa leads to a decrease in CH<sub>4</sub> production of ~10% (Arndt et al., 2022). Protozoa have been eliminated from the rumen using chemicals and lipids, by freezing rumen contents, or by isolating newborn animals (Newbold et al., 2015). However, it is very challenging to maintain protozoa-free animals in commercial production settings, as re-inoculation occurs very rapidly with cross-contamination between animals. Thus, defaunation has potential as a mitigation strategy if simple but permanent ways of defaunating animals could be developed.

### Biochar

Biochar, which is formed as a result of the pyrolysis (350–600°C treatment with limited oxygen) of various biomass sources, has been proposed as a CH<sub>4</sub> mitigation strategy because it enhances biofilm formation and electron transfer among members within microbial communities, including methanogens (Chen et al., 2014). However, most in vivo studies to date have shown no effect of biochar on CH<sub>4</sub> emissions (e.g., Terry et al., 2019; Sperber et al., 2021). It remains to be studied if the efficacy of biochar is influenced by biomass source as well as pyrolysis conditions and by secondary treatment of the biochar with acidic or alkali solutions.

### CH<sub>4</sub>-Oxidizing Device

A CH<sub>4</sub>-oxidizing device (ZELP, <https://www.zelp.co>) that attaches to animals to collect CH<sub>4</sub> is claimed to decrease CH<sub>4</sub> yield of steers between 26 and 53% (Budani et al., 2022). Although the concept is of much interest, more independently conducted studies are required.

## SUMMARY OF MITIGATION STRATEGIES

In all cases, the adoption potential of a given mitigation strategy depends on the production system, the farmer's objectives, and the regional or local conditions, including policies, incentives, and barriers; hence the need for numerous approaches. Strategies that differ in mode of action may have potential additive effects when combined; however, there is a paucity of research on the efficacy of combined mitigation approaches.

A summary evaluation of the various enteric CH<sub>4</sub> mitigation strategies proven in in vivo experiments and technically available, or very close to being technically available at present, is provided for typical confinement systems and partial grazing systems with feed supplementation (Table 1) and for grazing systems without supplementation (Table 2). Confinement systems include feedlots and dairies in which animals are penned or housed in dry lots or buildings. In these nongrazing systems, all feed ingested by the animals is provided by human operators. Feed ingredients are many, including cereal grains, oilseeds and meals, conserved forages, co-products, and premixes containing minerals, vitamins, and additives. The feeding frequency and management (i.e., TMR or feed components offered separately) is determined by the farm operator. On the other hand, partial grazing systems represent pasture-based production where animals also receive supplemental concentrates or conserved forages, with the proportion of total DMI consumed as fresh herbage versus supplemented feed varying throughout the year

depending on pasture availability. In grazing systems with no supplementation (other than possibly minerals and vitamins), animals exclusively ingest fresh herbage harvested by grazing; typically, there is minimal human intervention. These systems often require a large land base, as in the case of beef cattle and sheep ranching. Within each animal production system, there is ample variation depending on animal species and category, climate, eco-zone, and economic and societal factors.

A fundamental difference from the point of view of implementation of CH<sub>4</sub> mitigation strategies exists between confined and partial grazing systems on the one hand, and extensive production systems without supplementation on the other. Supplementation allows the daily delivery of feed ingredients such as chemical inhibitors of methanogenesis, algae, nitrate, or essential oils, as well as dietary changes involving feeding concentrate or lipids. Production systems with grazing ruminants with no supplementation represent a unique challenge for mitigation because delivery systems for dietary and rumen modification-based strategies are unavailable, and are therefore not listed in Table 2. Although confined and partial grazing systems are grouped together in Table 1 (because both systems allow supplementation of feed additives, concentrates, or lipids), it must be noted that considerably less research is available for partial grazing compared with confined systems, and the delivery mechanisms for feed-based strategies that are effective are very different across these 2 systems.

