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REVIEW ARTICLE

Research on enteric methane mitigation in Chile: Ten years of scientific insights

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ABSTRACT. Research on enteric methane abatement in Chile began approximately ten years ago and has steadily accelerated. A key challenge in developing strategies to decrease enteric methane emissions in Chile and other countries where ruminant production is largely pasture-based is that globally, most mitigation strategies have been developed and evaluated with confined ruminants fed total mixed rations, requiring adaptation and validation in pasture-based systems. In this review, we discuss *in vitro* and *in vivo* applied research of anti-methanogenic strategies conducted in Chile. The reviewed studies included the evaluation of grazing management, different genotypes and species of forages, seasonal forage crops, by-products, oilseeds, and algae supplementation, as well as the use of a chemical inhibitor of methanogenesis, 3-nitrooxypropanol, and the combination of more than one anti-methanogenic strategy. Global meta-analyses and reviews show that intensification of ruminant production consistently decreases methane output per unit of meat or milk and is often economically attractive to producers and acceptable to governments and consumers alike; however, it is generally associated with increased emissions of other greenhouse gases and can result in greater output of carbon dioxide equivalents. Results obtained in Chile with production intensification varied depending on the intensification strategy. We consider that at present, none of the anti-methanogenic strategies investigated thus far in Chile can be recommended for adoption in pasture-based systems. Important aspects to be addressed are the lack of published life cycle assessments, *in vivo* research in ruminants other than dairy cows, and a considerable proportion of research results available only as conference summaries. Efforts are ongoing to generate solutions to effectively and persistently decrease the emissions of greenhouse gases from Chilean ruminant livestock; however, more research is required to design, adapt, and evaluate enteric methane mitigation strategies applicable to pasture-based systems, which require greater funding from the public and private sectors.

Keywords: anti-methanogenic strategies, mitigation, grazing, pasture-based, research, situational analysis, future perspectives

INTRODUCTION

Increasing emissions of anthropogenic greenhouse gases [mainly carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)] have caused the average planetary temperature to rise by 1.1 °C in the 2011 – 2021 decade with respect to the 1850 – 1900 period, triggering extreme climatic events that negatively affect both natural systems and human populations worldwide (IPCC, 2023). There are currently ongoing efforts to limit the anthropogenic emissions of the main greenhouse gases to cap global warming to 1.5 °C with no or limited overshoot with respect to preindustrial temperatures to avoid the worst consequences of climate change in terms of frequency and severity of extreme climatic events, loss of ecosystems, food insecurity, negative impacts on human health, and social and economic disruptions (Hoegh-Guldberg *et al.*, 2018; IPCC, 2023). Compared to CO_2, CH_4 traps 28 times more heat in a 100-year period and has a much shorter lifetime in the atmosphere (Szopa *et al.*, 2021). Therefore, mitigating CH_4 emissions offers an opportunity to ameliorate global warming in the short term (IPCC, 2021; United Nations Environment Programme and Climate and Clean Air Coalition, 2021). However, the rate of increase in atmospheric CH_4 concentration has been rapidly accelerating since 2006, with a record in 2021 (Shindell *et al.*, 2024). In a concerted effort, 158 countries, including Chile, have agreed to decrease global CH_4 emissions by at least 30% by 2030, relative to 2020 levels (Global Methane Pledge, 2024).

Approximately one-third of anthropogenic CH_4 emissions originate from the rumen of domestic ruminants as a consequence of microbial digestion and fermentation of ingested feed, that is, enteric CH_4, which is released to the atmosphere mainly through eructation and respiration (Szopa *et al.*, 2021; United Nations Environment Programme and Climate and Clean Air Coalition, 2021). Whilst on the one hand it is highly important to decrease CH_4 emissions from ruminants, the global demand for livestock products, including ruminants meat and milk, is projected to continue to increase by 2050, particularly in developing economies

(FAO, 2023). The challenge of at the same time increasing ruminant production to satisfy human demands and decreasing enteric CH_4 emissions has prompted a global research effort to find solutions that can decrease CH_4 production in the rumen without negatively affecting ruminant production (Ungerfeld, 2022).

As the challenge of mitigating enteric CH_4 emissions from ruminant production has been gaining global attention (Beauchemin *et al*., 2020), Chile has been no exception. *In vitro* and *in vivo* studies to evaluate applied solutions to mitigate enteric CH_4 in Chilean ruminant production systems began at Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Remehue, Universidad Austral de Chile, and Universidad de Concepción in the early 2010s. A key challenge to develop strategies to control enteric CH_4 emissions in Chile and other countries where most ruminant production is pasture-based is that most enteric CH_4 mitigation strategies, although not all, have been designed and developed for confined ruminants fed total mixed rations (Vargas *et al*., 2022). It has been estimated that 27.5% of global emissions of enteric CH_4 are generated by ruminants in pasture-based systems, 70.3% come from mixed systems, and only 2.19% come from feedlots (FAO, 2022). However, up to 2020, only 7% of scientific papers on enteric CH_4 mitigation corresponded to studies in pasture-based systems, compared to 39% with confined ruminants (with the balance corresponding to *in vitro* studies, literature reviews, meta-analyses, and others) (Vargas *et al*., 2022).

