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Review

Opportunities and hurdles to the adoption and enhanced efficacy of feed additives towards pronounced mitigation of enteric methane emissions

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Abstract: This paper analyzes the mitigation of enteric methane (CH_4) emissions from ruminants with the use of feed additives inhibiting of rumen methanogenesis to limit global temperature increase to 1.5 °C. A mathematical simulation conducted herein predicted that pronounced inhibition of rumen methanogenesis with pure chemicals or bromoform-containing algae can contribute to limit global temperature increase by 2050 to 1.5 °C only if widely adopted at a global level and considering an efficacy higher than obtained in most studies. Currently, the most important limitations to the adoption of antimethanogenic feed additives are probably increased feeding cost without a consistent return in production efficiency, and achieving sustained delivery of inhibitors to the rumens of non-supplemented, extensively ranging animals. Economic incentives, and changes in rumen microbial metabolism caused by inhibiting methanogenesis, could potentially be used to make the methanogenesis inhibition intervention cost effective. Also, the composition of the methanogenic community, and rate of disappearance of inhibitors of methanogenesis in the rumen can influence the effective dose of the inhibitors, and hence the cost of their adoption. Possible means for sustained delivery of antimethanogenic compounds to extensively grazing animals are discussed. Limitations and knowledge gaps of these approaches, and future research directions, are examined.

Keywords: enteric methane; ruminants; mitigation; rumen; methanogenesis inhibition, feed additives; adoption; cost effectiveness

1. Enteric methane emissions and climate change

There is consensus that, in comparison to 2 °C or even higher levels of global temperature increase, limiting global temperature increase to 1.5 °C will diminish the frequency and severity of extreme climate events in the next decades [1]. Methane (CH_4) atmospheric concentration has doubled since industrial times and is currently second to carbon dioxide (CO_2) in causing global warming [2]. In addition to reaching net zero emissions of CO_2, achieving a strong, rapid and sustained decrease in CH_4 emissions is key to rapidly limit global warming [3]. This is largely due to CH_4´s relatively high global warming potential (28 times greater than CO_2 in a 100-year period), and relatively short life (9.25 ± 0.6 years) and perturbation time (12.4 ± 1.4 years) in the atmosphere [4]. Other benefits of decreasing CH_4 concentration in the atmosphere include preventing premature death due to ground level ozone pollution, and increasing crop yields [2].

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Global anthropogenic CH_4 emissions must be reduced by 40 to 45% by 2030 from 2015 levels to limit global warming to 1.5 °C in this century [2]. On the other hand, past and recent indicate continuous growth in the emissions of CH_4, with a recent acceleration [2, 4-5] and projected increases in the atmospheric concentration of CH_4 under the current scenario [2, 6]. Agriculture is a major source of short-term global warming through its emissions of CH_4 [4]. Enteric CH_4 emitted by domestic ruminants is the main source of agricultural CH_4 and accounts for about 30% of total CH_4 emissions from human activities [2, 7]. Emissions of CH_4 by livestock have increased by 51.4% between 1961 and 2018 [7]. The average necessary decrease in enteric CH_4 emissions between 2020 and 2030 across various socioeconomic scenarios and climate models, compatible with a maximal 1.5 °C increase in global temperature, was estimated to be of 20% [2]. The objectives of this paper are to critically examine through a mathematical simulation the possibilities of decreasing enteric CH_4 emissions through sustainable intensification of ruminant agriculture and the use of feed additives inhibitors of methanogenesis, and to analyze the opportunities and barriers to widespread adoption of inhibitors of methanogenesis for pronounced mitigation of enteric CH_4 emissions.

2. Intensification, productivity, and enteric methane emissions

Intensifying ruminant production increases feed intake and productivity of the individual animal. Feed intake is the main driver of CH_4 production [8]. Increased feed intake thus results in greater daily CH_4 emissions per animal. On the other hand, as animal productivity increases, a lesser proportion of dry matter intake (DMI) and of CH_4 emitted by an animal is associated with the animal´s maintenance requirements, which has been called the "dilution of maintenance" effect. The result is a decrease in CH_4 emitted per unit of milk [9] or meat [10] produced, or CH_4 intensity. Between the 2000-2004 and 2014-2018 quinquennials, CH_4 intensity of meat and milk from dairy cattle, buffalo, sheep, and goat protein decreased globally and in most regions, although this was more variable for beef. Despite the decreases in CH_4 intensity, total CH_4 emitted globally by ruminants increased in the same period of time [7]. Due to the forecasted increase in production of animal products, Chang et al. [7] projected a global increase in total emissions of livestock CH_4 (including pigs and poultry) of between 51 and 54% by 2050 relative to 2012 assuming constant CH_4 intensities. With decreasing CH_4 intensities due to improved production efficiency following past trends, total global emissions of CH_4 from livestock were estimated to increase less, by 15 to 21% between 2012 and 2050 [7]. A similar analysis for wool production in Western Australia also revealed a relationship between increased animal productivity, due mostly attributed to improvements in reproductive performance, and decreased CH_4 intensity, along with increased total emissions of CH_4 [11]. Therefore, whilst production intensification and resulting improvements in animal productivity and feed efficiency can ameliorate livestock CH_4 emissions relative to a scenario with constant CH_4 intensity, total CH_4 emissions from livestock will likely continue raising, as a result of the increases in human population and per capita consumption of animal products, especially in developing countries [12-13].

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It has been estimated that agricultural emissions of CH_4 must diminish between 24 and 47% by 2050 relative to a 2010 baseline in order to contain global temperature increase to 1.5 °C [14]. Given that the main source of agricultural CH_4 is livestock [15], it is reasonable to assume that enteric CH_4 will also need to be decreased between approximately 24 and 47% between 2010 and 2050. In the same period, the consumption of bovine and ovine meat, and dairy products, is expected to expand by 58, 78, and 58%, respectively [12]. It follows that, in order to decrease enteric CH_4 emissions by 24% by 2050 relative to 2010 levels, global CH_4 intensity of beef, lamb and milk production, would have to decrease by 52, 57, and 52%, respectively, in relation to its 2010 levels. Likewise, decreasing enteric CH_4 emissions by 47% between 2010 and 2050 would require decreasing global CH_4 emissions intensity of beef, lamb and milk production by 66, 70, and 66%, respectively (calculations not shown).

The same as with CH_4, intensifying animal production and improving animal productivity also decreases the emissions intensity of carbon dioxide equivalents [CO_2e; the sum of the main three greenhouse gases (GHG) CO_2, CH_4, and nitrous oxide (N_2O), each weighted by its heat-trapping capacity over a 100-years period], i.e. CO_2e per kilogram of animal product, or carbon footprint. In some cases, decreasing the emissions of CO_2e per kilogram of animal product has allowed to lower the total number of animals sufficiently so as to decrease the total emissions of CO_2e of the livestock industries [e.g., Capper et al. [9]]. However, in various other cases the decrease in the emissions of CO_2e per unit of animal product occurring as a consequence of intensification was insufficient to compensate for the increase in animal production, resulting therefore in increased total CO_2e emissions from milk and beef production [16]. Whilst producing meat and milk with a lower carbon footprint is a highly desirable goal, intensification of animal production alone is unlikely to stop the increase in total emissions of GHG from ruminant production, and much less to decrease it. Specific, additional measures to ameliorate the emissions of CH_4 and other GHG from the livestock industry are also needed.

3. Mitigation of enteric methane emissions

It is challenging to reconcile the objectives of decreasing total emissions of enteric CH_4 from ruminant production and at the same time increase the global supply of animal products. Therefore, several strategies to mitigate enteric CH_4 emissions from ruminants are being investigated: increasing feed efficiency, genetic selection of animals with lower CH_4 production, modifying diet formulation and concentrate and forage processing, grazing management, addition of oils to the diet, chemical inhibitors of methanogenesis, dietary inclusion of algae with antimethanogenic compounds, alternative electron acceptors, phytocompounds, defaunation (elimination of rumen protozoa), immunization against methanogens, early life interventions, and archaeal phages, among others. For more information, readers are referred to various excellent published reviews [17-24].

The effectiveness of all [25-27] or some [28-33] enteric CH_4 mitigation strategies currently available has been quantified through various meta-analyses. In their meta-analysis, Arndt et al. [26] identified increasing the individual animal feed intake (by on average 58%, without altering the composition of the diet) as the most effective strategy

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to decrease CH_4 intensity of milk production (by on average 16.7%) while simultaneously increasing animal productivity. Secondly, they identified the utilization of inhibitors of methanogenesis [including 3-nitrooxypropanol (3-NOP) and bromoform-containing red algae *Asparagopsis* spp.] as the most effective strategy to decrease total daily emissions of CH_4 per animal (by on average by 35.2%), and emissions of CH_4 per kilogram of milk produced (by on average 31.8%), without negatively affecting animal productivity [26].

Using the average decreases in CH_4 production found in their meta-analysis, Arndt et al. [26] estimated that the adoption of increased feed intake or inhibitors of methanogenesis, or both antimethanogenic measures in combination, could allow containing global temperature increase by 1.5 °C by 2030, but not by 2050, even if applied under an unrealistic scenario of global 100% adoption [26]. The conclusions of the analysis by Arndt et al. [26] illustrate the challenges and difficulties of increasing ruminant production while decreasing the emissions of enteric CH_4 and CO_2e.

4. Projection of global enteric methane emission under different scenarios of intensification and adoption of inhibitors of methanogenesis

A projection of emission of enteric CH_4 from beef, lamb, and bovine milk production, and their sum, between present time and 2050 was herein conducted, combining scenarios of production intensification and adoption of feed additives inhibiting rumen methanogenesis. The evolution of total global emissions of enteric CH_4 was simulated considering future increases in global production of beef, lamb, and bovine milk, and, dependig on each scenario, future decreases in CH_4 intensity of beef, lamb, and bovine milk production.

The analysis considered two different scenarios for intensification of production: i) constant production efficiency, assuming constant 2016-levels of CH_4 emission intensities of beef, lamb, and bovine milk as reported by Chang et al. [7] for the 2014-2018 quinquennial, and ii) improved production efficiency, assuming decreasing CH_4 emission intensities of beef, lamb, and bovine milk at constant rates. Constant annual rates of decline in CH_4 intensity of beef, lamb, and milk production were calculated from changes in global CH_4 intensities reported for those industries by Chang et al. [7] between the 2000-2004 and 2014-2018 quinquennials.