The application of each enteric CH<sub>4</sub> mitigation strategy for these production systems is based on a qualitative assessment, systematically analyzing the following aspects in Tables 1 and 2:

- (1) Expected CH<sub>4</sub> decrease range: The magnitude of the change in CH<sub>4</sub> production, both on an absolute (per animal per day) and intensity (per unit of animal product) basis. Application of some antimethanogenic strategies is expected to result in immediate mitigation; for example, use of specific feed ingredients or dietary changes, whereas others have more gradual effects over time; for example, intensification or breeding for low CH<sub>4</sub>. With respect to the latter, a 10-yr timeframe was assumed for evaluating the extent of mitigation in total CH<sub>4</sub> production and intensity.
- (2) Degree of confidence in the observed effects: Methane mitigation strategies vary considerably with regards to the number of in vivo experiments supporting the results. Thus, confidence can be low (<5 experiments), medium (between 5 and 10 experiments) or high (>10 experiments).
- (3) Effects on other emissions: Average measured or likely effects of the application of an enteric CH<sub>4</sub> mitigation strategy on the emissions of other GHG is assessed. Upstream changes may include the direct and indirect release of CO<sub>2</sub> and N<sub>2</sub>O in crop growth and manufacture of feeds, specific feed ingredients, or other products. Changes may also occur in emissions of CH<sub>4</sub> and N<sub>2</sub>O from manure, or enteric emissions of N<sub>2</sub>O. Changes in crop production and grazing management can affect carbon sequestration in soils. In some cases, changes in other GHG have been found to be minimal, whereas in others, an LCA is recommended for a defined production unit such as a farm, region, or country.
- (4) Animal productivity: Effects of the application of the enteric CH<sub>4</sub> mitigation strategy on meat and milk production and feed efficiency are assessed, with an emphasis on studies in which animal productivity and CH<sub>4</sub> production were simultaneously measured.
- (5) Risk: Concerns related to potential toxicity to animals, humans, residues in animal products, and the environment are considered.
- (6) Barriers to adoption on-farm: The main potential barriers to adoption of a mitigation strategy were considered, which can be highly variable among farms, regions, and countries. These include biological (accessibility, safety), economic (cost, lack of incentives), regulatory, environmental, and societal (resistance to change, availability of technical support, consumer acceptance) aspects. Barriers to mitigation technologies for livestock systems in high-income countries might be very different to barriers in low- and middle-income countries, because of differences in livestock systems, country infrastructure, and values of livestock producers.

Overall, the success of mitigation strategies needs to be based on value to the livestock producer. Local subject matter expertise and knowledge of the prioritized pain points of the livestock producer are important. For example, CH<sub>4</sub> mitigation may not be the priority of many livestock producers, but the co-benefits of a CH<sub>4</sub> mitigation strategy might be. Once a strategy becomes priority to the livestock producer, the strategy is more likely to be implemented and sustained as the livestock producers see the continuously added value to their farms.

**Table 1.** Summary of enteric methane mitigation strategies presently available for confined and partial grazing systems