In this review, we present and discuss *in vitro* and *in vivo* applied research conducted in Chile for the evaluation and development of anti-methanogenic strategies that can potentially be adopted by Chilean producers. We identify and discuss knowledge gaps and the applied research that we think needs to be conducted to develop practical solutions to mitigate enteric CH_4 in Chile. Basic research on rumen biochemistry and microbiology related to the fundamental aspects of CH_4 production in the rumen and its inhibition has also been conducted in Chile [for example, Ungerfeld (2013; 2015); Ungerfeld *et al*. (2024)]. Because this fundamental research is not specific to Chilean production systems, which are the focus of this review, we do not discuss this work herein.

As in any area of science, applied research on enteric CH_4 mitigation must meet rigorous scientific standards to provide society with credible knowledge. This ensures that governments, industry, the public, and other decision-makers can engage in informed discussions about the application of enteric CH_4 mitigation options to ruminant production. Claims of enteric CH_4 mitigation should only be seriously considered and discussed if published in peer-reviewed scientific journals to ensure scientific quality and credibility. Therefore, this review is primarily based on scientific papers on applied research on enteric CH_4 mitigation conducted in Chile. We also present and discuss conference papers as non-peer reviewed, preliminary information with the purpose of enlarging the national body of research results, which otherwise might be considered somewhat constrained to merit a review. However, we are cautious about non-peer-reviewed information, noting its incompleteness, and emphasize the importance of publishing research results in peer-reviewed journals. In this review, we do not discuss technical bulletins or claims appearing in commercial brochures, webpages, or outreach presentations.

RESEARCH ON ENTERIC METHANE MITIGATION IN CHILE: GENERAL ASPECTS

Research on the abatement of CH_4 emissions from ruminants has been increasing at an accelerating pace over the last two decades owing to growing concerns about the role of CH_4 emissions in global warming (Beauchemin *et al*., 2020). Several strategies for decreasing enteric CH_4 emissions are currently being investigated worldwide. These include supplementation with concentrates and other feedstuffs, improving forage quality, supplementation with oils and oilseeds, breeding for feed efficiency, use of legumes, supplementation with phytochemicals, supplementation with plants and algae containing secondary compounds, ionophores, inhibitors of CH_4 production, genetic selection of ruminants producing less CH_4, and vaccination against rumen methanogens (Beauchemin *et al*., 2022).

In Chile, research on enteric CH_4 mitigation strategies began at INIA Remehue in 2012 by setting up the sulfur hexafluoride (SF_6) tracer gas technique to determine CH_4 emissions in grazing dairy cows (Figure 1). The canisters and holsters used in this technique were later shown to cause minimal alterations in dairy cow behavior (Pereira *et al*., 2021). Research papers evaluating CH_4 emissions by scientists in Chile span from 2014 to 2025, with 21 studies conducted *in vitro* and 14 *in vivo* (Figure 2). There is a clear trend towards increasing research output, rising from one or two studies per year published in peer-reviewed journals or conference abstracts between 2014 and 2021 to three or more in 2022 and 2024, with the exception of 2023, where only one *in vivo* study was reported.

The drop in 2023 reflects the fact that, as all research is externally funded, the number of studies conducted and papers published can vary from year to year depending on the number of research projects funded and experiments being executed, independently of overall longer-term tendencies. Six institutions have been involved in this area of research; applied *in vitro* studies have been conducted primarily at Universidad de Concepción (57.2%), followed by INIA Remehue (19.0%) and Carillanca (14.3%), and Universidad Austral de Chile (9.52%). In contrast, *in vivo* research has been predominantly conducted by INIA Remehue (71.4%), with additional contributions from Universidad Austral de Chile (14.3%), Pontificia Universidad Católica (7.1%), and Universidad de Los Lagos (7.1%). Notably, all *in vivo* studies in which CH_4 production was measured were conducted exclusively in dairy cattle, highlighting a gap in research with other domestic ruminants. From a regional standpoint,