Chang et al. [7] estimated CH_4 intensities using three different methods; an average of the values reported with the three estimation methods was considered for CH_4 intensities of global lamb and milk production. As for beef production, Chang et al. [7] reported an increase in CH_4 intensity between the 2000-2004 and 2014-2018 quinquennials with one of their methods of estimation, and decreases with the other two, with an increasing CH_4 intensity on average. Assuming that global CH_4 intensity of beef production between 2010 and 2050 is likely to decrease as production increases [10, 34], only the two estimations reporting declining CH_4 intensity of global beef production were averaged for the projection herein conducted. Annual, constant rates of decline in CH_4 intensity were then calculated between 2002 (corresponding to the 2000-2004 quinquennial) and 2016 (corresponding to the 2014-2018 quinquennial; Table 1).

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Table 1. Estimated rates of change of production and methane (CH_4) intensity of beef, lamb, and milk, between 2010 and 2050.

Animal product	Metric	Period used for estimation	Total change (%)	Rate (%/yr)	Source used for estimation
Beef	Production	2010 – 2050	58	1.15	FAO [12]
Lamb	Production	2010 – 2050	78	1.45	FAO [12]
Milk	Production	2010 – 2050	58	1.15	FAO [12]
Beef	CH_4 intensity	2002 - 2016	-0.52	-0.037	Chang et al. [7][1]
Lamb	CH_4 intensity	2002 - 2016	-7.27	-0.54	Chang et al. [7][2]
Milk	CH_4 intensity	2002 - 2016	-9.55	-0.71	Chang et al. [7][2]

[1]Average of the two negative estimates.

[2]Average of three estimates.

Both scenarios of production efficiency (constant or declining CH_4 intensity) were combined with four different scenarios of adoption of feed additives inhibitors of methanogenesis, as the most potent strategy available to mitigate total emissions per animal of enteric CH_4 [25-26]: 0, 25, 50, or 100% worldwide adoption of feed additives inhibitors of methanogenesis in global production of beef, lamb, bovine milk, and all three combined. Percentages of global adoption refer to the percentage of global beef, lamb, and bovine milk produced with the use of inhibitors of methanogenesis, rather than to the percentage of total animals receiving the additives. The adoption of inhibitors of methanogenesis was simulated to take place gradually within a five years period beginning in 2023, and as a linear function of time, until reaching the maximum for each scenario of adoption.

Methane intensity of beef, lamb, or bovine milk in each year and scenario was multiplied by global production of the corresponding animal product to obtain total CH_4 emissions associated to each product. Initially, the use of inhibitors of methanogenesis in milk production was modelled as causing a 31.8% decrease in CH_4 intensity, as reported by Arndt et al. [26]. Arndt et al. [26] did not report an effect of inhibitors of methanogenesis on CH_4 intensity of meat production; for meat production, a 26.6% decrease in CH_4 intensity of beef and lamb production, as the average of the range (-13.2 to -39.9%) for the effect of 3-NOP reported by Almeida et al. [25] for CH_4 intensity of milk and body mass gain, was initially considered for the analysis. Stronger effect of methanogenesis inhibitors in dairy than in beef animals agrees with a meta-analysis conducted for 3-NOP [28].

From projected increases of production of beef, lamb, and milk production of 58, 78, and 58%, respectively, between 2010 and 2050 [12], constant yearly rates of increase of 1.15, 1.45, and 1.15% for beef, lamb, and milk production, respectively, were calculated (Table 1). Because Chang et al. [7] reported CH_4 intensity as kg CH_4 per kg of animal protein (of

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beef, lamb, or bovine milk), the future global production of each animal product was also calculated as kilograms of protein according to FAO [35].

Figures 1 to 4 depict the predicted evolution between present time and 2050 of enteric CH_4 emissions for different scenarios in which 0, 25, 50, or 100% of beef, lamb, and milk, or the sum of all three products, is produced with the use of inhibitors of rumen methanogenesis. The evolution of enteric CH_4 production for each scenario of adoption of inhibitors of methanogenesis was simulated under constant, 2016-levels of CH_4 intensity, or under decreasing CH_4 intensities, according to the rates in Table 1. The upper and lower targets of 24 and 47% decrease in enteric CH_4 emissions by 2050 relative to 2010 levels, as required to maintain global temperature increase within 1.5 °C [14], are shown as references. The database used for the simulations is available [36].

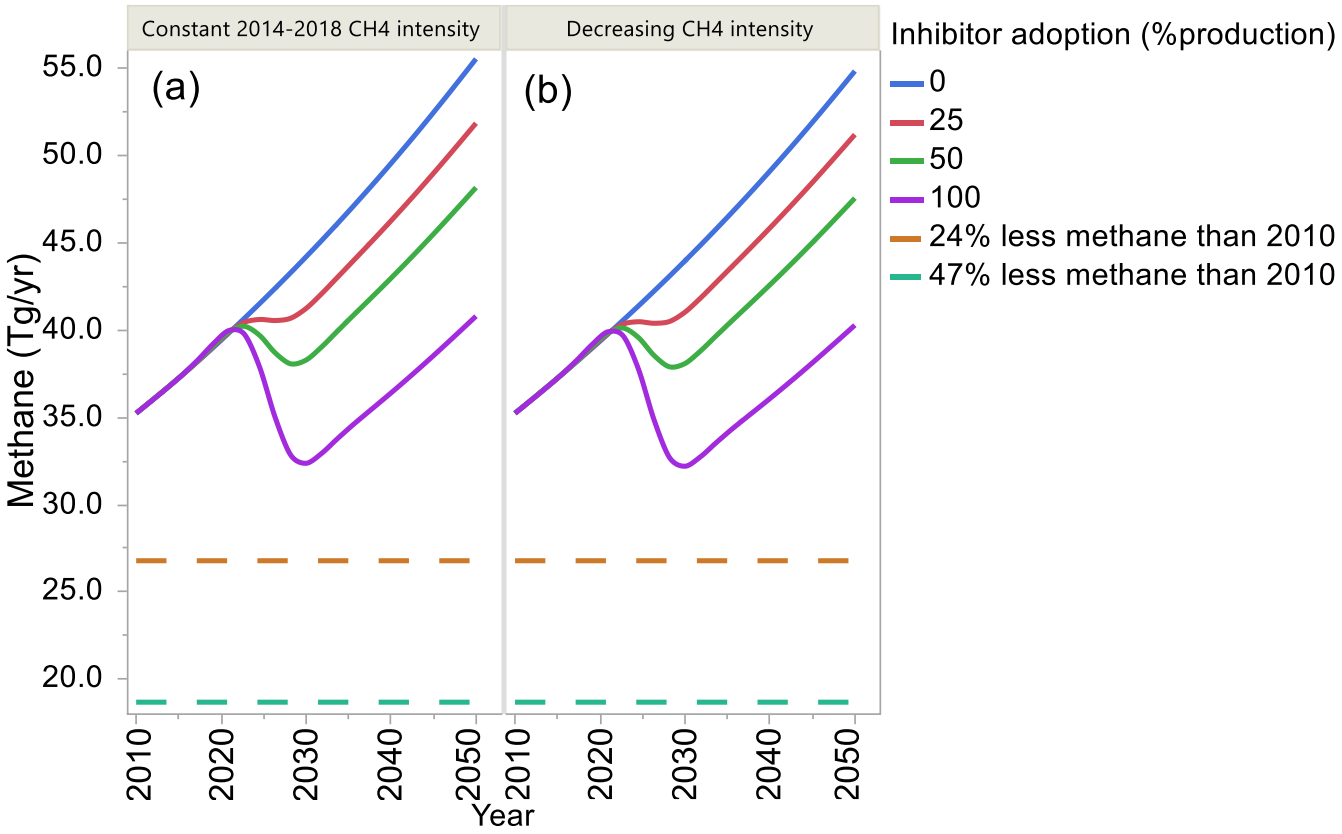

Figure 1. Predicted evolution of enteric methane (CH_4) emissions from global beef production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 26.6% is simulated to occur at 0, 25, 50, or 100% of global beef production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

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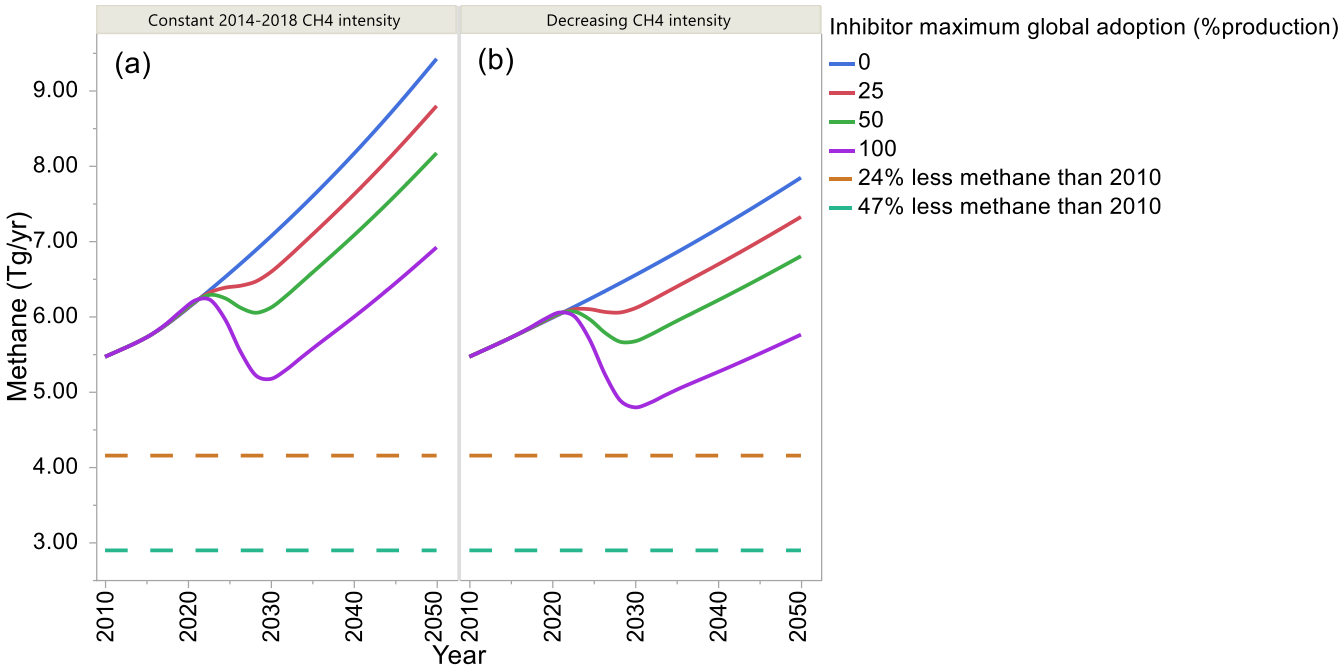

