Current Perspectives on Achieving Pronounced Enteric Methane Mitigation From Ruminant Production

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Limiting global warming to 1.5°C above pre-industrial levels by 2050 requires achieving net zero emissions of greenhouse gases by 2050 and a strong decrease in methane (CH_4) emissions. Our aim was to connect the global need for mitigation of the emissions of greenhouse gases and enteric CH_4 from ruminant production to basic research on the biological consequences of inhibiting rumen methanogenesis in order to better design strategies for pronounced mitigation of enteric CH_4 production without negative impacts on animal productivity or economic returns. Ruminant production worldwide has the challenge of decreasing its emissions of greenhouse gases while increasing the production of meat and milk to meet consumers demand. Production intensification decreases the emissions of greenhouse gases per unit of product, and in some instances has decreased total emissions, but in other instances has resulted in increased total emissions of greenhouse gases. We propose that decreasing total emission of greenhouse gases from ruminants in the next decades while simultaneously increasing meat and milk production will require strong inhibition of rumen methanogenesis. An aggressive approach to pronounced inhibition of enteric CH_4 emissions is technically possible through the use of chemical compounds and/or bromoform-containing algae, but aspects such as safety, availability, government approval, consumer acceptance, and impacts on productivity and economic returns must be satisfactorily addressed. Feeding these additives will increase the cost of ruminant diets, which can discourage their adoption. On the other hand, inhibiting rumen methanogenesis potentially saves energy for the host animal and causes profound changes in rumen fermentation and post-absorptive metabolism. Understanding the biological consequences of methanogenesis inhibition could allow designing strategies to optimize the intervention. We conducted meta-regressions using published studies with at least one treatment with >50% inhibition of CH_4 production to elucidate the responses of key rumen metabolites and

animal variables to methanogenesis inhibition, and understand possible consequences on post-absorptive metabolism. We propose possible avenues, attainable through the understanding of biological consequences of the methanogenesis inhibition intervention, to increase animal productivity or decrease feed costs when inhibiting methanogenesis.

Keywords: global warming, ruminant production, greenhouse gases, enteric methane, methanogenesis inhibition, metabolism, productivity, cost effectiveness

INTRODUCTION

All segments of society, including the agricultural industries, are challenged to mitigate greenhouse gas emissions to limit global warming to 1.5°C above pre-industrial levels by 2050 (IPCC, 2018, 2019). Net zero emissions is defined as a stage in which metric-weighted anthropogenic greenhouse gas emissions to the atmosphere are balanced by their removal (IPCC, 2021; Net Zero Climate, 2021). It is estimated that limiting global warming to a maximum of 1.5°C requires reaching net zero emissions globally by 2050 and concomitantly attaining deep reductions in methane (CH_4) emissions (Rogelj et al., 2018), with agriculture as a necessary component of emissions mitigation (Leahy et al., 2020). A net zero commitment by 2050 has been taken by more than 130 countries as well as many private sector companies (Black et al., 2021; United Nations Climate Action, 2021). Importantly, various large agribusiness companies and value chain organizations have set ambitious voluntary targets to decrease their emissions of greenhouse gases (**Table 1**). The 35 largest meat and dairy companies are responsible for 14% of total livestock emissions of greenhouse gases, and as their supply chains account for a major part of their total emissions, their targets of mitigation of emissions of greenhouse gases will impact livestock producers, who will thus need to decrease their emissions of greenhouse gases to sell their products (Leahy et al., 2020).

Decreasing the emissions of greenhouse gases from ruminant production is a challenging goal given the projected increased demand for ruminant meat and milk in future decades and the limited mitigation options currently available to producers (Beauchemin et al., 2020). Thus, avenues are being sought to decrease the emissions of greenhouse gases from ruminant production while simultaneously increasing production to meet the rising demand for animal products. To meet the challenge of decreasing the emissions of greenhouse gases while continuing to grow production, the focus has often been on decreasing greenhouse gas emissions intensity (i.e., lowering emissions per unit of product) through production intensification to increase animal productivity (Gerber et al., 2013a; Opio et al., 2013). Decreasing emissions intensity by increasing animal productivity is an attractive strategy in that it can increase farmers' profitability (Gerber et al., 2013a).

In this paper, we put forward the case that improved animal productivity alone is unlikely to achieve a substantial decrease in total emissions of greenhouse gases from ruminant production globally, and in many developed and developing countries and regions. Our first objective in this paper is to show that pronounced decreases in enteric CH_4 production per animal

are also necessary, and that moderate decreases of enteric CH_4 production per animal may be insufficient to mitigate total emissions of greenhouse gases from ruminant production. Methane is the main greenhouse gas emitted by ruminant production, accounting for about half of the 1.0°C increase in global temperature since pre-industrial times. Due to the short half-life of CH_4 in the atmosphere, mitigation of CH_4 emissions is considered the single most effective strategy to ameliorate global warming in the short term and limit temperature increase to 1.5°C (Beauchemin et al., 2020; European Commission, 2021; Global Methane Initiative, 2021). The evolution of CH_4

TABLE 1 | Voluntary commitments to reduce the emissions of greenhouse gases stated by various multinational agribusiness companies.

Company	Target established
ADM	Reduce emissions of greenhouse gases 25% by 2035 (2020 baseline)
Cargill Inc.	Reduce emissions of greenhouse gases per ton of product sold 30% by 2030 (2017 baseline)
Innovation Center for U.S. Dairy	Carbon neutral by 2050 for U.S. dairy industry
The Coca-Cola Co.	Reduce emissions of greenhouse gases 25% by 2030 (2015 baseline)
Danone SA	Net zero emissions by 2050
General Mills Inc.	Reduce emissions of greenhouse gases 30% by 2030 across value chain (2020 baseline); net zero emissions by 2050
JBS USA	Net zero by 2040 across value chain
Kellogg Co.	Reduce emissions of greenhouse gases from suppliers 50% by 2050 (2015 baseline)
McDonald's Corp.	Reduce emissions of greenhouse gases 31% by 2030 (2015 baseline)
Nestle SA	Carbon neutral by 2050
PepsiCo, Inc.	Net zero emissions by 2040; reduce emissions of greenhouse gases across direct operations 75% and from value chain 40% by 2030 (2015 baseline)
Smithfield Foods	Carbon neutral by 2030
Tyson Foods Inc.	Reduce emissions of greenhouse gases 30% by 2030 (2016 baseline); net zero emissions by 2050
Mars Inc.	Reduce emissions of greenhouse gases 27% by 2025 and 67% by 2050
Synlait Milk Limited	Reduce on-farm emissions per kilogram of milk solids by 35% and off-farm by 50% by 2028
Unilever PLC	Reduce emissions from the life-cycle of their products 50% per consumer use by 2030

Companies' webpages; Leahy et al. (2020).

emissions will affect the targets of carbon dioxide (CO_2) decrease necessary to limit global warming to 1.5°C (Reisinger et al., 2021).

Implementing measures that pronouncedly decrease enteric CH_4 production is technically possible but will likely increase feed costs and could negatively impact economic returns of ruminant production. Therefore, our second objective for this paper was to explore how strategies aimed at pronounced inhibition of rumen methanogenesis could be made cost effective. Designing effective antimethanogenic strategies attractive for adoption requires understanding the biological consequences of inhibiting rumen methanogenesis on rumen fermentation and animal metabolism. We conducted meta-regressions to relate key rumen metabolites and feed intake to CH_4 production using data from studies in which methanogenesis was inhibited by at least 50%. We discuss how changes in rumen and post-absorptive metabolism occurring as a consequence of pronounced inhibition of rumen methanogenesis might be manipulated toward the improvement of ruminant productivity, and ultimately cost effectiveness of CH_4 abatement. We finally identify knowledge gaps that need to be addressed by future research to achieve these goals.