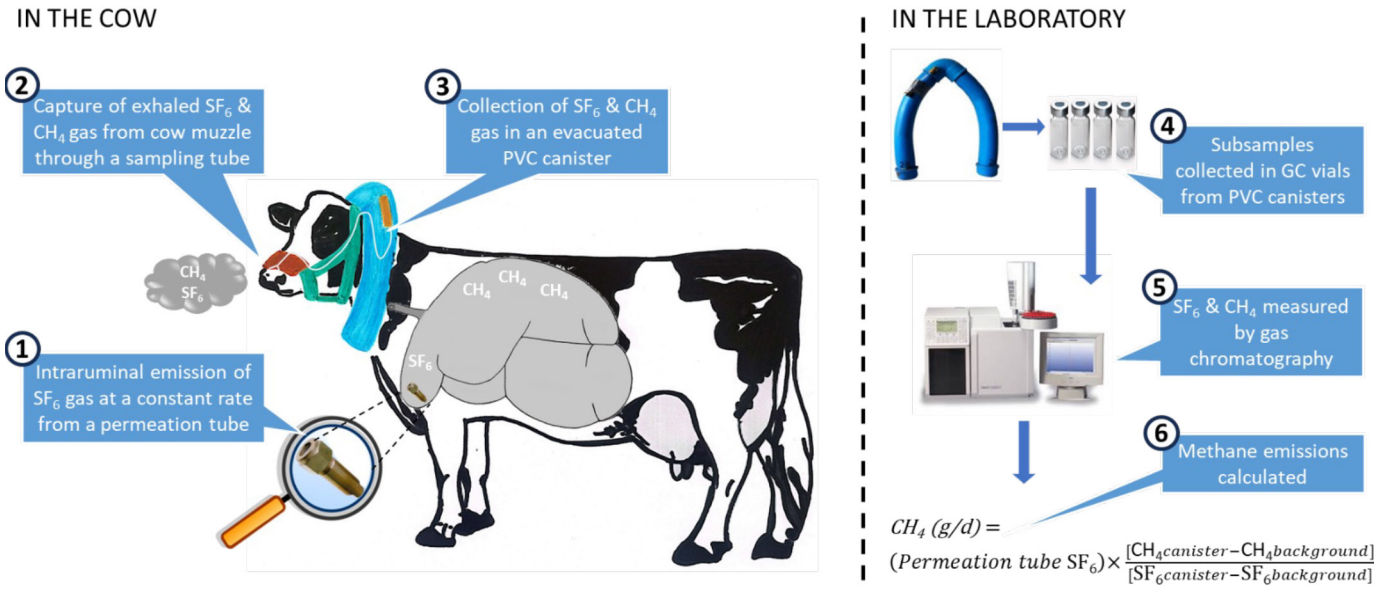

Figure 1.
Representation of the SF_6 gas tracer technique for measuring methane emissions from dairy cows. 1) Cows receive orally a pre-calibrated SF_6 permeation tube with a known release rate; 2) Exhaled gas is captured though a sampling unit consisting of an evacuated V-shaped PVC canister positioned on the cows' neck connected to a sampling line with a restricted flow continually sampling eructed and exhaled gases from near to the cow's muzzle; 3) Evacuated PVC canisters allow collection of a sample of gases emitted in a 24-h period and are replaced daily for a minimum of 5 d; 4) Canisters are pressurized to above atmospheric pressure with nitrogen, left to rest for at least 1 h, and then four subsamples of gas collected in canisters are obtained in chromatography vials; 5) Samples are analyzed in duplicate by gas chromatography; 6) Methane emissions are calculated as the product of the SF_6 permeation tube release rate and the ratio of CH_4:SF_6 concentration in samples, adjusted for background gas concentrations.