Figure 2. Predicted evolution of enteric methane (CH_4) emissions from global lamb production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 26.6% is simulated to occur at 0, 25, 50, or 100% of global lamb production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

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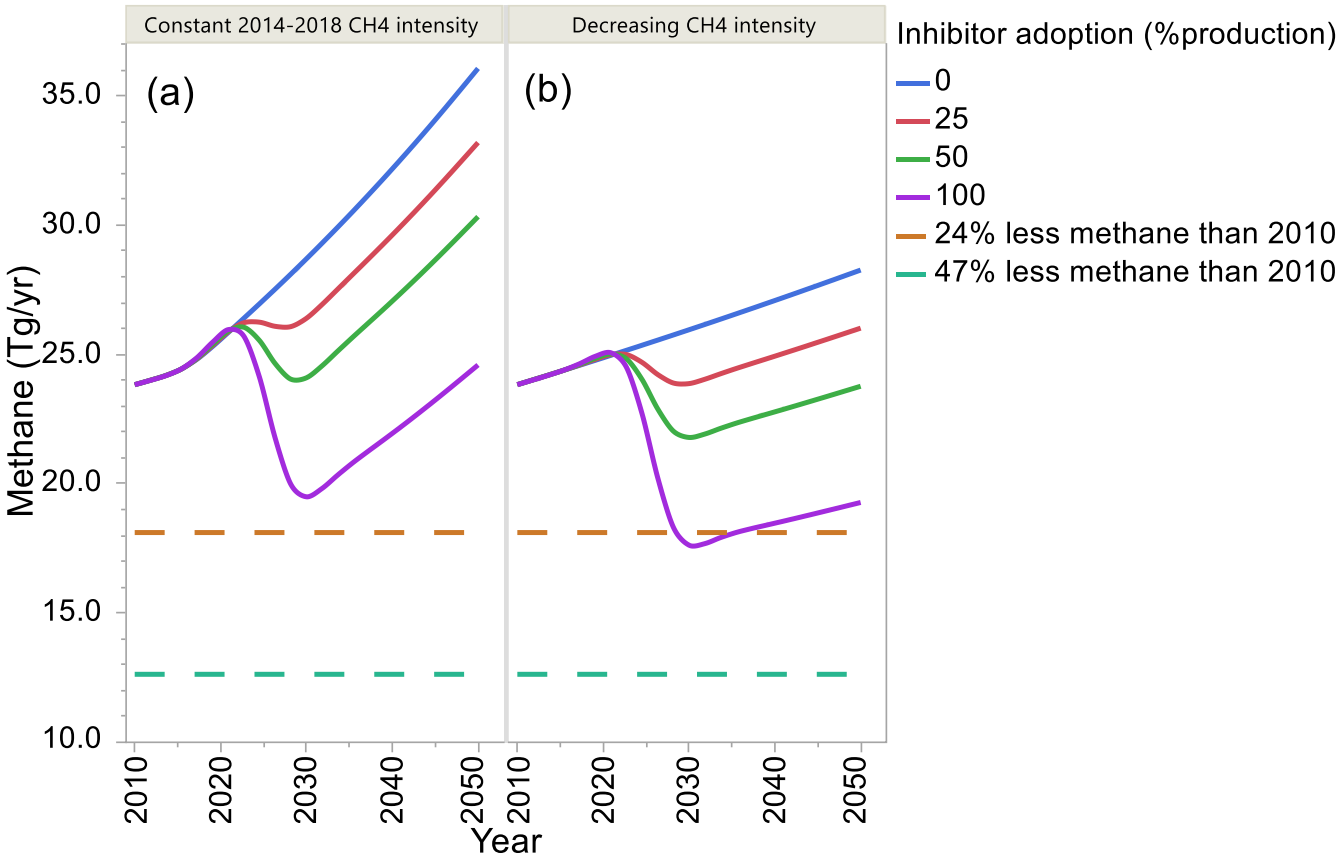

Figure 3. Predicted evolution of enteric methane (CH_4) emissions from global bovine milk production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 31.8% is simulated to occur at 0, 25, 50, or 100% of global lamb production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

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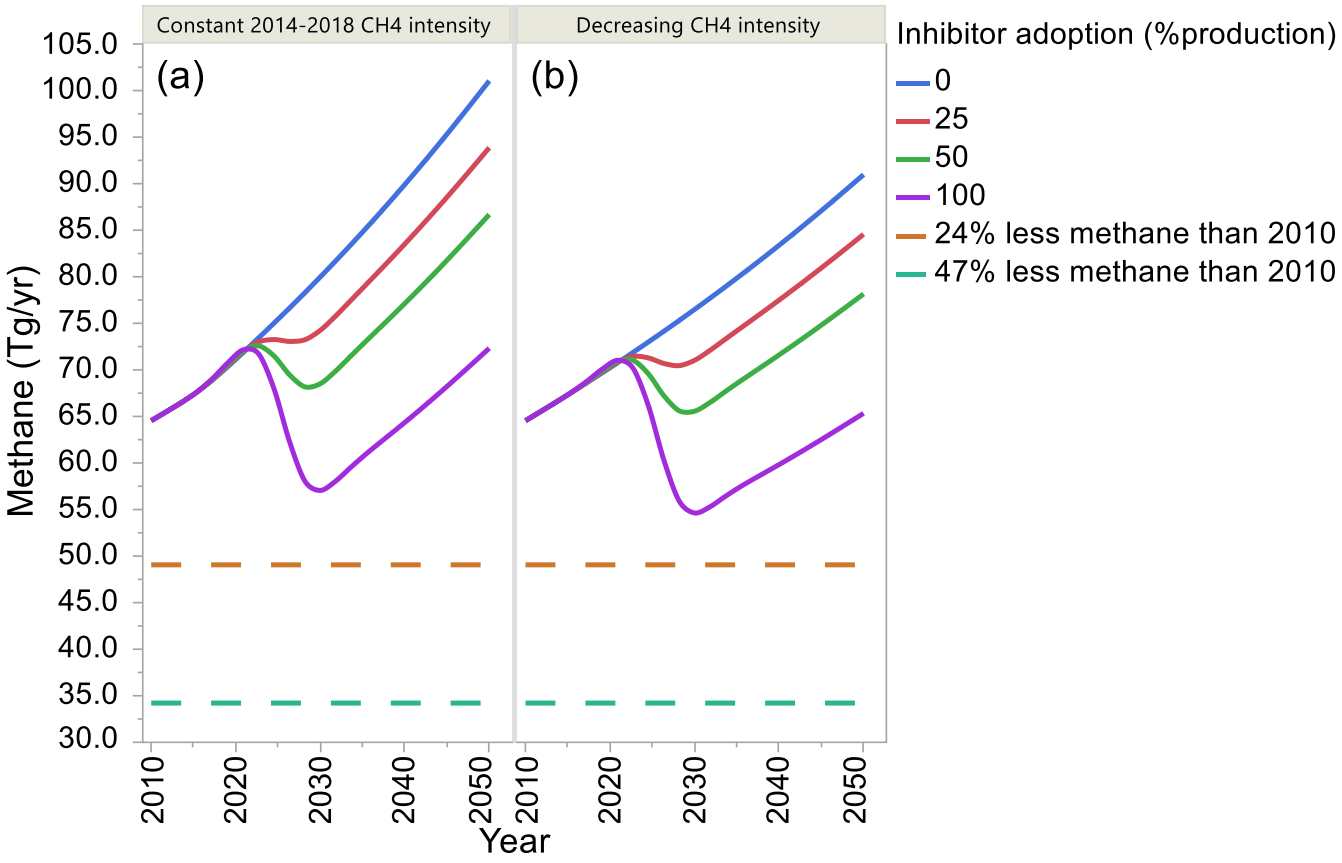

Figure 4. Predicted evolution of enteric methane (CH_4) emissions from global beef, lamb, and bovine milk production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 26.6% for beef and lamb and by 31.8% for milk production is simulated to occur at 0, 25, 50, or 100% of global production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

Considering a sustained decrease in CH_4 intensity resulting from improved animal productivity, no projected scenario of enteric CH_4 emissions from beef or lamb production would provide the required amelioration, even under an unrealistic 100% global adoption of inhibitors of methanogenesis (Figures 1 and 2). With 100% worldwide adoption of methanogenesis inhibitors, enteric CH_4 emissions from milk production would briefly decrease slightly below the upper 24% amelioration target around 2030, but would still not meet that target by 2050 (Figure 3). The same as beef and lamb production, total enteric CH_4 emissions from the sum of beef, lamb, and milk production was not be projected to meet the minimum 24% decrease in enteric CH_4 emissions at any point in time between 2023 and 2050, even with 100% worldwide adoption of inhibitors of methanogenesis (Figure 4).

5. Pronounced inhibition of rumen methanogenesis with feed additives

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Importantly, the use of feed additives inhibiting methanogenesis has allowed in some studies for considerably greater mitigation of enteric CH_4 production in comparison with the averages for methanogenesis inhibitors reported in the meta-analyses by Veneman et al. [27], Almeida et al. [25], and Arndt et al. [26]. Whilst in the vast majority of studies evaluating inhibitors of methanogenesis the extent of CH_4 decrease could be defined as moderate (i.e. ~30%), there have been various studies in which the inhibition of CH_4 production was considerably more profound (i.e. ~80% or more) [16]. Tables 2 and 3 summarize experiments in which the utilization of chemical inhibitors of methanogenesis or *Asparagopsis* spp. allowed decreasing CH_4 intensity of growth and fattening (Table 2) and milk production (Table 3) by 60% or more. There are studies not listed in Table 2 in which rumen methanogenesis was inhibited by 60% or more, but animal performance was not reported, and thus effects of methanogenesis inhibition on CH_4 intensity are not calculable (and thus cannot be used to calculate total emissions of CH_4); a more comprehensive list of experiments including treatments with inhibition of enteric CH_4 production equal or greater than 60% is presented in Supplementary Table 1, demonstrating that considerably more pronounced inhibition is possible. It should be noted that generally the extents of inhibition of CH_4 production on a daily per animal basis and of CH_4 intensity in Tables 2 and 3 are numerically very close. This is because, in most cases, inhibiting rumen methanogenesis does not cause important changes in animal performance [37]. It is worth noting that in 21 growth and fattening or maintenance experiments, but only in four two milk production experiments, a decrease in 60% of more in CH_4 production was obtained (Table S1), highlighting the need to investigate in promoting pronounced inhibition of rumen methanogenesis in dairy cows.