INTENSIFICATION OF RUMINANT PRODUCTION AND EMISSIONS OF GREENHOUSE GASES

The three major greenhouse gases emitted from animal production are CO_2, CH_4 and nitrous oxide (N_2O). They differ in their heat trapping effects and their lifetimes in the atmosphere. The heat trapping effect of a pulse emission of a mixture of CO_2, CH_4, and N_2O over a certain time period following the pulse emission can be calculated as CO_2 equivalents (CO_2e), i.e., the sum of the amount of each gas emitted weighted by its global warming potential integrated over the period of time considering its rate of disappearance. In this paper, we will consider global warming potentials over 100 yr, or GWP_{100} (Opio et al., 2013).

As product outputs (i.e., meat, milk) from individual animals increase due to improvements in genetics, nutrition, health and management, total (or absolute) emissions of enteric CH_4 and CO_2e per animal increase in parallel due to greater feed intake and digestion and manure output, along with greater upstream emissions of N_2O and fossil fuel generated CO_2 mainly from feed and farm inputs. However, emissions of CH_4 and of CO_2e per unit of product, or emissions intensity, decrease due to the dilution of animal maintenance requirements, better herd management and animal health, and greater productivity of feed crops (Capper et al., 2009; Capper, 2011; Gerber et al., 2011). We are interested in understanding how decreasing CO_2e emissions intensity of a production system relates to total CO_2e emissions, as atmospheric concentration of greenhouse gases and the extent of global warming depends on the latter. Total emissions of CO_2e in a certain period of time (e.g., emissions of CO_2e from a farm, industry, country or region, or global emissions, in 1 year), result from multiplying the emissions of CO_2e per unit of animal product by the total amount of animal product produced during that period. In this way, total emissions of CO_2e result

from multiplying the emissions of CO_2e per unit of product by the production per individual animal and the number of animals (**Supplementary Equation 1**).

An analysis of milk production from representative farms in 155 countries found that CO_2e emissions intensity decreased with increased milk production following an asymptotic function (Gerber et al., 2011). In extensive production systems, in which the potential to decrease CO_2e emissions intensity through increasing animal productivity is substantial (Gerber et al., 2011), intensification can accommodate for a simultaneous increase in production of milk or meat without an increase in total CO_2e emissions. However, it is less certain that decreasing emissions intensity alone will decrease total CO_2e emissions if production continues to expand (Gerber et al., 2013b). Production of beef, lamb and milk in developing countries is predicted to grow by 8.0, 15.1, and 26.2%, respectively, between 2021 and 2030 (OECD/FAO, 2021). If future decreases in CO_2e emissions intensities are similar to the rates of increase in production, greenhouse gas emissions from animal production will remain constant, i.e., without any mitigation (Leahy et al., 2020).

While in systems with low productivity, intensification decreases CO_2e emissions intensity, intensive systems may find it difficult to further intensify with simultaneous decrease in total emissions of CO_2e, to meet carbon neutrality goals (UN Climate Change News, 2021; United Nations Climate Action, 2021) (**Table 1**). Changes in total CO_2e emissions and CO_2e emissions intensity for the dairy, beef and lamb industries through different time periods have been estimated for various developed countries, states and provinces (Leslie et al., 2008; Capper et al., 2009; Capper, 2011; Jayasundara and Wagner-Riddle, 2013; Wiedemann et al., 2015; Legesse et al., 2016; Capper and Cady, 2020; Naranjo et al., 2020) and are summarized in **Table 2**. Based on those studies, we calculated constant fractional yearly rates of increase or decrease in total emissions of CO_2e and emissions of CO_2e per unit of product, expressed as percentages (**Supplementary Equation 2**). When not provided in the referenced papers, total CO_2e emissions were calculated from total food production and emissions intensity (**Supplementary Equation 1**).

A consistent pattern in **Table 2** is a decrease in CO_2e emissions intensity as production intensifies (Capper et al., 2009; Capper, 2011; Gerber et al., 2011) and producers use natural resources with greater efficiency (Gerber et al., 2013b). In contrast to CO_2e emissions per unit of product, the evolution of total CO_2e emissions was more variable, with decreases (Leslie et al., 2008; Capper et al., 2009; Capper, 2011; Jayasundara and Wagner-Riddle, 2013) but also increases in total CO_2e emissions (Leslie et al., 2008; Wiedemann et al., 2015; Legesse et al., 2016; Capper and Cady, 2020; Naranjo et al., 2020) in the periods and places studied. A global livestock assessment for the 1961–2010 period reported an increase in total CO_2e emissions from developing countries of 117% and a 23% decrease from developed countries (Caro et al., 2014); however, emissions of CO_2 associated to the use of fossil fuels and of N_2O resulting from the application of fertilizers, which are greater in high input production systems, were not considered in that analysis.

TABLE 2 | Fractional rates of change of total emissions and emissions intensity of carbon dioxide equivalents (CO_2e) of the dairy, beef and lamb industries in various regions.

References	Country or state	Product	Time period	r_{int} (%/yr)[a]	r_{total} (%/yr)[a]
Leslie et al. (2008)	New Zealand	Milk solids	1990–2005	−1.29	+3.62
Capper et al. (2009)	United States	Milk	1944–2007	−1.57	−0.85
Jayasundara and Wagner-Riddle (2013)	Ontario	Fat and protein-corrected milk	1991–2011	−1.23	−1.05
Capper and Cady (2020)	United States	Energy-corrected milk	2007–2017	−2.09	+0.12
Naranjo et al. (2020)	California	Energy-corrected milk	1964–2014	−1.22	+1.97
Capper (2011)	United States	Beef	1977–2007	−0.59	−0.21
Wiedemann et al. (2015)	Australia	Beef	1981–2010	−0.53	+0.80
Legesse et al. (2016)	Canada	Beef	1981–2011	−0.51	+0.83
Leslie et al. (2008)	New Zealand	Lamb	1990–2005	−1.40	−1.22

[a]r_{int} = Fractional rate of change in CO_2e emissions per unit of product (emissions intensity); r_{total} = Fractional rate of change in total CO_2e emissions (absolute emissions). Constant annual fractional rates of change were calculated (**Supplementary Equation 2**).
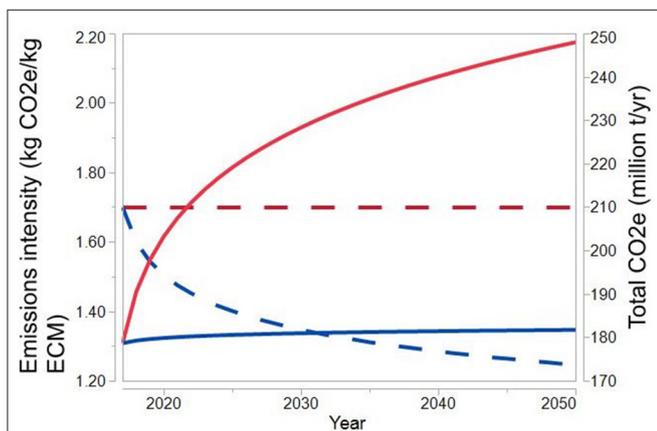
FIGURE 1 | Projected total CO_2e emissions and CO_2e emissions intensity [CO_2e emissions per kilogram of energy-corrected milk (ECM)] for the US dairy industry between 2017 and 2050. Total CO_2e emissions and total ECM production and CO_2e emissions per kg ECM for the baseline year 2017 are from Capper and Cady (2020). Rates of change of total CO_2e emissions and CO_2e emissions per kg ECM were calculated for the 2007–2017 period based on Capper and Cady (2020) using an asymptotic model and extrapolated to the 2017–2050 period. Dashed lines correspond to CO_2e emissions per kg ECM (CO_2e emissions intensity, left axis) and continuous lines correspond to total emissions of CO_2e (right axis). Red lines correspond to constant, 2017-levels, of CO_2e emissions per kg ECM; blue lines correspond to decreasing CO_2e emissions per kg ECM.