Strategy <sup>1</sup>	Expected CH <sub>4</sub> decrease range <sup>2</sup>		Effects on other emissions <sup>3</sup>	Animal productivity <sup>4</sup>	Risks <sup>5</sup>	Potential barriers for on-farm adoption
	g/d	g/kg of meat or milk				
Increased animal productivity	↑	↓ to ↓↓	↑↑↑ (UPS, MAN)	↑	0	Cost/lack of financial incentives; technical support
<i>Selection of low-CH<sub>4</sub>-producing animals</i>	↓	?	0	↑/↓	0	Accessibility; cost/lack of financial incentives; resistance to change; technical support
<b>Levels of feed and concentrate intake, concentrate sources, processing</b>	↑	↓↓	↑↑↑ (UPS, MAN)	↑	S-ANI	Cost/lack of financial incentives; technical support
<b>Lipid supplementation</b>	↓↓	↓ to ↓↓	↑↑↑ (UPS, MAN)	0 to ↑	0	Accessibility; cost/lack of financial incentives; technical support
<b>Increased forage digestibility</b>	↑	↓	↑/↓ (UPS, MAN)	↑	0	Cost/lack of financial incentives; resistance to change; technical support
<b>Perennial legumes</b>	↑/↓	↑/↓	↓/↑ (UPS, MAN)	↑/↓	0 <sup>6</sup> or S-ANI <sup>7</sup>	Accessibility; resistance to change; technical support
High-starch forages	↓	↓	↓/↑ (UPS, MAN)	0 to ↑	0	Accessibility; cost/lack of financial incentives; resistance to change; technical support
<b>High-sugar grasses</b>	↓	↓	0 to ↓ (MAN)	0	0	Accessibility; cost/lack of financial incentives; technical support
<b>Preservation and processing</b>	0 to ↑	0 to ↓	↑↑↑ (UPS, MAN)	0 to ↑	0	Accessibility; cost/lack of financial incentives; technical support
<b>Ionophores</b>	0 to ↓	↓	↑ (UPS)	↑	MAX	Accessibility; regulatory approval; consumer acceptance
<b>3-Nitrooxypropanol</b>	↓↓↓	↓↓↓	↑ (UPS)	↑/↓	MAX	Accessibility; cost/lack of financial incentives; regulatory approval; consumer acceptance
<i>Asparagopsis spp.</i>	↓↓↓	↓↓↓	↑ (UPS)	↑/↓	MAX, S-ANI, S-HUM, S-ENV	Accessibility; cost/lack of financial incentives; regulatory approval; safety for the animal; the consumer; and the environment
<b>Alternative electron acceptors (organic acids)</b>	↓↓	↑/↓	↑↑↑ (UPS)	↑/↓	0	Accessibility; cost/lack of financial incentives
<b>Alternative electron acceptors (nitrate)</b>	↓↓	↓	0 to ↑↑↑ (UPS, ANI, MAN)	0 to ↑	MAX, S-ANI	Accessibility; cost/lack of financial incentives; regulatory approval; safety for the animal; technical support
<b>Essential oils</b>	↓	0 to ↓	↑ (UPS)	?	MAX	Accessibility; cost/lack of financial incentives; technical support
<b>Tannins and saponins</b>	↓	↑/↓	↑/↓ (MAN)	↑/↓	MAX	Accessibility; cost/lack of financial incentives; technical support
<i>Direct-fed microbials</i>	0	0 to ↓	↑ (UPS)	0 to ↑	0	Accessibility; cost/lack of financial incentives; regulatory approval

<sup>1</sup>Degree of confidence: entries in italics indicate that few (<5) in vivo peer-reviewed published studies simultaneously evaluating CH<sub>4</sub> production and animal performance exist; entries in regular font indicate that 5 to 10 in vivo peer-reviewed published studies simultaneously evaluating CH<sub>4</sub> production and animal performance exist and no meta-analysis conducted solely on the particular strategy has been published; entries in bold indicate that >10 in vivo peer-reviewed published studies simultaneously evaluating CH<sub>4</sub> production and animal performance exist and at least one meta-analysis has been published.

<sup>2</sup>↓ = small decrease (≤15%); ↓↓ = medium decrease (15–24%); ↓↓↓ = large decrease (≥25%); 0 = minimal or no change; ↑ = increase; ↑/↓ = variable results; ? = more research is needed.

<sup>3</sup>↑↑↑ = large increases; ↑ = small increases; 0 = minimal or no change; ↓ = decreases; ↑/↓ = increases in some emissions and decreases in others, or net CO<sub>2</sub> fixation, can occur; sources of other emissions: UPS = changes in upstream emissions of CO<sub>2</sub> from fossil fuels use or N<sub>2</sub>O from application of fertilizers; ANI = increase in emissions of enteric N<sub>2</sub>O; MAN = increase in emissions of manure CH<sub>4</sub> and N<sub>2</sub>O.

<sup>4</sup>↑ = increase (of any magnitude); 0 = no change; ↑/↓ = variable results; ? = more research is needed.

<sup>5</sup>0 = no known risks; MAX = maximum dose exists; S-ANI = safety for the animal; S-ENV = safety for the environment; S-HUM = safety for humans.

<sup>6</sup>Conserved legumes or grazed, tannin-containing legumes.

<sup>7</sup>Grazed, non-tannin-containing legumes.