Modifying the previous analysis of prediction of enteric CH_4 emissions of section 4. by considering an average efficacy of inhibitors of methanogenesis of 60% decrease in CH_4 intensity of beef, lamb, and milk production, the enteric CH_4 amelioration required to maintain global temperature increase within 1.5 °C by 2050 could be met only if inhibitors of methanogenesis were employed in nearly 85% of beef production (Figure 5), ~75% of lamb production (Figure 6), and ~60% of milk production (Figure 7). Reaching a 24% decrease in combined enteric CH_4 emissions from beef, lamb, and milk production by 2050 would require a worldwide adoption slightly greater than 75% of inhibitors of methanogenesis with an efficacy of decreasing CH_4 intensity by 60% (Figure 8).

6. Opportunities and hurdles to increase adoption and efficacy of methanogenesis inhibitors

From the previous simulations, it appears that, under the likely scenario of improved productivity and decreasing CH_4 intensity [38], achieving the mitigation in enteric CH_4 from beef, lamb, and milk production required to maintain global temperature increase by 1.5 °C, needs attaining two factors: i) pronounced inhibition of rumen methanogenesis, i.e., use of inhibitors decreasing enteric CH_4 production by at least 60%, and if possible more, and ii) widespread adoption of inhibitors of methanogenesis in ruminant production.

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Table 2. In vivo growth and fattening experimental treatments resulting in 60% or more decrease in enteric methane (CH_4) production1.

Reference	Animal, diet	Inhibitor/algae (g/kg diet DM[2])	Experimental period (d)	Inhibition relative to Control treatment (% decrease in CH_4 animal^{-1} d^{-1})	Inhibition relative to Control treatment (% decrease in CH_4 kg ADG^{-1})	Performance		
						DMI	ADG	G:F
Trei et al. [39]	Lambs, mixed	2, 2, 2-trichloroacetamide (0.080)	90	67[3]	67[4]	NS[5]	NS	↑
Johnson et al. [40]	Steers, mixed	BCM (0.50)	28	~65[6]	~68[4]	NS	NS	-
Davies et al. [41]	Calves, mixed	ICI 13409 (0.20)	196	63[3]	66[4]	↓	↑	↑
Romero-Perez et al. [42]	Heifers, mixed	3-NOP (0.28)	112	59	60	R	NS	NS
Vyas et al. [43], finishing diet	Steers, high concentrate	3-NOP (0.2)	105	84	83	↓	↓	NS
Kinley et al. [44]	Steers, high concentrate	*Asparagopsis taxiformis* (1.8)	90	98	98	NS	↑	NS
Roque et al. [45]	Steers, high concentrate	*Asparagopsis taxiformis* (4.7)	63	82	83	↓	NS	NS
Alemu et al. [46]	Steers, high concentrate	3-NOP (0.108)	112	77	76[4]	↓[7]	↓[7]	↑[7]
Cristobal-Carballo et al. [47]	Calves, milk replacer, concentrate, partial mixed ration, pasture	Chloroform (0.050) plus 9, 10-anthraquinone (0.50)	84	90[3]	90	NS	NS	-

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[1]Only experiments reporting effects on performance and/or CH_4 intensity are presented. For a more complete list of experiments with at least one treatment with inhibition of CH_4 production equal or greater than 60% please refer to Table S1.

[2]Abbreviations: 3-NOP = 3-nitrooxypropanol; ADG = average daily body mass gain; BCM = bromochloromethane; CH_4 = methane; DM = dry matter; DMI = dry matter intake; G: F = body mass gain per kilogram of dry matter intake; ICI 13409 = 2,4-bis(trichloromethy1)-benzo [1, 3]dioxin-6-carboxylic acid.

[3]Methane concentration, rather than production, was measured, by rumenotomy [39, 48] or air expelled in a hood [41].

[4]Calculated from reported results.

[5]NS = effects reported as not significant ($P > 0.05$); ↑ = increase ($P < 0.05$); ↓ = decrease ($P < 0.05$); ↑ = tendency to increase ($0.05 \leq P < 0.10$); ↓ = tendency to decrease ($0.05 \leq P < 0.10$); - = not reported.

[6]Daily average, estimated from graph.

[7]K. Beauchemin, pers. comm.

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Table 3. In vivo milk production experimental treatments resulting in 60% or more decrease in enteric methane (CH_4) production[1].

Reference	Animal, diet	Inhibitor/algae (g/kg diet DM[2])	Experimental period (d)	Inhibition relative to Control treatment (% decrease in CH_4 animal^{-1} d^{-1})	Inhibition relative to Control treatment (% decrease in CH_4 kg FPCM^{-1})	Performance		
						DMI	MY	MY:F
Haisan et al. [49]	Cows, mixed	3-NOP (0.13)	28	60	61[3]	NS[4]	NS	NS
Roque et al. [50]	Cows, mixed	*Asparagopsis armata* (10)	21	67	61[3]	↓	↓	-

[1]Only experiments reporting effects on performance and/or CH_4 intensity are presented. For a more complete list of experiments with at least one treatment with inhibition of CH_4 production equal or greater than 60% please refer to Table S1.

[2]Abbreviations: 3-NOP = 3-nitrooxypropanol; CH_4 = methane; DM = dry matter; DMI = dry matter intake; MY = milk yield; MY: F = milk yield per kilogram of dry matter intake.

[3]Calculated from reported results.

[4]NS = no significant ($P > 0.05$) effects; ↑ = increase ($P < 0.05$); ↓ = decrease ($P < 0.05$); ↑ = tendency to increase ($0.05 \leq P < 0.10$); ↓ = tendency to decrease ($0.05 \leq P < 0.10$); - = not reported.

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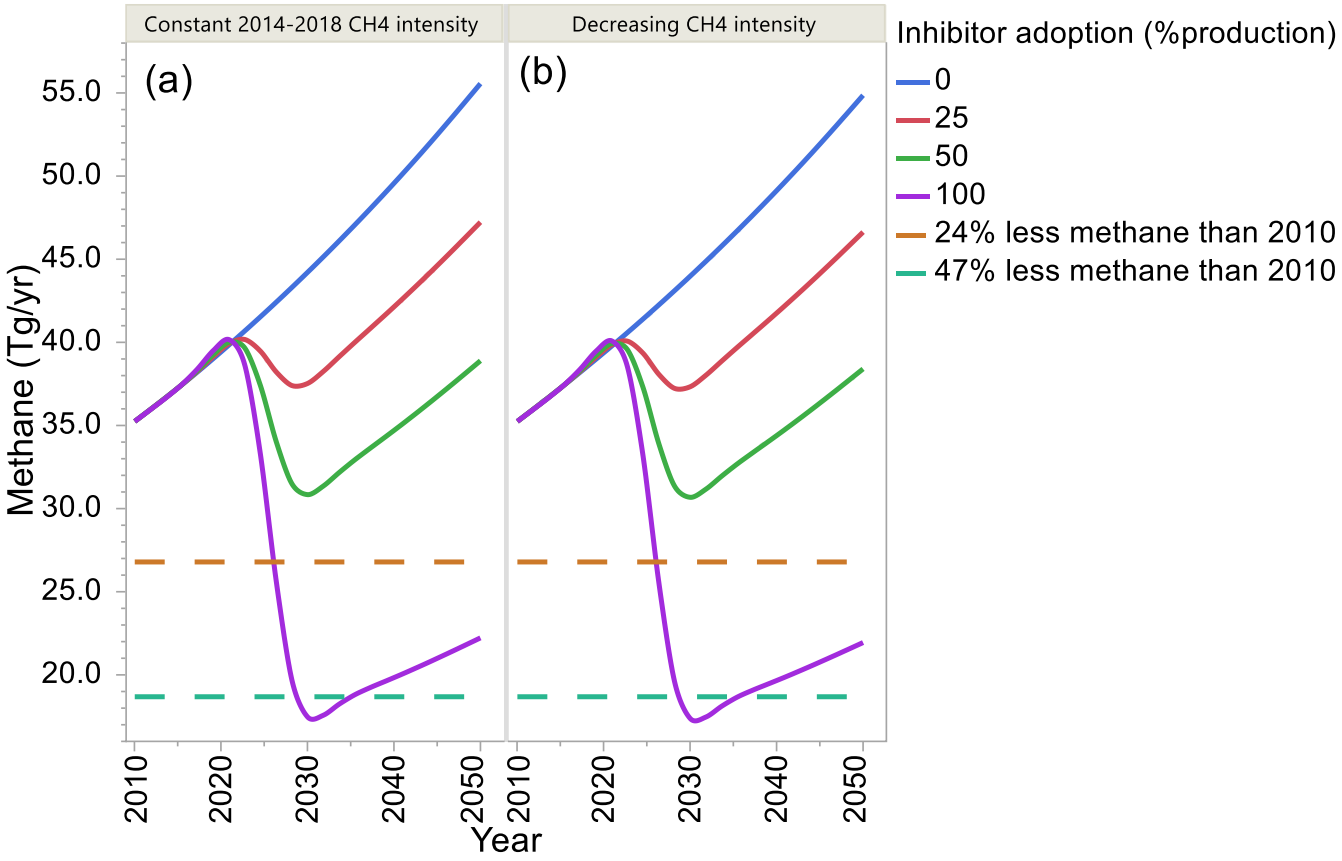

Figure 5. Predicted evolution of enteric methane (CH_4) emissions from global beef production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 60% is simulated to occur at 0, 25, 50, or 100% of global beef production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