Figure 1 predicts CO_2e emissions from the U.S. dairy industry toward 2050 using an asymptotic model (**Supplementary Equation 3**) to accommodate for an assumption of production growth slowing down in industrialized economies (Briunsma, 2003). The yearly rate of change in total CO_2e emissions and CO_2e emissions per kilogram of energy-corrected milk (ECM) was calculated from the estimations by Capper and Cady (2020) for the 2007–2017 period, and extrapolated to the 2017–2050 period. We selected the study by Capper and Cady (2020) for predicting the evolution of total CO_2e and CO_2e emissions per unit of product toward 2050 because it analyzed the latest time period in **Table 2**. We

projected total CO_2e emissions and emissions of CO_2e per unit of ECM for the U.S. dairy industry toward 2050 under two different scenarios: (i) at a constant CO_2e emissions intensity, as estimated by Capper and Cady (2020) for the year 2017, and (ii) at decreasing CO_2e emissions intensity with a rate calculated with **Supplementary Equation 3** for the 2007-2017 period of the Capper and Cady (2020) study.

The importance of decreasing CO_2e emissions intensity is illustrated by a predicted increase in total CO_2e emissions of 41% in **Figure 1** under a constant emissions intensity scenario, in comparison to a much lower 1.8% increase in total CO_2e emissions if CO_2e intensity continued to decrease in the 2017–2050 period at a similar pace as it did in the 2007–2017 period. However, according to these projections based on the 2007–2017 period, and if industry growth continues, even with decreasing emissions intensity, if there are no additional mitigation measures, the goal of carbon neutrality for the U.S. dairy industry by 2050 (**Table 1**) (Innovation Center for U.S. Dairy, 2020) would not be achieved, and there would even be a small increase in total CO_2e emissions by 2050.

In developing countries with low productivity systems, there is potential for greater decrease in emissions intensity through increasing animal productivity (Gerber et al., 2011). This approach can help both rural development and food security, but enhancing productivity can also incentivize the use of resources and expansion of land for agriculture, offsetting gains in emissions intensity (Leahy et al., 2020; Reisinger et al., 2021).

PRONOUNCED DECREASE OF ENTERIC METHANE EMISSIONS

Globally, enteric CH_4 emissions are the predominant source of greenhouse gases from ruminant production to the farm gate, comprising 46, 43, 60, and 55% of total CO_2e emissions for dairy, beef, buffalo and small ruminant production, respectively (Gerber et al., 2013b). A decrease in the emissions of enteric CH_4 of 20% by 2030 from a 2020 baseline was estimated as necessary to keep global temperature increase within 1.5°C (United Nations Environment Programme and Climate and

Clean Air Coalition, 2021). By 2050, a 24–47% (interquartile range) decrease in agricultural CH_4 emissions relative to 2010 is estimated as necessary to limit global temperature increase to 1.5°C (IPCC, 2018). Otherwise, the pressure to rapidly decrease CO_2 emissions will become even more stringent than what it is currently (Wollenberg et al., 2016; Leahy et al., 2020; Reisinger et al., 2021). If ruminant production expands to accommodate the global increases in the demand for meat and milk production (73 and 58%, respectively, by 2050; Beauchemin et al., 2020), CH_4 emissions per unit of meat and milk would have to decrease by 56–69% and 52–66%, respectively (calculations not shown) to meet the goal of decreasing enteric CH_4 emissions by 24 to 47% during the same time period (relative to 2010), in accordance with mitigation targets set for agricultural CH_4. However, with current trends agricultural CH_4 emissions are projected to increase by 30% by 2050 relative to 2010 (Reisinger et al., 2021). The large increases in global meat and milk production required to meet human population demands are difficult to accommodate with the enteric CH_4 amelioration goals unless measures to strongly decrease CH_4 production from individual animals are introduced in livestock production systems.

Various strategies to decrease enteric CH_4 emissions from ruminants are being investigated, among them, dietary changes such as additions of concentrates and oils, changes in forage composition and quality, chemical inhibitors of rumen methanogenesis, ionophores, alternative electron acceptors to CO_2, plant secondary compounds, algae containing compounds inhibiting methanogenesis, selection of low-CH_4 producing animals, vaccination against methanogens, archaeal phages, and others (Martin et al., 2010; Cottle et al., 2011; Hristov et al., 2013a; Knapp et al., 2014; Goopy, 2019; Beauchemin et al., 2020; Terry et al., 2020). Measures that decrease absolute enteric CH_4 emissions or CH_4 emissions intensity moderately (e.g., <20%) are likely to be offset partially or totally by the predicted increases in animal numbers and/or total animal production. For net zero goals of CO_2e emissions from the ruminant production sector to be realized or at least approached, emissions of enteric CH_4 as well as fossil fuel CO_2 and N_2O emissions and manure CH_4, would have to be decreased substantially. Strategies that can mitigate enteric CH_4 production pronouncedly should at the same time not significantly increase upstream and downstream emissions of fossil fuel CO_2, N_2O, and manure CH_4.

Meta-analyses have identified the dietary inclusion of chemical inhibitors of methanogenesis and the bromoform-containing, red algae *Asparagopsis* spp., as the most effective strategies to decrease both total enteric CH_4 emissions per animal and enteric CH_4 emissions expressed per unit of dry matter intake (DMI), or CH_4 yield (Veneman et al., 2016; Almeida et al., 2021; Arndt et al., 2021). Although on average the decrease in enteric CH_4 yield (CH_4 produced per unit of DMI) caused by chemical inhibitors of methanogenesis was 25% (Veneman et al., 2016), 34% (Arndt et al., 2021) or 23% (Almeida et al., 2021), the antimethanogenic effects of chemical inhibitors are dose-dependent (Mitsumori et al., 2012; Martinez-Fernandez et al., 2016; Dijkstra et al., 2018), and considerably greater decreases in CH_4 production e.g., >80% are possible (**Table 3**). Similarly, the decrease in CH_4 production and yield by *Asparagopsis* spp. is

related to its dose inclusion (Li et al., 2016; Roque et al., 2019, 2021; Kinley et al., 2020), and considerably greater inhibition of CH_4 production than the 49% average (Almeida et al., 2021) has been reported (**Table 3**). Whilst all studies in **Table 3** were conducted with beef and sheep, slightly less but still severe inhibition of methanogenesis (i.e., between 60 and 70%) has been reported in studies conducted with lactating dairy cows (Haisan et al., 2014; Roque et al., 2019).