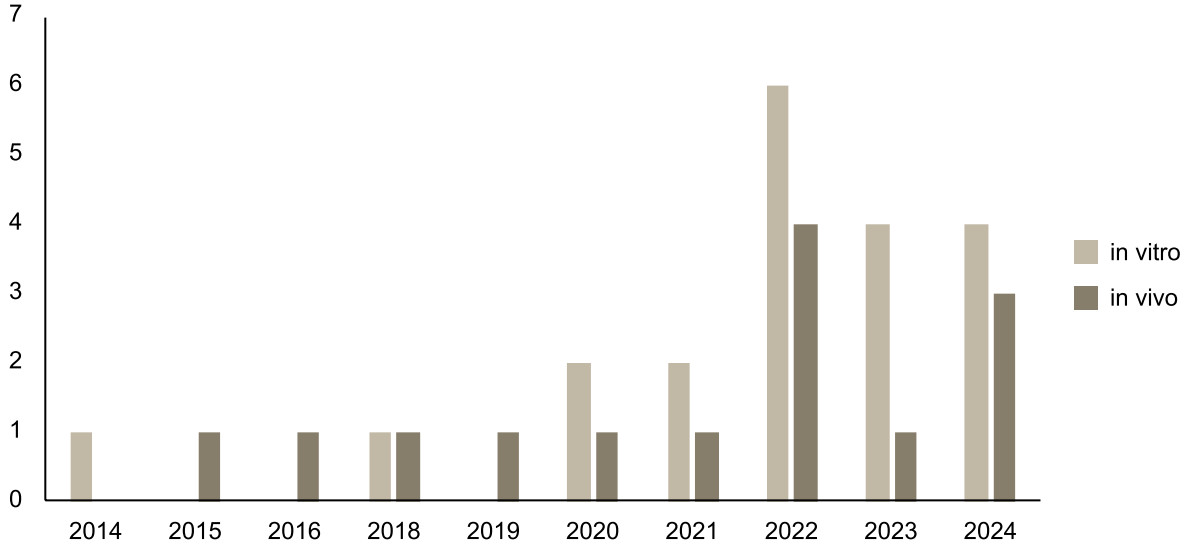

Figure 2.
Total number of publications on applied *in vivo* and *in vitro* studies on enteric methane mitigation in Chile, including peer-reviewed scientific papers and conference proceedings.

92.9% of the *in vivo* research was conducted in the center-south of the country under a humid and cool climate, and only 7.1% was conducted in the central region with a Mediterranean climate. This would not create a bias for research conducted to date with dairy cows, as most dairy cows and milk production are concentrated in the Los Ríos and Los Lagos regions in the center-south region of the country (ODEPA, 2024).

Of the *in vivo* studies, nine were conducted under confined conditions, four under pasture-based conditions, and in one case, information was not available. In 13 of 14 *in vivo* studies, CH_4 emissions were measured using the SF_6 technique, with the remaining study using a GreenFeed unit (C-Lock Inc.). The establishment and widespread use of the SF_6 technique responds to its affordability and suitability for measuring CH_4 emissions from grazing ruminants (Hammond *et al*., 2016; Hristov *et al*., 2025). Most importantly, only 57.1% of the studies were published in peer-reviewed scientific journals, with the remaining 42.9% available only as conference proceedings, most of them available at the national level only, and published in Spanish (86.7%). Various strategies to mitigate enteric CH_4 emissions have been investigated in Chile, including production intensification through dietary modifications, type of forage, supplementation of oilseeds, by-products and their extracts, supplementation of algae, and chemical inhibitors of CH_4 production. In the following sections, we present and discuss the research results grouped by type of anti-methanogenic strategy. In order to clearly distinguish preliminary findings available as conference summaries from peer-reviewed papers published in scientific journals, only peer-reviewed papers are listed in Tables 1 and 2.

Intensification through dietary modifications

Intensifying ruminant production through improvements in nutrition, management, genetics, and health decreases the output of CH_4 per kilogram of ruminant meat and milk, that is, CH_4 intensity (Beauchemin *et al*., 2020). This is largely because of the dilution of the maintenance requirements (Capper, 2011). However, intensification usually increases the total CH_4 emissions per animal (Arndt *et al*., 2022; Beauchemin *et al*., 2022). If the decrease in CH_4 emissions per kilogram of meat or milk of ruminant products is accompanied by a larger increase in the total output of meat or milk, the total enteric CH_4 emissions will increase (Leahy *et al*., 2020). Chang *et al*. (2021) showed that, despite a decrease in global CH_4 intensity, global absolute emissions of enteric CH_4 increased between 2000 and 2018, with regional increases occurring in South Asia, Latin America and the Caribbean, Sub-Saharan Africa, North Africa, and the Near East. Furthermore, intensification most often increases upstream emissions of CO_2 and N_2O from feed production and downstream emissions of manure CH_4 and N_2O, as more manure is produced (Beauchemin *et al*., 2022). Therefore, the overall effects of anti-methanogenic interventions on the entire production system must be evaluated by modelling life-cycle assessments (LCA) (Beauchemin *et al*., 2020; Beauchemin *et al*., 2022).

An examination of case studies on beef, lamb, and milk production in nine countries and regions revealed that production intensification consistently decreased the output of CO_2 equivalents (CO_2e) per kilogram of ruminant meat or milk, but changes in the total output of CO_2e were variable (Ungerfeld *et al*., 2022). In agreement with global trends, it is estimated that CO_2e emission intensity in Chile has consistently decreased for beef, lamb, and milk production between 2004 and 2021 along with increasing production intensification [Figure 3; FAO (2024)]. Due to a steady decrease in total cattle numbers coupled to the decrease in CO_2e emissions intensity that has been taking place, total CO_2e emissions from beef and dairy cattle decreased steadily in Chile between 2000 and 2021, stabilizing in 2022 [Figure 4; data from Basoa *et al*. (2024)]. The decrease in the total cattle numbers is, in turn, attributed to declining profits (Basoa *et al*., 2024).