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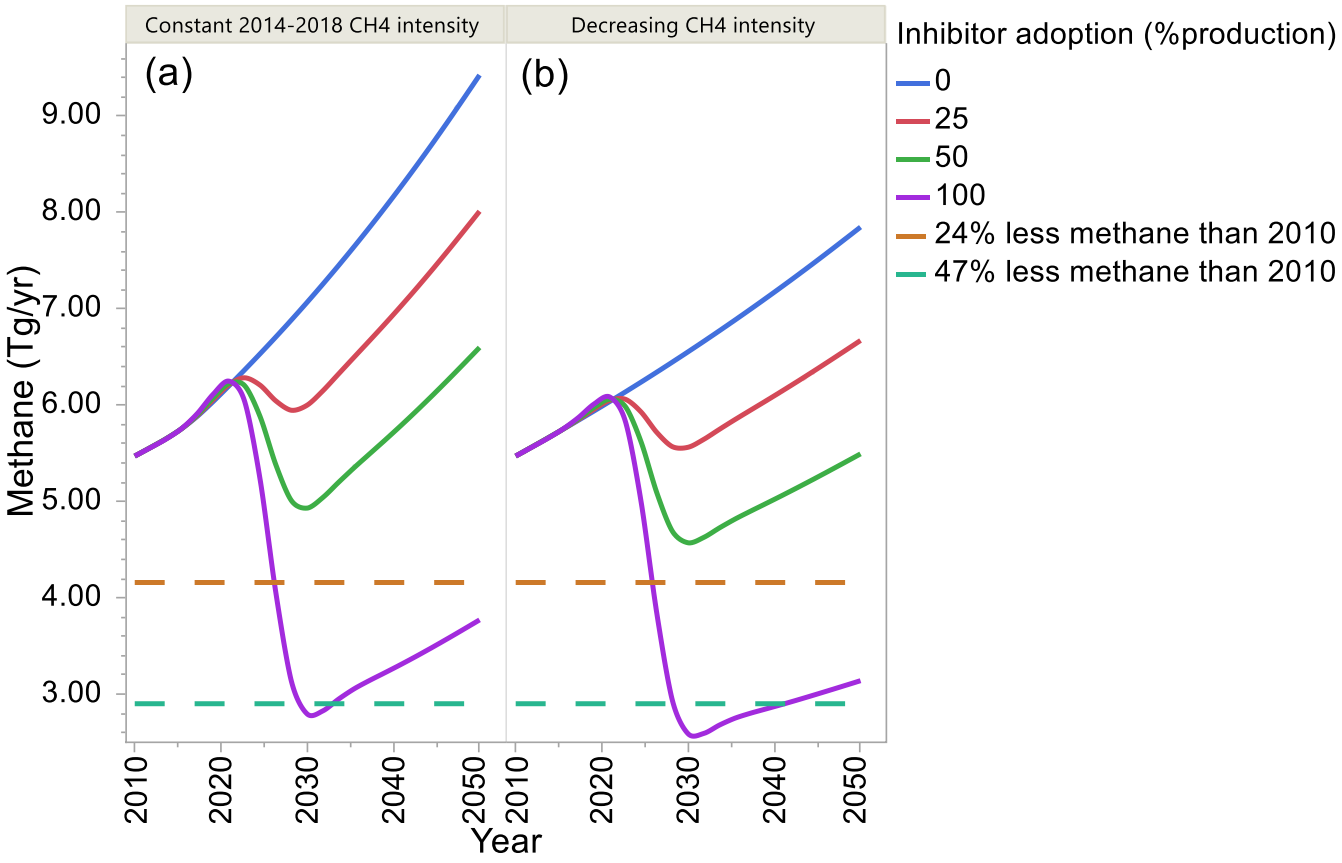

Figure 6. Predicted evolution of enteric methane (CH_4) emissions from global lamb production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 60% is simulated to occur at 0, 25, 50, or 100% of global lamb production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

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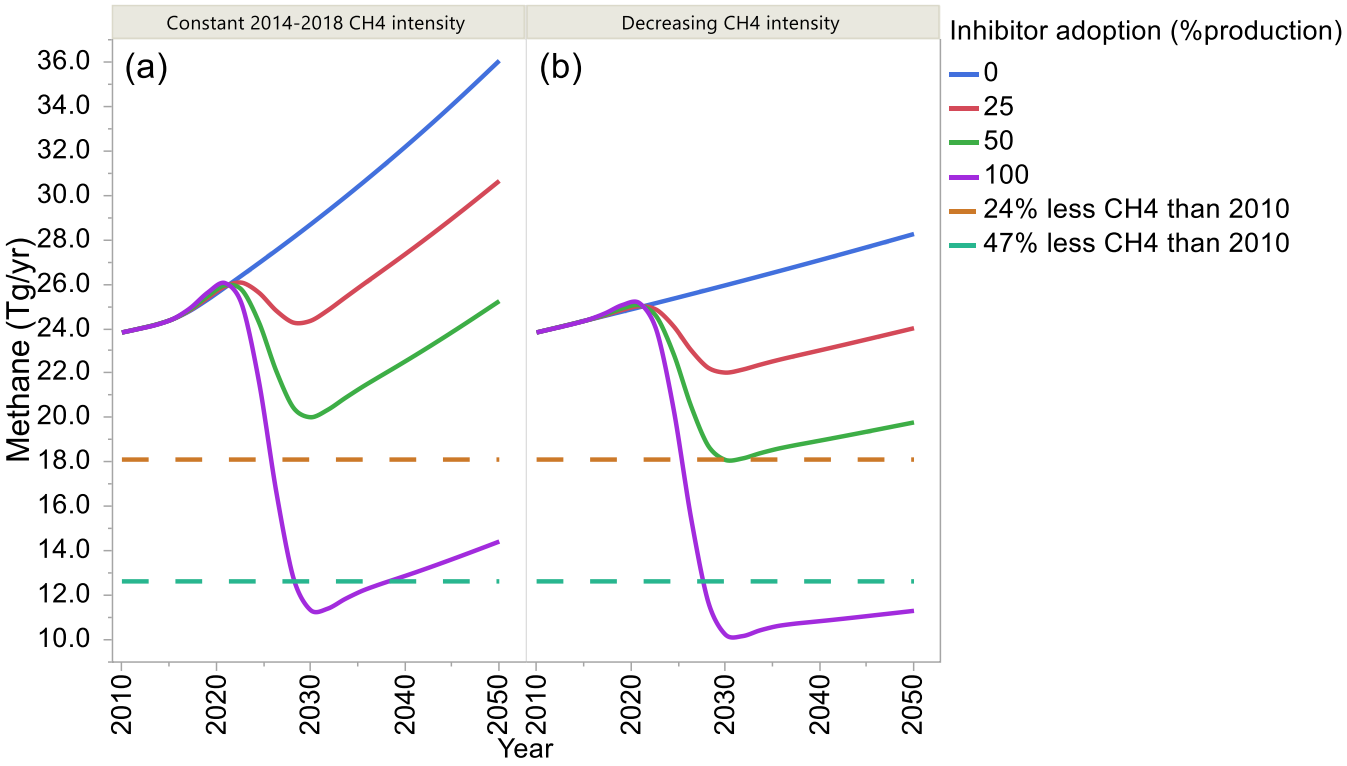

Figure 7. Predicted evolution of enteric methane (CH_4) emissions from global bovine milk production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 60% is simulated to occur at 0, 25, 50, or 100% of global lamb production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

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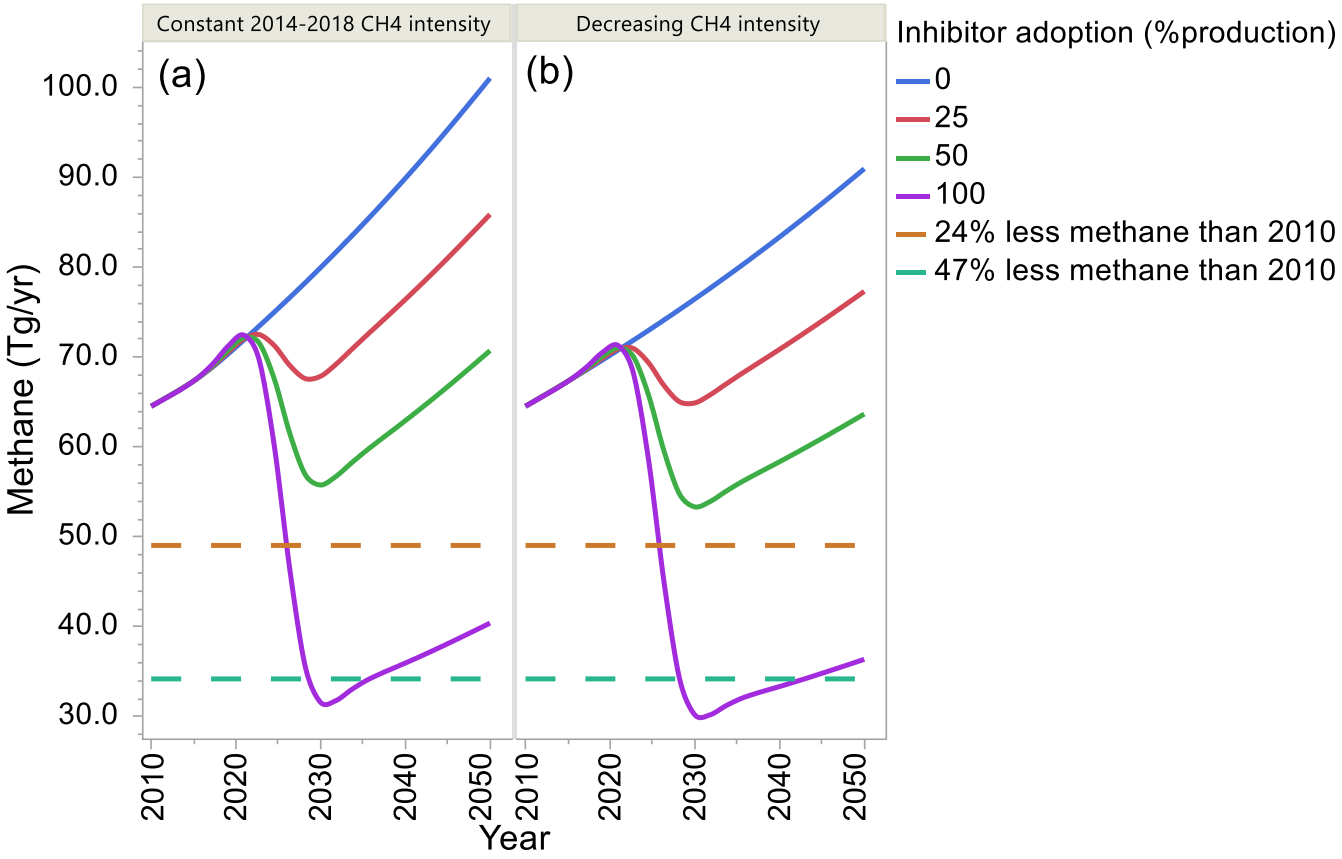

Figure 8. Predicted evolution of enteric methane (CH_4) emissions from global beef, lamb, and bovine milk production between 2010 and 2050 considering (a) constant CH_4 intensity and (b) declining CH_4 intensity according to Table 1. Adoption of feed additives inhibitors of methanogenesis decreasing CH_4 intensity by 60% for beef, lamb and milk production is simulated to occur at 0, 25, 50, or 100% of global production. Decreases in enteric CH_4 emissions of 24 and 47% relative to 2010 required to maintain global temperature increase within 1.5 °C according to different socioeconomic scenarios and climate models [14] are shown in dashed lines.