Currently, chemical inhibitors of methanogenesis and bromoform-containing algae are the most potent proven means to mitigate the emissions of enteric CH_4. Equally important, dosing animals with the small amounts required of these additives can largely dilute the upstream emissions of CO_2e from fossil fuels required to produce and transport those additives. It must be considered, however, that solutions to decrease CH_4 emissions should not negatively affect animal productivity and health, must be cost effective, feasible for adoption in different production systems, safe for the environment and humans, must be approved by government agencies, and must be acceptable to consumers. The inclusion of chemical inhibitors of methanogenesis or bromoform-containing algae in animal feeds will increase feeding costs, and, all else unchanged, could decrease the economic benefits of animal production, which is regarded as critical for wide adoption of CH_4 mitigation strategies by producers (Hristov et al., 2013b).

It has long been known that CH_4 formation in the rumen and its release to the atmosphere is an energy loss for ruminants (Ritzman and Benedict, 1938). Whilst scientists have attempted to inhibit rumen methanogenesis to improve energy efficiency (Czerkawski and Breckenridge, 1975), this theoretical potential has not been realized through consistent improvements in the efficiency of milk production or animal growth and fattening (Ungerfeld, 2018). Herein, we propose avenues to make the methanogenesis inhibition intervention cost effective through the understanding of its biological consequences. First, we examine through meta-regressions the effects of pronounced inhibition of rumen methanogenesis on rumen and animal variables, discuss possible consequences on post-absorptive metabolism, and identify knowledge gaps that still need to be filled through research (sections Meta-Regressions and Thermodynamic Calculations and Consequences of Inhibiting Enteric Methane Production). In section Cost Effectiveness of Inhibiting Rumen Methanogenesis, we discuss how understanding those biological responses can potentially be used to improve the cost effectiveness of the methanogenesis inhibition intervention.

META-REGRESSIONS AND THERMODYNAMIC CALCULATIONS

We analyzed responses in rumen fermentation and animal variables to the inhibition of enteric CH_4 production with chemical inhibitors or bromoform-containing algae to understand possible changes in the absorption and utilization of metabolites, which can affect the responses of animal production to the methanogenesis inhibition intervention. Chemical

TABLE 3 | *In vivo* experiments reporting at least one treatment resulting in 80% or more inhibition of rumen methanogenesis.

References	Animal, diet	Inhibitor/algae (g/kg diet DM)	Experimental period (d)	Inhibition relative to Control treatment (% decrease in CH_4 $animal^{-1}$ d^{-1})	Effects on performance, digestion or metabolism[a]
Johnson (1972)	Sheep, mixed, high intake experiment	Hemiacetal of chloral and starch (2.2)	7	82	DMI[b], energy and N digestibility and energy and N in urine and N retention unaffected. Increased H_2 emission
Sawyer et al. (1974)	Sheep, mixed	Bromochloromethane (0.1–0.3)	26	83–86	No effects on performance or fiber digestibility. Increased DM, N and energy digestibility. No changes in VFA profile[c]
Mathers and Miller (1982)	Sheep, mixed	Chloral hydrate (intraruminal, equivalent to 1 to 4)	10	96	Digestibility unaffected. Decrease in acetate to propionate molar ratio[c]
McCrabb et al. (1997)	Steers, roughage	Bromochlorometane (0.26)	28	CH_4 not detected	No difference in body mass[c]
Tomkins and Hunter (2004)	Steers, high concentrate	Bromochloromethane (0.15–0.30)	28	88–91	Performance unaffected[c]
Mitsumori et al. (2012)	Goats, mixed	Bromochloromethane (2.6)	22	91	No effects on digestibility. Increased H_2 emission and decreased acetate to propionate molar ratio
Vyas et al. (2016)	Steers, high concentrate	3-Nitrooxypropanol (0.2)	105	84	Tendency to decrease DMI and body mass gain. Increased H_2 emission
Li et al. (2016)	Sheep, roughage	*Asparagopsis taxiformis* (27)	72	81	DMI and body mass gain unaffected. Decreased total VFA concentration and acetate to propionate molar ratio. Rumen mucosa inflammation[c]
Kinley et al. (2020)	Steers, high concentrate	*A. taxiformis* (1.8)	90	98	Feed intake unaffected, improved body mass gain. Increased H_2 emission
Roque et al. (2021)	Steers, mixed and high concentrate	*A. taxiformis* (2.3 and 4.7)	21 and 63	87 and 82	DMI tended to decrease, body mass gain was unaffected and feed conversion efficiency tended to improve. Increased H_2 emission
Cristobal-Carballo et al. (2021)	Calves, milk replacer, concentrate, partial mixed ration, pasture	Chloroform (0.050) plus 9, 10-anthraquinone (0.50)	84	90	Performance largely unaffected

[a]*Only significant effects are presented;* [b]*Abbreviations: DM, dry matter; DMI, dry matter intake; VFA, volatile fatty acids;* [c]*H_2 emission not reported.*

inhibitors and bromoform-containing algae are included in minimal amounts in animal feed, so that the supply of nutrients by those additives is non-existent or negligible, and therefore the effects of methanogenesis inhibition are not confounded with changes in diet composition. A data base of *in vivo* studies in which enteric CH_4 yield was inhibited by 50% or more through the use of chemical compounds or algae was compiled (Ungerfeld, 2021) (**Supplementary Table 1**). For the meta-regressions, we lowered the 80% methanogenesis inhibition threshold of **Table 3** to 50% methanogenesis inhibition to include more studies, because some of the studies in **Table 3** did not report all of the responses of interest. The experiments by Johnson (1972), Martinez-Fernandez et al. (2016) and Roque et al. (2021) contained more than one control, as they compared the effects of methanogenesis inhibitors across two levels of DMI (Johnson, 1972) or more than one diet (Martinez-Fernandez et al., 2016; Roque et al., 2021). In those three studies, each set of treatments was separately regressed against its corresponding

control. Response variables studied were DMI, energy exhaled in dihydrogen (H_2), rumen acetate to propionate molar ratio, rumen propionate molar percentage in total volatile fatty acids (VFA), and rumen molar concentrations of total VFA, acetate, propionate and butyrate.

Animal productivity can be strongly affected by DMI. We first studied the relationship between rumen methanogenesis inhibition and DMI by regressing DMI against the linear and quadratic terms of CH_4 production, the random effect of the experiment, and the interaction between the random effect of the experiment and the linear effect of CH_4 production.

A typical effect of the inhibition of rumen methanogenesis is an increase in the release of H_2 in eructation and respiration (Czerkawski, 1986). Similarly to CH_4, the release of H_2 also represents a loss of energy, hence it is important to understand what proportion of the energy spared in CH_4 not formed when methanogenesis is inhibited is lost to the atmosphere as H_2. Energy loss in exhaled H_2 was modeled as

a function of the random effect of the experiment, the linear effect of energy in exhaled CH_4, and the interaction between the random effect of the experiment and the linear effect of energy in exhaled CH_4. Because the relationship was non-linear, square root transformations of both the regressor and response variables were conducted. Heats of combustion of H_2 and CH_4 were obtained from Domalski (1972).