In the first *in vivo* study on enteric CH_4 mitigation conducted in Chile, Muñoz *et al*. (2015) compared supplementing dairy cows in early lactation grazing perennial ryegrass in spring with 1 or 5 kg/d of a commercial concentrate. They found that intensifying production by boosting the amount of concentrate offered to dairy cows increased milk production, although CH_4 yield (CH_4 emitted per kilogram of dry matter intake, DMI) and CH_4 intensity remained unchanged, and total emissions of CH_4 increased (Table 1). A meta-analysis by Arndt *et al*. (2022) reported no effects of supplementing concentrates to grazing ruminants on total CH_4 emissions or CH_4 yield, and a tendency towards decreased CH_4 intensity. It is difficult to compare studies evaluating the supplementation of concentrates to grazing dairy cows, as animal responses, especially grass intake, are influenced by the amount and type of concentrate supplemented, and the availability and quality of grass (Pulido *et al*., 2009). Possibly, high availability of good quality spring grass in the study by Muñoz *et al*. (2015) resulted in a predominantly substitutive effect of the supplemented concentrate on grass, as evidenced by the lack of effects on total DMI, limiting milk production, and CH_4 intensity. A follow-up study evaluated the response of late-lactation cows fed grass and conserved forage to extra concentrate supplementation (Muñoz *et al*., 2018). Supplementing with 8 vs. 4 kg/d of concentrate increased total DMI but had no effect on milk yield in late lactation, increased total daily CH_4 emissions by cow, decreased CH_4 yield, and did not affect CH_4 intensity (Table 1). It was concluded that because late-lactation cows respond little in milk production to increased concentrate supplementation, providing extra concentrate is ineffective at decreasing CH_4 intensity in this dairy cow category.

Improving the nutritional quality of grazed grass increases pasture intake and ruminant meat and milk production, thus decreasing CH_4 intensity (Arndt *et al*., 2022). This is because a greater rate of digestion and lower rumen retention time increases DMI (Allen, 1996) and animal productivity, diluting

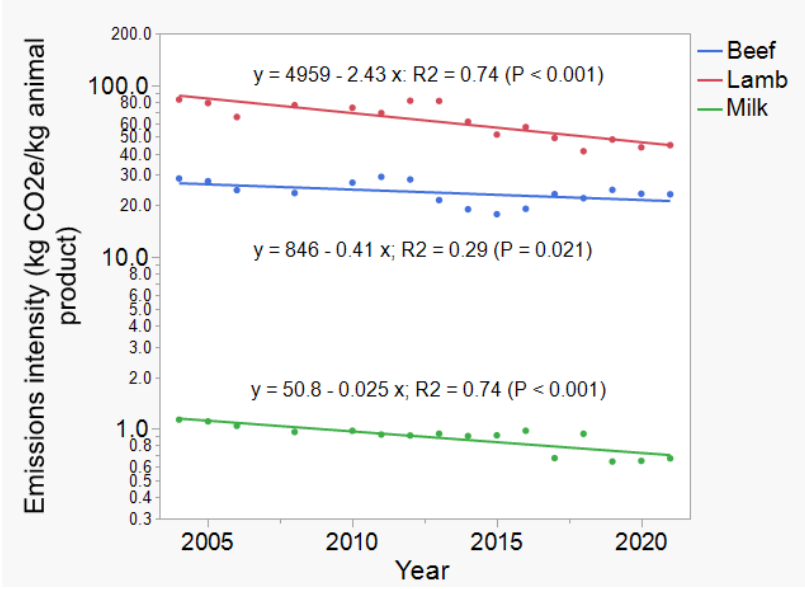

Figure 3.
Evolution of the emissions of CO_2e per kilogram of beef, lamb, and milk (CO_2e intensity) in Chile between 2004 and 2021. Data are from FAO (2024).

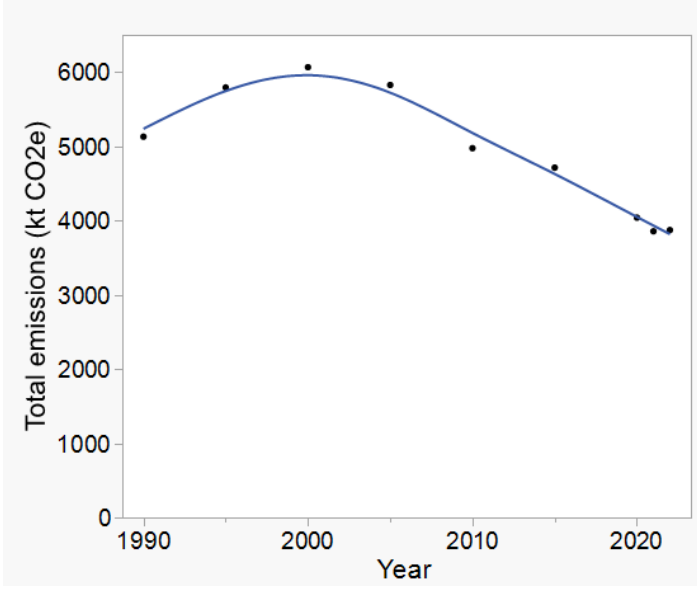

Figure 4.
Evolution of total emissions of CO_2e from cattle (beef and dairy) in Chile between 1990 and 2022. Data are from Basoa *et al.* (2024) .