## CONCLUSIONS

There is increasing pressure on ruminant livestock producers to lower enteric CH<sub>4</sub> emissions from meat and milk production, in terms of both absolute daily emissions and emissions relative to animal product outputs (e.g., meat, milk, wool). The unique ability of ruminants to consume forages and high-fiber co-products and the wide use of grazing systems, makes reducing CH<sub>4</sub> emissions on a global scale extremely challenging. Several enteric CH<sub>4</sub> mitigation solutions are now technically available, but many barriers to their implementation remain. Continued innovation is needed to develop additional technologies that accommodate the large variation in global ruminant production systems.

Intensification of animal production through improved feeding and management is recognized as the most immediate and universally applicable means of decreasing CH<sub>4</sub> emissions intensity (relative to animal products), but absolute CH<sub>4</sub> emissions may not always decrease. Considerable research on lipid supplementation indicates that, where applicable and affordable, it can be an effective CH<sub>4</sub> mitigant. 3-Nitrooxypropanol has been approved by some government agencies and is now available in some locations; however, its adoption will depend on costs, incentives, and consumer acceptance. Red seaweeds with considerable mitigation potential such as *Asparagopsis* still need to be thoroughly assessed for safety, their production and feeding evaluated through LCA, and the economics of their inclusion in ruminant diets assessed. Although a large amount of research has been conducted on plant secondary compounds such as tannins, saponins, and essential oils, their efficacy for CH<sub>4</sub> mitigation has been inconsistent and further research is required before they can be used as reliable mitigation tools. Supplementing nitrate can be an option in low-protein basal diets where feed intake can be carefully controlled, but economic incentives are needed for nitrate to be preferred over urea as a source of nonprotein N. Progress has been made in understanding the heritability of CH<sub>4</sub> production metrics, their relationship with productive traits, and possible proxies for estimating CH<sub>4</sub> toward selection for low-CH<sub>4</sub>-producing animals; this approach can be particularly important as it is applicable to both intensive and extensive production systems. Various other technologies that are at earlier stages of development may also have potential to mitigate CH<sub>4</sub> in intensive and extensive grazing systems, such as vaccines and early-life interventions.

Although research continues to provide mitigation options, many challenges limit adoption by commercial ruminant production, particularly in extensively managed pasture-based systems, where it is difficult to

provide the required dose of specific feed ingredients through an appropriate delivery mechanism. In general, considerably less research on CH<sub>4</sub> mitigation has been conducted in grazing systems, although they comprise a major proportion of global CH<sub>4</sub> emissions. It is telling that not a single additive manufacturer has identified the development of inhibitors of methanogenesis suitable for grazing systems as an extremely high priority (Hegarty et al., 2021). Precision feeding of concentrates or salt blocks containing chemical inhibitors of methanogenesis or nitrate may play a role in the future to ensure accurate, efficient, and safe delivery of these types of additives.

Another major issue is affordability; livestock farmers need greater information on the cost of mitigation, impacts on animal productivity, and opportunities to diversify their income by producing branded products or participating in carbon offset markets. Regulatory approval requirements for some promising feed ingredients may slow their adoption, and lack of consumer acceptance of some of them may preclude their use for CH<sub>4</sub> mitigation. Incentives may be needed to encourage adoption because, in most cases, decreased CH<sub>4</sub> production has not increased animal performance. Additionally, many enteric CH<sub>4</sub> mitigation technologies have only been evaluated at the research scale, with limited consideration for differences in production systems at the farm scale. Safety and consumer acceptance also need to be considered. Communicating with consumer associations at an early or mid stage of research and development may help facilitate consumer acceptance. Specialized technical support capacity is required to assist farms in estimating current CH<sub>4</sub> emissions and identifying where changes can be made using options that are tailored toward their specific production system. Regardless of the system, management practices need to be evaluated from the perspective of their net impact on total GHG emissions using an LCA approach to ensure that mitigating enteric CH<sub>4</sub> does not increase total GHG emissions arising from the entire production system. Consideration needs to be given globally to the effect of CH<sub>4</sub> mitigation on climate change in particular, given the short atmospheric half-life of CH<sub>4</sub> and its impact on warming. This is particularly important relative to the potential reductions in CH<sub>4</sub> atmospheric concentrations and effects on temperature associated with CH<sub>4</sub> mitigation in short-term time horizons.