To understand the effects of methanogenesis inhibition on VFA and possible consequences on post-absorptive metabolism, the rumen acetate to propionate molar ratio, propionate molar percentage, and molar concentration of total VFA, acetate, propionate and butyrate were likewise regressed against the linear and quadratic terms of CH_4 production, the random effect of the experiment, and the interaction between the random effect of the experiment and the linear effect of CH_4 production. Responses were adjusted for DMI, which was included as a co-variable to avoid possible confounding effects of alterations of DMI on response variables when inhibiting rumen methanogenesis (Ungerfeld, 2018). Individual VFA concentrations were calculated by multiplying total VFA concentration by each individual VFA molar percentage.

In all models fit, quadratic terms and interactions with $P \geq 0.10$ were removed and the reduced model refitted. A standard procedure in meta-regression is weighting each treatment mean by the reciprocal of its standard error normalized to unity (Sauvant et al., 2008). Standard errors of H_2 emission, total VFA concentration, and propionate molar percentage were not available for all of the experiments meta-regressed; for those response variables, models built with both weighted and non-weighted treatment means are reported. In the case of acetate, propionate and butyrate concentration, standard errors were not available for any experiment, as treatment means for those variables were calculated from total VFA concentration and each individual VFA molar percentage; thus, only models built with non-weighted treatment means are reported for acetate, propionate and butyrate concentration.

JMP® (2016) was used in all statistical analyses.

We also estimated if accumulation of H_2 might affect fermentation through the inhibition of NADH oxidation to NAD^+. Gibbs energy change (ΔG) of NADH oxidation in confurcation with reduced ferredoxin to produce H_2 ($NADH + Fd_{red}^{2-} + 3\,H^+ \rightarrow NAD^+ + Fd_{ox} + 2\,H_2$) was calculated based on $\Delta G° = -102$ KJ/mol (Van Lingen et al., 2016), $R = 8.3145$ L kPa mol^{-1} K^{-1}, $T = 312$ K, $NAD^+/NADH = 2.56$ (Hino and Russell, 1985), $Fd_{ox}/Fd_{red}^{2-} = 0.1$ (Buckel and Thauer, 2013) and intracellular pH $= 7.0$ (Van Lingen et al., 2016).

CONSEQUENCES OF INHIBITING ENTERIC METHANE PRODUCTION

Inhibiting methanogenesis cannot be considered an isolated intervention. It causes profound changes in the flows of metabolic hydrogen in rumen fermentation, and eventually in the animal's post-absorptive metabolism (Ungerfeld, 2018, 2020). It is important to understand the consequences of inhibiting rumen methanogenesis to design strategies to adapt

and optimize the animal nutritional management to take advantage of physiological changes occurring. The ultimate goal is to achieve a pronounced and sustained decrease in enteric CH_4 production while maintaining or improving animal productivity and profitability.

Because CH_4 release to the atmosphere is a loss of energy for ruminants, it has been proposed that inhibiting enteric CH_4 production can improve ruminant productivity (Czerkawski and Breckenridge, 1975). However, a previous meta-analysis found that inhibiting enteric CH_4 production did not consistently enhance animal productivity, which was partially attributed to the decrease in CH_4 production not being sufficiently pronounced for energy savings to be evident (Ungerfeld, 2018; Beauchemin et al., 2020). A recent large scale trial with growing cattle receiving 3-NOP found a tendency toward improved feed efficiency associated to a 26% decrease in CH_4 emissions (Alemu et al., 2021). The latter result suggests that a large number of animals may be required for experiments to have statistical power to detect significant gains in productivity when CH_4 decrease and energy savings are moderate. Likewise, some studies with much fewer animals but a pronounced inhibition of enteric CH_4 production reported increases in the efficiency of feed conversion to body mass gain (Davies et al., 1982; McCrabb et al., 1997; Vyas et al., 2016; Kinley et al., 2020; Roque et al., 2021). In this regard, both the environmental need to achieve a strong mitigation in the emissions of enteric CH_4 as a greenhouse gas (section Pronounced Decrease of Enteric Methane Emissions) and the theoretical expectations that energy savings from CH_4 emissions could translate into benefits in animal productivity, point toward seeking pronounced inhibition of rumen methanogenesis as a goal for ruminant production.

In our meta-analysis, inhibition of rumen methanogenesis by 50% or more was overall associated with lower DMI ($P = 0.020$; **Figure 2**), in agreement with a previous meta-analysis comprising a broader range of extent of methanogenesis inhibition (Ungerfeld, 2018). A positive relationship between CH_4 production and DMI within a large intercontinental database has been reported (Niu et al., 2018). In that study, however, differences in DMI were the result of different diets, hence it was DMI that drove CH_4 production as a response. In the present analysis, diet composition was virtually constant within each experiment, therefore changes in DMI were the result of the addition of very small amounts of antimethanogenic additives, and not of changes in diet composition.

We found an interaction between CH_4 production and the random effect of the experiment on DMI ($P = 0.022$; **Figure 2**). In order to understand the interaction between methanogenesis inhibition and the experiment effect on DMI, the random effect of the experiment, both as a main effect and in its interaction with energy losses in CH_4, was replaced by: (i) the fixed effect of the type of animal (sheep, goats, heifers, steers or dairy cows), (ii) the fixed effect of the percentage of concentrate in the diet, or (iii) the fixed effect of the type of inhibitor, including halogenated compounds as a group [hemiacetal of chloral and starch, chloral hydrate, chloroform, bromochloromethane, and bromoform contained in *Asparagopsis* spp. (Stefenoni et al.,

FIGURE 2 | Relationship between dry matter intake (DMI, kg/d) and methane production (CH_4, mol/d) by experiment. The x-axis is $\log_{10}$-scaled for better visualization of low CH_4 experiments. The adjusted model included the random effect of the experiment (exp) and its interaction with DMI: (1) non-weighted treatment means ($N = 64$): DMI $= 6.80$ (± 1.10; $P < 0.001$) + exp, random ($P = 0.003$) + 0.14 (± 0.054; $P = 0.020$) CH_4 + exp × CH_4, random ($P = 0.022$); $R^2 = 0.99$; (2) weighted treatment means ($N = 46$): DMI $= 7.93$ (± 1.32; $P < 0.001$) + exp, random ($P = 0.012$) + 0.14 (± 0.052; $P = 0.021$) CH_4 + exp × CH_4, random ($P = 0.033$); $R^2 = 0.99$.

FIGURE 3 | Energy losses as exhaled dihydrogen (H_2, MJ/d) as a function of energy losses as exhaled methane (CH_4, MJ/d) by experiment. The adjusted model included the random effect of the experiment (exp) and both response and regressor were root-square transformed: (1) non-weighted treatment means ($N = 48$): $\sqrt{H_2} = 1.23$ (± 0.18; $P < 0.001$) + exp ($P = 0.057$) − 0.38 (± 0.065; $P < 0.001$) $\sqrt{CH_4}$; (2) weighted treatment means ($N = 33$): $\sqrt{H_2} = 1.13$ (± 0.17; $P < 0.001$) + exp ($P = 0.40$) − 0.28 (± 0.070; $P < 0.001$) $\sqrt{CH_4}$.

2021)] or 3-nitrooxypropanol (3-hydroxypropyl nitrate, or 3-NOP). There were no interactions between CH_4 production and the type of animal ($P = 0.74$) or the percentage of concentrate in the diet ($P = 0.23$); however, the decrease in DMI with methanogenesis inhibition was more pronounced when CH_4 production was inhibited by halogenated compounds than with 3-NOP ($P < 0.001$; results not shown). Palatability aspects might contribute to explain the interaction, as low palatability has been reported in some studies with *Asparagopsis* spp. (Muizelaar et al., 2021; Stefenoni et al., 2021), and in contrast, no palatability issues were found with 3-NOP (Lee et al., 2020).