maintenance requirements (Capper *et al.*, 2009). A study by Muñoz *et al.* (2016) compared CH_4 production in dairy cows managed under an optimal pre-grazing pasture mass of 2,200 kg/ha with a supra-optimal pre-grazing pasture mass of 5,500 kg DM/ha. Lower pre-grazing pasture mass resulted in higher grass quality, as assessed by lower neutral detergent fiber and higher crude protein (CP) concentration and *in vitro* digestibility. The higher quality pasture resulted in greater DMI and milk yield and lower CH_4 yield and intensity; however, despite the large difference in pre-grazing pasture mass, the optimal grazing management with lower pasture mass did not mitigate the total daily emissions of CH_4 per cow (Table 1). Likely, similar total CH_4 production occurred because the lower CH_4 yield was compensated by a greater

intake of higher quality grass, allowed by lower NDF content and lower rumen filling capacity (Allen, 1996), resulting in greater fermentation and more CH_4. This result agrees with the meta-analysis by Arndt *et al.* (2022), who found that increasing grass quality decreased CH_4 yield and intensity, but resulted in a numerical increase in absolute CH_4 emissions. Importantly, the higher quality pasture had more nitrogen (N) at a level that exceeded dairy cows' requirements, as reflected by increased milk urea N (Muñoz *et al.*, 2016). Greater N content in the pasture, along with greater grass digestibility, can result in greater rates of ammonium release in the rumen and absorption through the rumen wall, and greater elimination of urea in urine (Wallace *et al.*, 1997; Bach *et al.*, 2005). Once in the soil, urea is rapidly hydrolyzed to ammonia. Ammonia oxidation can contaminate water courses as nitrate, and also produces N_2O, a very potent greenhouse gas when released to the atmosphere (Hristov *et al.*, 2013; Arndt *et al.*, 2022).

In another study, Muñoz *et al.* (2022a) evaluated under a 2 × 2 factorial arrangement two pre-grazing pasture masses, combined with 6 kg/d of a corn- or wheat-based

Table 1.
Summary of *in vivo* research of enteric methane mitigation with dairy cows conducted in Chile published in peer-reviewed papers.

Reference	Type of anti-methanogenic strategy	Treatments	DMI§	Methane			Milk production	Comments
				Absolute	Yield	Intensity		
Muñoz *et al.* (2015)	Intensification through increasing concentrate supplementation	1 vs. 5 kg/d concentrate	0†	↑	0	0	↑	High grass availability and quality
Muñoz *et al.* (2016)	Intensification through improving grass quality	High vs. low pre-grazing pasture mass	↑	0	↓	↓	↑	Higher quality pasture had excess N content
Muñoz *et al.* (2018)	Intensification through increasing concentrate supplementation	4 vs. 8 kg/d concentrate	↑‡	↑	↓	0	0	Late lactation cows did not respond to extra concentrate
Muñoz *et al.* (2019)	Oilseeds supplementation	Inert fat vs. unprocessed cottonseed, rapeseed, or linseed in a TMR§	0 or ↓⁋	0 or ↓⁋	0⁋	0⁋	0	Oilseeds affected N excretion and milk fatty acid profile
Enriquez-Hidalgo *et al.* (2020)	Dietary reformulation	Replacing alfalfa hay and corn silage with fresh ryegrass and berseem clover in a TMR	↓	0	0	0	↓	Lower crude protein in the TMR resulted in less N voided in urine
Muñoz *et al.* (2021)	Oilseeds supplementation	Long-term effects of unprocessed cottonseed, rapeseed, or linseed in grazing dairy cows	0 or ↓	0	0 or ↓	0	0 or ↓	Cottonseed moderately but not persistently decreased methane
Garcia *et al.* (2022)	Methanogenesis inhibitor	Supplementation with 3-NOP§ or its carrier with or without urea inclusion	↓	↓	↓	↓	↓	An unintended dietary change affected especially DMI and milk production
Muñoz *et al.* (2024)	Methanogenesis inhibitor	Supplementation with 3-NOP§ or its carrier with or without cottonseeds	↓	0 or ↓	0	0	0	Time gap between 3-NOP supplementation and grass ingestion caused mild methanogenesis inhibition