With regards to the first point, i.e., extent of inhibition, the experiments and treatments resulting in pronounced inhibition of rumen methanogenesis presented in Tables 2 and 3 and Supplementary Table 1, and in Ungerfeld et al. [16], do not constitute a majority of studies of chemical inhibition of methanogenesis. Importantly however, meta-analyses also show that the extent of inhibition of methanogenesis by 3-NOP is positively related to dose [28-29, 31]. The effectiveness of *Asparagopsis* at inhibiting methanogenesis has also been reported to be positively related to its dose [44-45, 50-52] and content of bromoform [53]. The implication of this is that the extent of inhibition of rumen methanogenesis by feed additives is potentially greater than the averages obtained in meta-analyses if high-end doses are used.

It must be noted, however, that responses to dose of 3-NOP have not been linear in all individual studies evaluating multiple doses of 3-NOP [46, 54-56], and it is possible that under some conditions the inhibition of methanogenesis may plateau at doses lower

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than maximal. It is important to understand the reasons, other than the maximal dose examined in each study, behind the variation among studies in the linearity and the magnitude of the response of CH_4 production to dose of inhibitors. For example, the response of CH_4 decrease to 3-NOP in beef and dairy animals has been shown to decrease with increasing dietary NDF [28]. Conversely, using a very high dose of 1,200 mg 3-NOP/kg of substrate DM in semicontinuous culture, Schilde et al. [57] decreased CH_4 production by 97% without observing interactions between the dose of 3-NOP and the percentage of concentrate in the substrate incubated. Aspects such as diet and animal, affecting the composition of the methanogenic community, may affect the magnitude of the responses to inhibitors of methanogenesis.

With regards to the second point, the adoption of feed additives inhibitors of methanogenesis towards pronounced mitigation of enteric CH_4 requires of various aspects, apart from effectiveness and persistency, to be satisfied. Feed additives should not have negative effects on animal productivity, be safe for animals, consumers and the environment, the decreases in enteric CH_4 emissions should not be compensated by upstream or downstream emissions of other GHG, additives must be possible to implement in the production systems being considered, must be approved by government agencies, be acceptable to consumers, and be economically attractive for producers to adopt.

3-Nitrooxypropanol is regarded as safe within recommended and experimentally evaluated doses [58-59]. Increasing the dose of 3-NOP would minimally increase upstream emissions of fossil fuel CO_2 associated to manufacturing and transporting 3-NOP, as because of 3-NOP low levels of inclusion in diets, fossil fuel CO_2 associated to manufacturing and transporting 3-NOP is rather small in terms of CO_2e compared to CO_2e not emitted as CH_4 (calculations not shown).

Supplementing high doses of *Asparagopsis* can decrease DMI and milk production [50, 52] and cause rumen mucosa abnormalities and inflammation [51, 60]. Conversely, in other studies supplementing *Asparagopsis* improved growth and feed efficiency [44-45]. Bromoform, the main CH_4-suppressing compound in *Asparagopsis*, is a suspected carcinogen and stratospheric ozone-depleting agent [53], although the potential global ozone depletion caused by hypothetical global adoption of *Asparagopsis* was estimated to be relatively small [61]. Supplementing *Asparagopsis* has not resulted in passage of bromoform to meat, milk organs or feces [44-45, 50-52], with the exception of the first experimental day with non-adapted cows in the study by Muizelaar et al. [60]. However, supplementing *Asparagopsis* resulted in the passage of iodine and bromide to milk [52], and iodine to meat [45]. Bromoform is rapidly degraded by rumen cultures, mainly to dibromomethane [62], which is considered less toxic than bromoform [63]. An alternative might be the use of pure bromoform or dibromomethane, which would allow the exact dosing of the active compound independently of the content of bromoform in *Asparagopsis*. In addition, dosing pure bromoform (or dibromomethane) would avoid potential problems of excess iodine passing to milk and meat, although it would still result in bromide accumulation in milk.

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7. Cost effectiveness of pronounced mitigation of enteric methane emissions

Successful adoption of antimethanogenic strategies requires that they are economically attractive to producers [64]. The inclusion of 3-NOP, *Asparagopsis*, or any other inhibitor of methanogenesis developed in the future, in ruminant diets for achieving pronounced inhibition of rumen methanogenesis would raise feed costs, and, everything else unchanged, would make the use of antimethanogenic feed additives economically unattractive. In the rest of this paper, I will discuss some possible means to overcome the added costs of antimethanogenic feed additives:

1. Economic incentives;
2. Methanogenesis inhibition increasing feed efficiency;
3. Adjusting basal diet composition to the inhibition of methanogenesis;
4. Improving the effectiveness of inhibitors of methanogenesis

7.1. Economic incentives

It could be possible to overcome an increase in feed costs resulting from the inclusion of antimethanogenic feed additives in ruminant diets through establishing premium prices to producers for meat and milk from animals fed inhibitors of methanogenesis and emitting less CH_4. Ultimately, consumers would be rewarding environmentally friendly-labelled meat or milk produced with lesser emissions of GHG. However, the relative size of these type of niche markets at a global scale is uncertain [65]. Most of the growth in production of animal products is expected to occur in the developing world [12], where niche markets for food products are generally marginal. The payment of a premium prize for meat or milk products to encourage the adoption of inhibitors of methanogenesis may be feasible only in a relatively minor proportion of ruminant production markets situated in developed economies. Furthermore, higher prices of meat and milk associated with lower enteric CH_4 and CO_2e emissions might increase total CO_2e by further stimulating production in countries that already have a relatively high consumption of animal products.

Methane taxes are also a possibility to implement to stimulate the adoption of antimethanogenic measures, including feed additives inhibiting methanogenesis. A rising global tax has been proposed as an effective measure to mitigate emissions of CH_4. Models have predicted that responses in anthropogenic CH_4 abatement resulting from taxation would be largest in the fossil fuels sector, followed by agriculture, and lastly the waste sector; in the ranking of modelled responses to CH_4 taxation by subsectors, enteric fermentation is second to coal [2, 66]. The same as with the premium prices directed towards lowering enteric CH_4 emissions, it may be questionable whether enteric CH_4 taxation schemes would be implemented in developing economies increasing their production of ruminant products, at least in the short- and medium-term.

7.2. Methanogenesis inhibition increasing feed efficiency

Ruminant nutritionists have long known that CH_4 formation in the rumen is a loss of energy for the host animal and thus an inefficient process for ruminant production: "In the ruminant the waste products of digestion include the unused feed residues in the feces

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and relatively large amounts of methane gas, which is formed as a result of fermentation and serves no useful purpose" [67]. The realization of CH_4 formation in the rumen as an energy loss motivated in the 1960s and following decades various studies seeking to inhibit methanogenesis in rumen fermentation to improve the efficiency of ruminant production. Blaxter and Czerkawski [68] proposed that "The almost saprophytic role which methanogenic organisms appear to play in the rumen, obtaining their energy from the end products of the microbial fermentation of carbohydrates and amino-acids, and producing waste products of methane and heat in large amounts, suggests very strongly that if their activity could be reduced without an impairment of cellulolysis, an increase in the productivity of ruminants could be obtained". It was not until the 1980s and 90s when the concern about livestock CH_4 emissions causing global warming [69-70] started to shift the main goal of the research on the inhibition of rumen methanogenesis from improving energy efficiency to decreasing the environmental impacts of ruminant livestock [e.g., Johnson and Johnson [71], McCrabb et al. [72]].

Because ruminants lose between 2 and 12% of ingested gross energy as CH_4 [71], the possibility of enhancing feed efficiency through conserving energy otherwise lost as CH_4 could be thought as a strong incentive to pay for the utilization of feed additives to inhibit rumen methanogenesis. However, examination of the evidence does not lead to conclude about a consistent improvement in feed efficiency or animal performance when rumen methanogenesis is inhibited ([37]; Tables 1 and 2 and Table S1).

It has been suggested that the moderate inhibition of CH_4 production observed in most studies may not translate into large enough savings of net energy to elicit significant differences in feed efficiency or animal productivity [16-17]. Experiments with much larger number of animals would be thus needed to detect significant differences in feed efficiency; in that regard, Alemu et al. [73] working with 4048 steers in a commercial feedlot, reported a tendency towards a 2.5% increase in feed efficiency when CH_4 production was moderately inhibited by 26%. It seems reasonable to think that energy savings resulting from moderate decreases in CH_4 production may not be detected in most experiments with much smaller number of animals and lower statistical power. However, experiments with pronounced decreases in CH_4 production, still do not show consistent improvement in feed efficiency or animal performance (Tables 1 and 2 and Table S1).

Another factor that may contribute to explain why inhibiting rumen methanogenesis does not consistently benefit animal productivity is that not all of the energy spared from CH_4 formation is incorporated into products nutritionally useful for the ruminant host animal. Most notably, inhibiting CH_4 production typically results in release of dihydrogen (H_2) [74], which in the typical rumen fermentation with functional methanogenesis is a fermentation intermediate found at low concentration, and the main electron donor for CH_4 production [75]. Accumulation of H_2 is potentially problematic because H_2 expelled is in itself a loss of energy. In 14 experiments in which the inhibition of CH_4 production was greater than 50%, energy lost as H_2 followed a non-linear and variable relationship with energy lost in CH_4. Energy losses as H_2 could be described as moderate. For example, energy losses in H_2 at 80% inhibition of methanogenesis accounted for 11% [CI_{95} = 4.5, 17%] of the energy saved in CH_4 not formed. There has been speculation about incorporating

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H_2 into useful metabolic pathways through the use of electron acceptors or hydrogenotrophic microorganisms when methanogenesis is inhibited [76]. Benefits to animal productivity may not only depend on the sheer energy savings of H_2 incorporation, but also on the significance and metabolic fate of the absorbed sink of metabolic hydrogen for each type of animal, depending on its nutritional requirements [77].