Dihydrogen is a central metabolite in rumen fermentation that is rapidly produced and consumed chiefly by methanogenesis, but also by other pathways such as propionate formation. Because of its fast turnover rate, only traces of H_2 are found in the typical rumen fermentation (Hungate, 1967; Ungerfeld, 2020). Scientists conducting the first experiments in which rumen methanogenesis was inhibited *in vitro* or *in vivo* noticed the occurrence of an atypical accumulation of H_2 (Bauchop, 1967; Rufener and Wolin, 1968; Trei et al., 1971). In the 15 experiments analyzed herein including at least one treatment in which CH_4 production was inhibited by 50% or more, the response in energy losses as H_2 as function of energy in CH_4 was non-linear and variable (**Figure 3**). Losses of energy in H_2 as a proportion of energy saved in CH_4 not formed increased with methanogenesis inhibition. For example, energy losses as H_2 predicted by the model at 50, 80 and 100% inhibition of methanogenesis represented 7.1% ($CI_{95} = [1.8, 12\%]$), 11% ($CI_{95} = [4.5, 17\%]$) and 19% ($CI_{95} = [9.2, 29\%]$), respectively, of the energy spared from CH_4 production with respect to Control treatments.
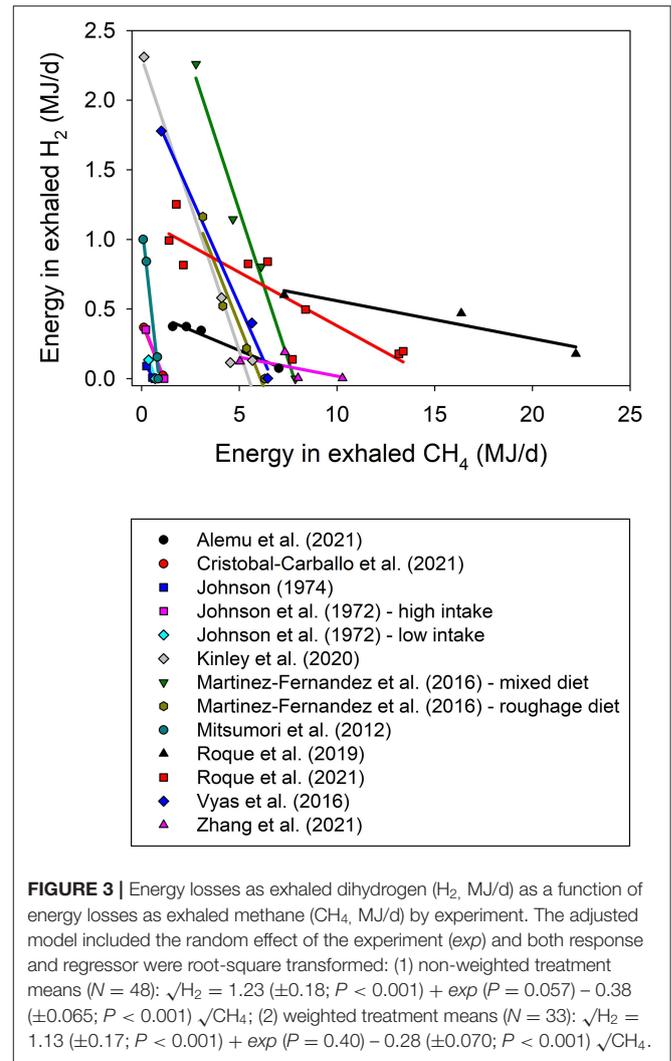
Apart from causing a loss of energy as exhaled H_2, elevated H_2 concentration in the rumen can inhibit NADH re-oxidation and hence fermentation (Wolin et al., 1997). We examined

FIGURE 4 | Relationship between total volatile fatty acid concentration (VFA, mM) in the rumen and methane production (CH_4, mol/d) by experiment. The x-axis is $\log_{10}$-scaled for better visualization of low CH_4 experiments. The adjusted model included the random effect of the experiment (exp) and dry matter intake (DMI, kg/d): (1) non-weighed treatment means ($N = 37$): total VFA = 75.5 (±7.73; $P < 0.001$) + exp, random ($P = 0.067$) + 1.48 (±1.06; $P = 0.18$) DMI + 0.74 (±0.69; $P = 0.29$) CH_4; $R^2 = 0.84$; (2) weighted treatment means ($N = 33$): total VFA = 74.0 (±6.69; $P < 0.001$) + exp, random ($P = 0.24$) + 2.08 (±1.13; $P = 0.086$) DMI + 0.62 (±097; $P = 0.53$) CH_4; $R^2 = 0.71$.

FIGURE 5 | Relationship between the rumen acetate to propionate concentration molar ratio (Ac/Pr, mM/mM) and methane production (CH_4, mol/d) by experiment. The x-axis is $\log_{10}$-scaled for better visualization of low CH_4 experiments. The adjusted model included the random effect of the experiment (exp) and dry matter intake (DMI, kg/d): (1) non-weighted treatment means ($N = 35$): Ac/Pr = 2.53 (±0.26; $P < 0.001$) + exp, random ($P = 0.10$) − 0.11 (±0.043; $P = 0.021$) DMI + 0.13 (±0.034; $P < 0.001$) CH_4; $R^2 = 0.82$; (2) weighted treatment means ($N = 27$): Ac/Pr = 2.98 (±0.39; $P < 0.001$) + exp, random ($P = 0.15$) − 0.14 (±0.050; $P = 0.019$) DMI + 0.12 (±0.036; $P = 0.003$) CH_4; $R^2 = 0.85$.

the theoretical and empirical evidence of the possibility of H_2 accumulation inhibiting fermentation and digestion. When rumen methanogenesis is inhibited, concentration of dissolved H_2 increases from its typical range of 0.2–30 μM (Ungerfeld, 2020) to up to 550 μM (Zhang et al., 2020). NADH-ferredoxin confurcation appears as a predominant mechanism of NADH re-oxidation to H_2 in the rumen as genes encoding for reversible bifurcating hydrogenases are widespread in rumen microorganisms and are highly transcribed (Greening et al., 2019). Given that, we estimated ΔG of NADH oxidation in confurcation with reduced ferredoxin as a function of dissolved H_2 concentration. At 550 μM dissolved H_2, the reaction would still appear to be weakly exergonic ($\Delta G = -18.5$ kJ; **Supplementary Figure 2**). *In situ* fiber degradation at 550 μM H_2 was not compromised (Zhang et al., 2020). Similarly, other studies and meta-analyses have not found effects of the inhibition of methanogenesis on apparent digestibility in the overall tract (Jayanegara et al., 2018; Ungerfeld, 2018; Kim et al., 2020) and *in situ* (Nolan et al., 2010; Martínez-Fernández et al., 2014).

Total VFA concentration can also be a proxy to evaluate the effects of inhibiting rumen methanogenesis on fermentation. Total VFA concentration in the rumen was unaffected by CH_4 production adjusted by DMI ($P = 0.29$; **Figure 4**). However, the use of total VFA concentration, rather than VFA actual production, as a proxy of fermentation has limitations as VFA concentration is not a sole result of VFA production but it is also affected by VFA absorption, passage, incorporation into microbial biomass, and changes in rumen volume (Dijkstra et al., 1993; Kristensen, 2001; Hall et al., 2015). Even though, digestion and fermentation did not seem to be impaired by the inhibition of methanogenesis and H_2 accumulation, it is recommendable to generate results on actual VFA production and true nutrient digestibility to better understand the consequences of elevated H_2 concentration resulting from methanogenesis inhibition on fermentation and digestion.