†↑ = increase, 0 = no change, ↓ = decrease compared to control treatments; ‡numerical increase, as individual intake was not determined in that study; ⁋In comparison with the control treatment; §Abbreviations: TMR = total mixed ration; 3-NOP = 3-nitrooxypropanol (Bovaer®).

concentrate, on enteric CH_4 emissions from dairy cows. They reported that the greatest total CH_4 emissions and CH_4 intensity occurred with the wheat and high pre-grazing pasture mass combination, so that pasture management effectively decreased CH_4 with wheat but not with corn. Previous work on non-grazing dairy cows showed that wheat produced less total CH_4 than corn (Moate *et al*., 2017), although this could not be confirmed by Muñoz *et al.* (2022a). At this point, it is difficult to explain the interaction between pre-grazing pasture mass and the type of concentrate. An analysis of rumen variables and other physiological aspects may be required to understand how this interaction occurred.

Pasture growth, quality, and nutrient composition, and hence CH_4 emissions from grazing ruminants, are influenced by season (Delagarde *et al*., 2000; Elgersma *et al*., 2005). Thus, it may be strategic to prioritize the adoption of anti-methanogenic strategies during seasons in which the greatest CH_4 production occurs. Thus, another study evaluated the effects on CH_4 emissions of feeding dairy cows exclusively with a cut and carried ryegrass pasture harvested daily across early and advanced autumn and early and advanced spring (Muñoz *et al*., 2022b). There was seasonal variation between periods within autumn and spring on CH_4 emissions. Absolute CH_4 emissions in autumn were higher early in the season, associated with higher forage quality and greater intake, but there were no differences between early and advanced spring (Table 1). Methane intensity in spring was lower early in the season and was associated with higher forage quality and milk production, but CH_4 intensity did not differ between early and advanced autumn (Muñoz *et al*., 2022b). This study confirmed that pasture quality, particularly its digestibility and nutrient concentration, significantly affects dairy cow productivity and CH_4 emissions.

As a conclusion about intensifying dairy production as an anti-methanogenic strategy, overall and partially contrary to existing global analyses (Gerber *et al*., 2011; Arndt *et al*., 2022), increasing concentrate supplementation and improving pasture quality has not consistently resulted in decreased CH_4 intensity in all the studies conducted in Chile. In agreement with previous research (Arndt *et al*., 2022; Beauchemin *et al*., 2022), total CH_4 emissions per dairy cow were unaffected or increased when dairy production was intensified by increasing concentrate supplementation or pasture quality was improved.

Type of forage

Given the advantages that ruminants have in terms of transforming human-inedible feedstuff such as grazed forage into milk and meat, it has been proposed that an important CH_4 mitigation option in pasture-based systems should be forage manipulation itself (Pacheco & Keim, 2018). Perennial ryegrass is an important forage crop in Chile and other temperate regions worldwide. Keim *et al.* (2014) evaluated the *in vitro* fermentation of four Chilean pastures differing amply in botanical composition and proportion of perennial ryegrass, which varied between 16 and 754 g/kg DM, in three regrowth periods. No differences in CH_4 production and CH_4 proportion of total gas produced were observed.

Ryegrass with high sugar content has been proposed as a strategy to decrease CH_4 emissions from grazing ruminants (Beauchemin *et al*., 2022). It has been reported that grazing frequency, N fertilization and ryegrass cultivar can modify the sugars to CP ratio (Bryant *et al*., 2012; Loaiza *et al*., 2017), which influences CH_4 production (Vera-Aguilera *et al*., 2022). Rivero *et al.* (2020) combined a reduced grazing frequency with a low N fertilization rate and a high-sugar tetraploid ryegrass cultivar to achieve a high sugar ryegrass, increasing the sugars to CP ratio by 52 and 89% in the spring and autumn, respectively, in comparison with the conventional management and cultivar. Incubating the different forages so obtained in *in vitro* batch cultures, they found that the high-sugar treatment harvested in autumn and spring produced 10 and 5% less CH_4 in total gas, respectively. In another *in vitro* study, Vera-Aguilera *et al.* (2022) reported a broad range of *in vitro* CH_4 production among 13 different ryegrass genotypes at vegetative stage, with the least methanogenic genotype producing 52% less CH_4 per gram of organic matter disappeared than the most methanogenic genotype. In their study, the range in sugar content was relatively narrow (17.3 – 21.5% DM), and, although CH_4 production was negatively associated with sugar concentration, the association was weak. Instead, CH_4 production per gram of organic matter disappeared was negatively associated with organic matter disappearance (Vera-Aguilera *et al*., 2023a).