Accumulation of H_2 resulting from inhibiting methanogenesis can impair re-oxidation of microbial NADH, increasing the NADH/NAD^+ ratio [78]. Insufficient availability of NAD^+ can theoretically halt fermentation [79]. Inhibiting methanogenesis in rumen in vitro cultures induced H_2 accumulation and decreased fermentation as estimated from production of volatile fatty acids (VFA) [80]. In vivo, however, there is no conclusive evidence of negative effects of inhibiting methanogenesis on apparent digestibility, or on VFA concentration adjusted by changes in DMI. It should be noted, however, that VFA concentration does not necessarily reflect VFA production. Changes in VFA production might be compensated by changes in rates of VFA absorption or passage, incorporation into microbial biomass, and changes in rumen volume; effects of inhibiting methanogenesis on actual production of VFA have not been determined [16, 37].

Apart from H_2, inhibition of methanogenesis in vitro [81] and in vivo [e.g., Martinez-Fernandez et al. [82], Martinez-Fernandez et al. [83], Melgar et al. [84]] has also resulted in increased concentration of other electron carriers intermediate in rumen fermentation: formate, lactate, and ethanol. Lactate formed in propionate absorption through the rumen wall is used as a substrate for gluconeogenesis [85]. Direct absorption of lactate from the rumen seems to be influenced by adaptation to high concentrate diets [86]. Whether lactate could be absorbed from the rumen and utilized for gluconeogenesis in methanogenesis-inhibited animals is unknown, but it seems reasonable to think it may. However, increases observed in lactate concentration in methanogenesis-inhibited rumens are much lower than what is observed in acidotic rumens, and, if absorbed, it may likely have a relatively small influence on the ruminant´s energy budget.

Results about responses of formate and ethanol to methanogenesis inhibition are scarce. Formate can reach about 6-12 mM concentration in methanogenesis-inhibited rumens [82-83]; in other studies, it accumulated to a much lower concentration below 1 mM [84]. Ethanol concentration has been found to increase with methanogenesis inhibition, yet to relatively low levels [84, 87]. It is unknown how the kinetics of formation and disappearance of formate and ethanol, respond to pronounced methanogenesis inhibition, and their metabolic fate, so as to establish the importance of these metabolites in the flow of carbon and metabolic hydrogen in the methanogenesis-inhibited fermentation, and fundamentally, their significance for the animal´s energy budget.

7.3. Adjusting basal diet composition to the inhibition of methanogenesis

Inhibiting methanogenesis is not an isolated intervention and causes profound changes in rumen fermentation. Alterations in both catabolic and anabolic processes may result in increased absorption of some nutrients and may open opportunities to decrease the needs for them to be supplied by the basal diet.

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Inhibiting rumen methanogenesis decreases the acetate to propionate concentration ratio, as predicted by the elevation of H_2 concentration [88] and confirmed in meta-analysis of in vivo experiments [16]. Although propionate concentration adjusted by DMI did not respond to methanogenesis inhibition [16], it is still possible that if increases in propionate production occur they may have gone unnoticed when measuring propionate concentration, because of compensatory changes occurring in propionate absorption. It is important to understand the responses of propionate production and absorption to methanogenesis inhibition because propionate is the main substrate for gluconeogenesis in ruminants [89]. If a positive response in propionate production to inhibiting methanogenesis could be shown experimentally through the use of labelled propionate, it would be important to understand the fate of the extra propionate absorbed and its metabolic consequences.

The meta-analysis by Loncke et al. [90] found that the formation of glucose from the sum of propionate, amino acids, and lactate, increased at decreasing rates as their flow to the liver increased. This response suggests that glucogenic precursors may exceed the animal´s demands for glucose as their availability increase. If inhibiting methanogenesis can be shown to increase propionate production in the rumen, perhaps basal diets could be modified to include less concentrates as glucogenic precursors, and still match the animal requirements for glucose. This could allow decreasing feed costs in regions where concentrates are expensive. It might also prevent the decrease in DMI observed when CH_4 production is inhibited, if the drop in DMI observed when inhibiting rumen methanogenesis [16, 37] is caused by greater propionate oxidation in the liver acting as satiety signal [91]. In regions of the world where cereal grains are not used for feeding ruminants, inhibiting methanogenesis might allow enhancing gluconeogenesis with forage-only diets.

Accumulated H_2 resulting from inhibiting rumen methanogenesis can also be incorporated into reductive acetogenesis, the reduction of CO_2 with H_2 to acetate. Proof of concept in vitro experiments in which reductive acetogens were added to methanogenesis-inhibited in vitro fermentation were successful at incorporating H_2 into acetate formation [92-94]. Raju [95] showed the occurrence of reductive acetogenesis in sheep rumens and its increase when methanogenesis was inhibited by acetylene. Because of the higher H_2-threshold of reductive acetogens compared to the methanogens so far cultivated, it is expected that reductive acetogens could decrease H_2 accumulation but H_2 concentration may still be higher compared to rumen fermentation with functional methanogenesis [76]. Animal production implications of enhancing reductive acetogenesis as a sink of metabolic hydrogen have been discussed [77].

Consequences of inhibiting rumen methanogenesis on microbial anabolism have received little attention. Incorporation of ammonium into the synthesis of microbial amino acids was stimulated by the methanogenesis inhibitor 9, 10-anthraquinone in rumen cultures growing on starch but not on cellulose [96]. If this finding could be confirmed in vivo and with a broader range of inhibitors of methanogenesis and real diets fed to ruminants, it may be possible to replace greater proportions of expensive plant protein

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supplements with urea, again lowering feed costs and favoring cost effectiveness of the use of inhibitors of methanogenesis.

The effects of inhibiting methanogenesis on rumen metabolism of fatty acids have potential implications for the quality of ruminant products. Decreases in milk fat percentage of vaccenic and rumenic acids, and mono- and polyunsaturated fatty acids observed when inhibiting rumen methanogenesis [52, 55-56, 84, 97-98] suggests an increase in biohydrogenation, perhaps stimulated by increased availability of reduced cofactors. This would be an undesirable consequence of the methanogenesis inhibition intervention, as the fatty acids profile in ruminant products would be richer in saturated fatty acids. Perhaps the decrease in mono- and polyunsaturated fatty acids could be lessened by adding to the diet sources rich in linolenic acid, such as fresh forages or linseed, when inhibiting rumen methanogenesis.

7.4. Improving the effectiveness of inhibitors of methanogenesis

Increasing the effectiveness of methanogenesis inhibitors can represent an avenue to decrease their cost, i.e. achieving more pronounced inhibition with current average doses, or the same extent of inhibition with lower doses than current averages. Variation in the effectiveness of 3-NOP has been empirically shown to be related to the type of animal and dietary fiber content [28]. A more mechanistic understanding of the effects of 3-NOP on different methanogens [99-100], as well as elucidating the mechanisms that contribute to the resistance of methanogens to inhibitors [101], can help designing means to improve their efficacy and cost effectiveness.

Differential sensitivity among different methanogens grown in pure culture to 3-NOP [102] and to the chemical inhibitor of methanogenesis 2-bromoethanesulfonate (BES) [95, 103] has been reported before. Both 3-NOP [102] and BES [104] inhibit methanogenesis as structural analogues of methyl-coenzyme-M, a methylated cofactor involved in the last step of methanogenesis. Whilst inhibition caused by 3-NOP is persistent [42-43, 55], inhibition caused by BES in sheep lasted for only 3 d, after which methane production returned to pretreatment levels [105].

Methanobrevibacter ruminantium M1, which has lost three genes required to synthesize coenzyme M [106] and therefore requires coenzyme M included in its growth medium [107], took up coenzyme M from the medium with a high-affinity transport system. Conversely, *Mbr. ruminantium* PS, which synthesizes coenzyme M, took up coenzyme M with a rate of less than 10% than *Mbr. ruminantium* M1 [108]. Mutants of *Methanococcus voltae* resistant to BES had a considerably reduced capacity to transport BES into the cell [109]. Inhibition of methane production by BES in *Mbr. ruminantium* M1 and *Methanosarcina* spp. could be diminished or reversed by addition of coenzyme M to the medium, demonstrating a competition for transport between coenzyme M and BES [108, 110]. The same as with BES, it appears conceivable that differences among methanogens in sensitivity to 3-NOP could also be related to transport of 3-NOP into the cell and the ability of methanogen species to synthesize coenzyme M. It is possible that the effectiveness of 3-NOP at inhibiting methanogenesis is influenced, among other factors,

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by the proportion of coenzyme M-synthesizing methanogens in the rumen methanogenic community, and by the concentration of coenzyme M in rumen fluid.

In vivo work has also revealed shifts in the methanogenic community when inhibitors of methanogenesis are supplemented. Chloroform inhibited *Mbr. gottschalkii* more than *Mbr. ruminantium* [83], and both chloroform and 3-NOP were more inhibitory to hydrogenotrophic *Methanobrevibacter* spp. than to methylotrophic *Methanosphaera* spp. [87, 100, 111]. Those results agree with Duin et al. [102], who found that *M. ruminantium* was the most sensitive to 3-NOP of the methanogens they evaluated, *Msp. stadtmanae* was more resistant, and methylotrophic *Ms. barkeri* and hydrogenotrophic *Methanomicrobium mobile* were the most resistant. On the other hand, 3-NOP decreased the abundance of *M. mobile* in rumen fluid [111]. Ungerfeld et al. [103] also found that methylotrophic *Ms. mazeii* was more resistant to BES and other inhibitors than *Mbr. ruminantium*, with *Mmb. mobile* being intermediate. It seems then that some chemical inhibitors evaluated may preferentially target hydrogenotrophic, over methylotrophic, methanogens. It is of much interest to understand if variation in the sensitivity of methanogens to particular chemical inhibitors is related to their metabolic pathway of CH_4 formation, i.e., hydrogenotrophic vs. methylotrophic methanogenesis, as the dietary content of methyl group precursors can influence the make-up of the methanogenic community and the relative importance of both methanogenic pathways. Furthermore, as H_2 thresholds differ among hydrogenotrophic, methyl-reducing, and methyl-fermenting methanogens [112], differential effects of 3-NOP on the different groups could have implications for the extent of H_2 accumulation and release occurring as a consequence of inhibiting rumen methanogenesis.