Inhibiting methanogenesis shifts rumen fermentation from acetate to propionate (**Figure 5** and **Supplementary Figure 2**), which has been explained based on the Monod function

FIGURE 6 | Relationship between rumen propionate concentration (Pr, mM) and methane production (CH_4, mol/d) by experiment. The x-axis is $\log_{10}$-scaled for better visualization of low CH_4 experiments. The adjusted model included the random effect of the experiment (*exp*) and dry matter intake (DMI, kg/d): $Pr = 18.7$ (± 4.86; $P < 0.001$) $+ exp$, random ($P = 0.062$) $+ 1.06$ (± 0.64; $P = 0.13$) DMI $- 0.45$ (± 0.28; $P = 0.12$) CH_4; $R^2 = 0.94$ ($N = 33$). Treatment means are not weighted by the reciprocal of the standard error (SEM) normalized to unity, as SEM were not reported.

of microbial growth and displacement of thermodynamic equilibrium between acetate and propionate (Janssen, 2010). Even though the acetate to propionate molar ratio decreased with methanogenesis inhibition ($P < 0.001$; **Figure 5**), no clear overall relationship between CH_4 production adjusted by DMI and rumen propionate concentration was observed in the present analysis ($P = 0.12$; **Figure 6**). There was a linear decline in acetate concentration with decreased CH_4 production adjusted by DMI ($P = 0.015$; **Supplementary Figure 3**), and no association with rumen butyrate concentration ($P = 0.95$; **Supplementary Figure 4**).

The effects of inhibiting rumen methanogenesis on total and individual VFA concentration *in vivo* have been reported in numerous studies, but changes in VFA production and absorption caused by the inhibition of methanogenesis have not been characterized. Individual VFA have different post-absorptive effects, and changes in the absorbed VFA profile could have profound effects on ruminant post-absorptive metabolism (DiCostanzo et al., 1999). It would be important to determine the *in vivo* response of propionate production to methanogenesis inhibition, because propionate is the principal glucose precursor

in ruminants (Aschenbach et al., 2010; Larsen and Kristensen, 2013). The response of gluconeogenesis to a potential increase in propionate absorption from the rumen would depend on both the animal's requirements for glucose and the liver uptake of other glucogenic precursors (Loncke et al., 2020). Acetate and propionate have differential effects on the synthesis of milk fat and protein, as shown in intraruminal infusion experiments (Ørskov et al., 1969; Sheperd and Combs, 1998; Maxin et al., 2011; Urrutia and Harvatine, 2017). Also, propionate is a satiety signal in ruminants and an increase in propionate absorption may thus decrease voluntary intake (Allen, 2014). A stimulation of satiety may partially explain the decrease in DMI with methanogenesis inhibition found herein with experiments with >50% inhibition, and previously with a greater range of methanogenesis inhibition (Ungerfeld, 2018).

If the decrease in rumen acetate concentration resulting from methanogenesis inhibition reflected lower acetate production, an ensuing lower flow of acetate absorption could be a potential concern as it would be expected to decrease milk fat content (Maxin et al., 2011; Urrutia and Harvatine, 2017). However, experimental evidence with 3-NOP showed, on the contrary, a tendency to increase milk fat content (Kim et al., 2020). In any case, if some chemical inhibitors of methanogenesis cause decreases in milk fat content, a possibility to overcome this problem, at least partially, could be dosing reductive acetogens to harness accumulated H_2 in acetate production (Ungerfeld, 2020).

An increase in plasma glucose concentration in dairy cows with rumen methanogenesis inhibited by 3-NOP might reflect an increase in propionate absorption (Haisan et al., 2017). Conversely, the lack of effect of 3-NOP on plasma glucose concentration observed by Melgar et al. (2020a) may agree with the numerical lack of effect of 3-NOP on propionate concentration (as estimated from total VFA concentration and propionate molar percentage) in that study, if lack of change in propionate concentration in turn indicated lack of effect of 3-NOP on propionate production. Melgar et al. (2020a) suggested that the lower plasma insulin concentration that they observed concomitant with unchanged circulating glucose could be the result of favored glucose partitioning toward milk production in detriment of other tissues competing for glucose uptake.

Less research has examined the consequences of inhibiting rumen methanogenesis on microbial anabolism and its implications for the synthesis of microbial protein, the major source of amino acids for ruminants. Inhibition of methanogenesis *in vitro* causes a severe and consistent decrease in the recovery of metabolic hydrogen in propionate, butyrate, CH_4 and H_2, and it was speculated that some of the missing reducing equivalents might be accounted for by incorporation of ammonium into carbon chains to synthesize microbial amino acids (Ungerfeld, 2015). Inhibiting methanogenesis in *in vitro* rumen cultures has increased microbial nitrogen production in some studies (Guo et al., 2007; Ungerfeld et al., 2007) but not in others (Russell and Martin, 1984; Ungerfeld et al., 2019). In another *in vitro* study, methanogenesis inhibition stimulated microbial de novo synthesis of amino acids from ammonium

with a starch substrate, but not with a less fermentable cellulose substrate (Ungerfeld et al., 2020). Mitigation of enteric CH_4 may indirectly affect nitrogen metabolism of the animal, but more research is needed to understand the changes elicited by methanogenesis inhibition.

Effects of inhibiting rumen methanogenesis on microbial long chain fatty acid metabolism have received little attention, and have mostly focused on microbial biohydrogenation of dietary long chain fatty acids rather than on microbial synthesis of long chain fatty acids, a process highly demanding of metabolic hydrogen (Ungerfeld, 2015). Czerkawski et al. (1975) supplemented sheep with linseed oil, and observed a stimulation of the incorporation of ^{14}C-labeled acetate into bacterial lipids, although CH_4 production was not reported in that study. Inhibiting rumen methanogenesis in sheep and *in vitro* with carbon tetrachloride, chloroform or amichloral inhibited biohydrogenation of unsaturated long chain fatty acids, in particular the reductive steps (Kemp and Lander, 1976). In another study with sheep, the methanogenesis inhibitor chloral hydrate had little effect on long chain fatty acid composition of digesta flowing to the small intestine (Mathers and Miller, 1982). 3-Nitrooxypropanol supplemented to heifers did not affect total long chain fatty acids in rumen contents but increased content of saturated fatty acids (Zhang et al., 2021).

Rumen biohydrogenation of long chain fatty acids has important implications for milk fat synthesis (Dewanckele et al., 2020) and fat composition of milk (Nguyen et al., 2019) and meat (Scollan et al., 2017). An increase in the percentage of de novo synthesized short- and medium-chain fatty acids in milk fat of dairy cows and goats supplemented with 3-NOP or bromochloromethane has been reported, as well as less consistent decreases in vaccenic and rumenic acids, and total mono- and polyunsaturated fatty acids, and increases in total saturated fatty acids (Abecia et al., 2012; Hristov et al., 2015; Melgar et al., 2020a,b, 2021). Some of those changes, along with the changes in long chain fatty acids in rumen contents observed by Zhang et al. (2021) might suggest a stimulation of the last steps of rumen biohydrogenation by increased availability of metabolic hydrogen resulting from the inhibition of methanogenesis.