## ACKNOWLEDGMENTS

This work was supported by the Food and Agriculture Organization of the United Nations (FAO) Livestock Environmental Assessment and Performance (LEAP) Partnership (GCP/GLO/369/MUL; Rome, Italy).

**Table 2.** Summary of enteric methane mitigation strategies presently available for grazing systems without feed supplementation

Strategy <sup>1</sup>	Expected CH <sub>4</sub> decrease range <sup>2</sup>		Effects on other emissions <sup>3</sup>	Animal productivity <sup>4</sup>	Risk <sup>5</sup>	Potential barriers for on-farm adoption
	g/d	g/kg meat or milk				
Increased animal productivity	↑	↓ to ↓↓↓	↑↑↑ (UPS, MAN)	↑	0	Cost/lack of financial incentives; resistance to change; technical support
<i>Selection of low-CH<sub>4</sub>-producing animals</i>	↓	?	0	↑/↓	0	Accessibility; cost/lack of financial incentives; resistance to change; technical support
<i>Increased forage digestibility</i>	↑	↓	↑/↓ (UPS, MAN)	↑	0	Cost/lack of financial incentives; resistance to change; technical support
Perennial legumes	↑/↓	0	↑/↓ (UPS, MAN)	↑/↓	0 <sup>6</sup> or S-ANI <sup>7</sup>	Accessibility; resistance to change; safety for the animal; technical support
High-sugar grasses	↓	↓	0 to ↓ (MAN)	0	0	Accessibility; climate; cost/lack of financial incentives; resistance to change; technical support
Pastures and grazing management	↑/↓	0 to ↓	↑/↓ (MAN)	↑	0	Cost/lack of financial incentives; resistance to change; technical support
Tannins and saponins (in grazed forages)	↓	↓	↑/↓ (MAN)	↑/↓	MAX	Accessibility; cost/lack of financial incentives; resistance to change; technical support
<i>Alternative electron acceptors (nitrate)</i>	?	?	0 to ↑↑↑ (UPS, ANI, MAN)	↓ <sup>8</sup>	MAX, S-ANI	Accessibility; cost/lack of financial incentives; regulatory approval; safety for the animal; technical support

<sup>1</sup>Degree of confidence: entries in italics indicate that few (<5) in vivo peer-reviewed published studies simultaneously evaluating CH<sub>4</sub> production and animal performance exist; entries in regular font indicate that 5 to 10 in vivo peer-reviewed published studies simultaneously evaluating CH<sub>4</sub> production and animal performance exist and no meta-analysis conducted solely on the particular strategy has been published.

<sup>2</sup>↓ = small decrease (≤15%); ↓↓ = medium decrease (15–24%); ↓↓↓ = large decrease (≥25%); 0 = minimal or no change; ↑ = increase; ↑/↓ = variable results; ? = more research is needed.

<sup>3</sup>↑↑↑ = large increases; ↑ = small increases; 0 = minimal or no change; ↓ = decreases; ↓/↓ = increases in some emissions and decreases in others occur; Sources of other emissions: UPS = changes in upstream emissions of CO<sub>2</sub> from fossil fuels use or N<sub>2</sub>O from application of fertilizers; ANI = increase in emissions of enteric N<sub>2</sub>O; MAN = increase in emissions of manure CH<sub>4</sub> and N<sub>2</sub>O.

<sup>4</sup>↑ = increase (of any magnitude); 0 = no change; ↑/↓ = variable results; ? = more research is needed.

<sup>5</sup>0 = no known risks; MAX = maximum dose exists; S-ANI = safety for the animal; S-ENV = safety for the environment; S-HUM = safety for humans.

<sup>6</sup>Tannin-containing legumes.

<sup>7</sup>Non-tannin-containing legumes.

<sup>8</sup>Compared with nitrogen supplementation as urea.

Article processing charges were funded by Agencia Nacional de Investigación y Desarrollo (Project Fondecyt 1190574; Santiago, Chile). The views expressed in this publication are those of the authors and do not necessarily reflect the views or policies of the Food and Agriculture Organization of the United Nations. The authors have not stated any conflicts of interest.

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