Antimethanogenic compounds differ in their mechanisms of action. For example, as discussed 3-NOP [102] and BES [104] inhibit methyl-coenzyme M reductase through being structural analogues of methyl-coenzyme M, a cofactor present in all known methanogens. Methane halogenated analogues such as chloroform or bromoform react with cobamides and block the transfer of a methyl group from tetrahydromethanopterin to coenzyme M [113]. Derivatives of *p*-aminobenzoic acid inhibit the synthesis of methanogenic cofactor tetrahydromethanopterin [114], and statins inhibit methanogens growth by impairing membrane lipids synthesis [115]. Through the understanding of these mechanisms of action, it may be possible to design different combinations or rotations of antimethanogenic compounds to target specific methanogenic communities varying in composition depending on diet and animal. Because methanogens less inhibited by a particular compound may partially occupy the niche and share of methanogenesis precursors left by those methanogens inhibited more severely, it is conceivable that combining antimethanogenic compounds targeting different methanogens could result in synergic effects.

Apart from microbiological factors related to the sensitivity of different methanogens to inhibitors, the effectiveness of inhibitors of methanogenesis is also influenced by the daily pattern of inhibitor concentration in the rumen. Rumen concentration of inhibitors of methanogenesis is affected by the mode of administration, the time elapsed since the last feeding episode, the rates of feed ingestion and rumen fluid outflow, changes in

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rumen volume, and rates of metabolism and absorption of each specific compound. Almost all 3-NOP is metabolized to 1, 3-propanediol within 24 h, and about 50% within 7 h [102]. Absorption of 3-NOP occurred in orally dosed rats, with plasma concentration peaking at 5 – 15 min after dosing [59], with no published results being available for ruminants. van Lingen et al. [116] modelled a peak in rumen concentration of 3-NOP of 0.055 mM 1.5 h after feeding, which gradually fell to 0 mM at about 12 h after feeding in animals fed twice per day a diet with 121 mg 3-NOP/kg DM. Bromoform was degraded in rumen in vitro cultures by 70 and 90% after 30 min and 3 h incubation, respectively [62].

Predicted fluctuations in 3-NOP concentration in the rumen agree with the diurnal pattern of CH_4 emissions of animals fed once [56] or twice a day [46, 73]. Dosing 3-NOP through the rumen cannula resulted in a relatively strong but short inhibition of methanogenesis, ultimately resulting in less than 10% decrease in daily methane production, presumably because of rapid washout of 3-NOP from the rumen [117]. Delivering methanogenesis inhibitors into the rumen in slow release forms may result in more sustained concentration and greater effectiveness at inhibiting CH_4 production, even in animals fed total mixed rations. On the other hand, slow release forms would likely increase manufacturing costs.

8. Adoption of inhibitors of methanogenesis in extensive systems

Globally, 37.4% of total enteric CH_4 emissions are generated by ruminants on pastures, 60.5% in mixed systems, and only 2.10% from animals in feedlots [35]. Mixed systems can in turn comprise an ample range of production systems, from low-cost, extensive systems based on different proportions of pastures and agricultural and agro-industrial by-products, to total mixed rations based on conserved forages and concentrates used in intensive dairy production operations with confined animals. Assuming that most intensive dairy operations using total mixed rations are located in North America and Europe, it can be estimated using data from FAO [35] that a total of 6.94% of enteric CH_4 globally could be emitted from ruminants in intensive production systems with confined animals (calculations not shown), the rest corresponding to extensive and semi-extensive systems.

For every peer-reviewed published study on enteric CH_4 mitigation conducted with grazing animals, 5.6 were conducted with confined animals (calculated from Vargas et al. [118]). Therefore, investment in research and development in the mitigation of enteric CH_4 production under intensive production conditions appears to be overrepresented relatively to the contribution of confined production systems to global enteric CH_4 emissions, and, conversely, information is lacking on enteric CH_4 mitigation in extensive and semi-extensive production systems. In particular, very little research has been conducted in extensive systems without any supplementation. This is especially important because, as discussed, antimethanogenic feed additives are the most potent means of decreasing enteric CH_4 emissions from ruminants [16, 25-26, 119], and they have been developed and evaluated to be delivered in feed supplemented to animals. Other means of delivery of antimethanogenic feed additives would have to be developed for extensively ranging animals without feed supplementation, such as salt and molasses lick

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blocks [120], very slow release forms, or in drinking water [16]. Also, perhaps genes encoding for bromoform biosynthesis in *Asparagopsis* spp. could be genetically engineered in forages, or directly in ruminants to deliver bromoform to the rumen in saliva. The latter possibilities, however, would require enough bromide content in the soil and in ingested forages, may have environmental implications, may affect the fitness and performance of bromoform-synthesizing plants or animals, and could be both technically and economically difficult.

Other strategies to mitigate enteric CH_4 production in extensively ranging animals not receiving supplementation are being investigated, such as the inclusion of tannin-containing legumes in pastures [121], the selection of grazing animals producing less CH_4 [122], early life interventions with potential long-lasting effects [47, 123], and immunization against rumen methanogens [124]. It is of much interest to continue research in those antimethanogenic strategies that could be applied to free-ranging animals, although so far mitigation of enteric CH_4 has been mild or moderate, results have sometimes been contradictory, and implications to animal productivity in different production systems need to be studied.

9. Final remarks

Enteric CH_4 emissions from ruminants is a moving target for mitigation. This is because, as ruminant production increases, the decrease in total enteric CH_4 emissions necessary to contain global warming augments with time. Simulations herein conducted of projected enteric CH_4 emissions under constant or decreasing CH_4 intensity, and different extents of adoption and effectiveness of inhibitors of methanogenesis, yielded two main conclusions: attaining decreases in enteric CH_4 emissions compatible to the maximum 1.5 °C increase in global temperature requires antimethanogenic feed additives to be widely adopted worldwide, and to consistently attain pronounced inhibition of rumen methanogenesis, i.e., beyond average inhibition so far obtained in most studies.

Worldwide adoption of antimethanogenic feed additives in turn requires ensuring animal, consumer, and environmental safety, approval by government agencies, acceptance by consumers, worldwide distribution of approved inhibitors and adequate technical support, finding effective and practical solutions to deliver the inhibitors to grazing animals, and cost-effectiveness of the inclusion of inhibitors in animal diets. Also, life cycle assessments should be conducted to ensure that decreases in the emission of enteric CH_4 are not offset by equal or greater increases in the emissions of other GHG.

Use of antimethanogenic feed additives will increase feeding costs, which, everything else being the same, will discourage their adoption. This is especially true for extensive production systems that rely solely on grazing or on the use of low cost by-products. For commercial extensive production systems such as ranching, keeping low costs of production is key to remain competitive in the business, whereas the possibilities for smallholders and pastoralists to increase production costs are limited by lack of finance and technology, access to markets, volatility of prices, and risks to sheer subsistence. In extensive production systems, and also in intensive and semi-extensive operations, the

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adoption of an antimethanogenic feed additive would likely be unattractive unless coupled to some benefit.

A premium price offered for meat and milk associated with lower CH_4 emissions could be attractive to producers so as to encourage the use of antimethanogenic feed additives. The development and growth of niche markets ready to pay a higher price for environmentally produced livestock products is conceivable in developed economies, but may be less likely to occur in developing countries, where most of the growth in animal production is forecasted to take place. This mechanism can contribute to catalyze the global adoption of antimethanogenic feed additives in the initial phases, although may be limited once high income markets ready to pay an extra price for animal products associated with lower emissions of enteric CH_4 have widely adopted the use of feed additives inhibiting methanogenesis. Methane taxes can be another economic incentive for CH_4 mitigation, but again, may be preferentially implemented in developed economies, at least initially.

Research on understanding the alterations in rumen and whole animal physiology and metabolism caused by inhibiting rumen methanogenesis may unveil new avenues for improving feed efficiency or lower the cost of basal diets. This path, however, is relatively long-term, and its results are uncertain. Continuing, and accelerating efforts in this direction is important and should be boosted, but should also be accompanied by other more immediate actions towards enhancing adoption and effectiveness of antimethanogenic feed additives, such as premium prices for low-enteric CH_4 animal products and CH_4 taxation, as well as implementation proven antimethanogenic strategies causing moderate or mild decreases in emissions of enteric CH_4 and CO_2e which can at the same time be productively and economically attractive, at least in some production systems. For example, ionophores cause a mild decrease in CH_4 production [125] and they also independently decrease CH_4 intensity by improving feed efficiency [126].

The simulations of projected global enteric CH_4 emissions conducted also showed that meeting enteric CH_4 mitigation targets to maintain global temperature increase within 1.5 °C will require pronounced, rather than moderate, inhibition of rumen methanogenesis. Increasing the dose of inhibitors can increase effectiveness, but will also increase the costs of production of meat and milk. Research has shown differences among pure cultures of methanogens in their sensitivities to different chemical inhibitors [95, 102-103]. In vivo work is unraveling the effects of inhibitors of methanogenesis on the methanogenic community composition and activity, as well as the effect of inhibitors on the kinetics of H_2 transactions through studying changes in abundance of hydrogenases genes and transcripts [99-100, 127-128]. Understanding differential sensitivities to inhibitors among methanogens may allow designing antimethanogenic strategies based on combinations or rotations of inhibitors that could act in synergism. If equally effective, rotation of inhibitors may be a more economical option than their combination.

There is a need for feasible, safe, and cost-effective technological solutions for pronounced mitigation of enteric CH_4 emissions. At the same time, the tension between decreasing enteric CH_4 emissions from ruminant production while at the same time

increasing ruminant production should also lead to critically examine the side of the equation which has been assumed as fixed in this analysis, that is, the demand for ruminant products. This is a complex aspect, because ruminant production has ample implications beyond emissions of GHG. Nutritional implications are important, as increases in the consumption of nutrient-rich ruminant products are largely forecasted to occur in developing countries where per capita consumption of animal products is presently low. There are also important economic, social, and environmental roles of ruminant production and its integration within crop production and its ability to use of non-arable land. Reduction in the consumption of ruminant products where it exceeds the nutritional requirements of human populations, in the use of arable land and human edible food in ruminant production, and of food waste, are additional means to ameliorate enteric CH_4 emissions.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: In vivo experimental treatments resulting in 60% or more decrease in enteric methane (CH_4) production.

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