COST EFFECTIVENESS OF INHIBITING RUMEN METHANOGENESIS

Inclusion of chemical inhibitors of methanogenesis or antimethanogenic algae in ruminant diets will represent an additional feeding cost. For the adoption of these additives by producers, their addition to ruminant diets will have to be made cost-effective through economic incentives, improvements in animal productivity, favorable physiological changes, or combinations of those possibilities. It is also possible that widespread adoption of antimethanogenic additives will allow scaling up their production thereby decreasing their cost.

Climate friendly labeling of animal products can allow reaching high value niche markets, although the range of consumers willing to pay a premium price may not be broad enough (Leahy et al., 2020). Economic incentives to farmers have been used successfully to decrease greenhouse gas emissions. For example, in France a scheme of Payment for Environmental Services to producers was introduced to stimulate the use of omega-3 rich feed resources such as linseed and grass fodders, resulting in an estimated 11% decrease in CO_2e emissions (Le Gloux et al., 2021). A rising global tax to CH_4 emissions has also been proposed (United Nations Environment Programme and Climate and Clean Air Coalition, 2021). Possible carbon pricing and taxation schemes to livestock production and regulatory policies have been discussed (FAO, 2019).

Improving cost effectiveness of inhibiting rumen methanogenesis could result from the incorporation of increased H_2 emission into additional propionate formation or reductive acetogenesis (Ungerfeld, 2013, 2020). An increase in propionate contribution to gluconeogenesis may save other glucose precursors, such as glucogenic amino acids, for alternative uses. Alternatively, basal diets could perhaps be formulated to be less glucogenic when rumen CH_4 production is inhibited, and still meet animal requirements for glucose (Ungerfeld, 2018). This reformulation of diets could decrease the use of concentrates in regions or countries where they are relatively expensive or little available, and contribute to making the methanogenesis inhibition intervention cost effective even if animal productivity is not improved. It is true that diets with less concentrates are more methanogenic (Johnson and Johnson, 1995; Janssen, 2010), but potent inhibitors of methanogenesis can override the diet effect and cause a strong inhibition in enteric CH_4 even with high roughage diets (McCrabb et al., 1997; Martinez-Fernandez et al., 2016; Roque et al., 2021). Research is needed on the optimization of the flows of metabolic hydrogen in rumen fermentation and on understanding and matching changes in absorbed VFA to basal diet composition and animal requirements.

If inhibiting methanogenesis stimulates synthesis of microbial amino acids with fermentable diets, it may be possible to replace expensive plant protein supplements with urea (Ungerfeld et al., 2020), which might also help the cost-effectiveness of the intervention.

OTHER ASPECTS

Most CH_4 mitigation strategies have been developed for intensive production systems in industrialized economies (Harrison et al., 2021), and a critical point to implementation at the farm level is that both chemical inhibitors of methanogenesis and bromoform-containing algae have demonstrated CH_4 mitigation effects when incorporated into the ration and supplemented to animals on a daily basis. Globally, 47% of total enteric CH_4 is produced by ruminants in grazing systems, with approximately one-half of grazing enteric CH_4 emissions coming from non-dairy ruminants [calculated from FAO (2017)]. In particular, beef and sheep are often raised under extensive grazing, especially in developing countries. The possibilities of supplementing those animals with inhibitors mixed with concentrates may be limited as the animals are typically not managed daily, or concentrates

may not be available or may be expensive. It would be useful to develop means to deliver inhibitors of methanogenesis into the rumens of extensively grazing animals on a daily or continuous basis. Some possibilities could be slow-release rumen devices lasting for several months, inclusion of additives in blocks of salt and molasses, delivery in drinking water, or genetically modifying forages to produce inhibitors of methanogenesis. The latter possibility, however, may have environmental implications as inhibitors synthesized by plants would be less controllable compared to feeding the inhibitors directly to the animals.

Apart from the use of chemical inhibitors of methanogenesis and bromoform-containing algae, another possible approach to enhance the inhibitory effects of antimethanogenic interventions is to combine various strategies which, when applied alone may exert mild or moderate effects on enteric CH_4 production, but may achieve stronger effects when combined (**Supplementary Table 2**). The strongest effects of combinations of two antimethanogenic strategies in **Supplementary Table 2** occurred when nitrate (Van Zijderveld et al., 2010; Guyader et al., 2015, 2016) or 3-NOP (Smith, 2017; Vyas et al., 2018) were included. We are aware of only one study combining more than two antimethanogenic strategies: the combination of lauric and myristic acids, linseed oil and calcium fumarate decreased CH_4 production by only 10% in lactating dairy cows (van Zijderveld et al., 2011). Although in none of the studies in **Supplementary Table 2** the decrease in enteric CH_4 production was as large as in experiments in **Table 3** with chemical inhibitors and bromoform-containing algae, this line of research has yet to study various approaches to combining antimethanogenic strategies. Thus, it is still possible that stronger decreases in enteric CH_4 could be obtained in the future through combinations of treatments.

Mitigating greenhouse gases emissions from ruminant production requires a multi-factorial approach, including interventions to decrease enteric CH_4 production, replacing fossil fuels used in feed production with renewable sources of energy, using manure to generate biogas and recycling nutrients to soils to reduce the use of or replace chemical fertilizers (FAO, 2019). Carbon sequestration in soil is regarded as a tool to potentially offset enteric CH_4 emissions from grazing ruminants, especially in developing countries and locations with degraded soils. However, soil carbon content cannot increase indefinitely (Harrison et al., 2021) and therefore carbon sequestration in soil alone cannot offset raising emissions of greenhouse gases in the long term. Amelioration in the emissions of greenhouse gases from livestock can also be approached through the demand side by decreased consumption and waste. However, livestock production has important economic, social, cultural and environmental roles that must also be considered. Nutritionally balanced substitutions of livestock products may not be feasible for all human populations. Also, shifting pastures to croplands will result in oxidation of soil organic matter and increased CO_2 emissions (Leahy et al., 2020; Reisinger et al., 2021).

CONCLUSIONS

We propose that substantial decreases in the emissions of enteric CH_4 from individual animals are required to decrease global enteric CH_4 emissions from livestock, in view of the expected global growth of animal production. Chemical inhibitors of methanogenesis and bromoform-containing algae can both cause pronounced inhibition of rumen methanogenesis, but there are some challenges to their widespread adoption. Safety to animals, consumers and the environment has to be ensured, as well as approval by government agencies and acceptance by consumers. Incorporation of additives in ruminant diets has to be made profitable by an increased price paid to producers for animal products and/or enhanced productivity derived from optimizing animal feeding for the methanogenesis inhibition intervention. Research is needed to fully understand the consequences of methanogenesis inhibition on rumen fermentation and post-absorptive metabolism so that nutritional strategies can be designed that optimize the flow of absorbed nutrients altered by the methanogenesis inhibition intervention to match animal requirements and potentially improve animal productivity and efficiency.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found at: Open Science Framework (https://osf.io/xsfe9/?view_only=5bc486ae58314284a5e09b6243e8bcc3) doi: 10.17605/OSF.IO/XSFE9.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the present research presents re-analysis (meta-analysis) of previously published research results with ruminants. The present research did not involve any experimentation.

AUTHOR CONTRIBUTIONS

EU conceived the hypothesis, conducted the research, and wrote the original draft. KB and CM contributed ideas, reviewed, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2021.795200/full#supplementary-material

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