In the following year, Vera-Aguilera *et al.* (2023b) compared the two most and two least methanogenic ryegrass genotypes reported by Vera-Aguilera *et al.* (2022) harvested from the same plots of the previous year at three phenological stages: vegetative, elongation, and reproductive. No interaction was found between the phenological stage and genotype. As ryegrasses matured, they produced more CH_4, in agreement with the observed increase in neutral detergent fiber and decrease in sugar content (Janssen, 2010). Unlike the ryegrasses harvested in the previous year (Vera-Aguilera *et al*., 2022), ryegrass genotypes did not differ in CH_4 production per gram of organic matter disappeared, although they numerically ranked as they had in the previous year. Vera-Aguilera *et al.* (2022) harvested ryegrass plants from the same plots and used the same collection and processing methods in 2021 and 2022; hence, the lack of significant differences in the methanogenic capacity of ryegrass genotypes may have been related to differences in climate conditions and plant maturation between both years. More research across different years and locations is needed to better interpret the results and to identify and generalize the main factors causing variation among ryegrass genotypes in their methanogenic capacity.

Legumes are digested faster in the rumen than grasses, and hence have a more rapid rumen passage

rate (Beauchemin *et al*., 2022), which can result in a rumen fermentation profile lower in CH_4 (Janssen, 2010). Higher passage rate is also associated with increased feed intake, which lowers CH_4 intensity, although the effects of legume inclusion on CH_4 production are highly influenced by the phenological stage of the forages being compared and the type of legume (Beauchemin *et al*., 2022). In this regard, results regarding whether non-tannin-containing temperate legumes can effectively ameliorate CH_4 production from ruminants are inconsistent (Vargas *et al*., 2022). Muñoz *et al*. (2022c) compared ryegrass and alfalfa hay constituting 40% of the dry matter (DM) of a total mixed ration fed to dairy cows. Although there was a tendency towards greater absolute CH_4 emissions in cows fed alfalfa hay associated with greater feed intake, there were no differences in CH_4 yield or intensity. Feeding alfalfa hay resulted in greater N excretion in urine (Muñoz *et al*., 2022c), which, as discussed above, can result in augmented N_2O emissions from soils.

Enriquez-Hidalgo *et al*. (2020) replaced 40% of alfalfa hay and corn silage DM in a total mixed ration fed to dairy cows with a mixture of predominantly fresh ryegrass and lower proportions of berseem clover. There were no changes in total CH_4 emissions, CH_4 yield, or CH_4 intensity, although DMI and milk yield decreased when fresh ryegrass and berseem clover replaced alfalfa hay and corn silage in the total mixed ration (Table 1).

Chilean dairy producers are increasingly searching for drought-resistant crops to supplement cow diets during dry summers. Studies in New Zealand have shown that sheep fed forage rape produced less CH_4 than those fed perennial ryegrass (Sun *et al*., 2016). These authors suggested that this decrease could be attributed to a higher concentration of readily fermentable carbohydrates, lower rumen pH, changes in the rumen microbiome, and faster digesta passage rate. Chicory has also been proposed as a potential CH_4 mitigating forage due to its higher concentration of condensed tannins (Niderkorn *et al*., 2019). The inclusion of forage rape and chicory in dairy cow diets has been evaluated as an option in Chile to supplement forage during the summer, in cows receiving grass silage and a concentrate basal diet (Keim *et al*., 2024). In that study, forage rape, chicory, or an irrigated ryegrass pasture was offered to dairy cows at an inclusion level of 25% of the diet DM. Dry matter intake and N excretion were lower in cows fed chicory, whereas milk and protein yields were higher in cows fed forage rape. Forage rape and chicory showed potential benefits compared to irrigated ryegrass pasture in terms of milk production and nitrogen efficiency, although they did not decrease CH_4 emissions (Keim *et al*., 2024). In a follow-up study, the effects of supplemental chicory or forage rape (45% of the diet DM) on CH_4 emissions were compared in dairy cows fed a basal grass silage and concentrate diet. A reduction of 8% in total CH_4, 6% in CH_4 intensity, and 32% in milk urea concentration was observed in cows supplemented with chicory compared to the forage rape-supplemented group (León *et al*., 2024). Therefore, for summer feeding, chicory appears as an alternative to forage rape to maintain milk production while mitigating CH_4 production and N excretion.

Supplementation of oilseeds

The inclusion of oils and oilseeds in ruminant diets induces a moderate decrease in CH_4 production and can increase ruminant productivity owing to the supply of extra energy (Beauchemin *et al*., 2022). Research on *in vitro* semi-continuous cultures conducted in a Rusitec system evaluated the inclusion of ground linseed at 15% DM in combination with glycerol at 5 or 10% DM (Table 2) (Gutierrez-Gomez *et al*., 2020). The authors hypothesized that linseed, as a source of lipids, and glycerol, a reported propionogenic biofuel by-product, could act additively to decrease CH_4 production. Linseed inclusion resulted in a 30% decrease in total CH_4 production and CH_4 per gram of digested DM. However, glycerol failed to further decrease CH_4 production, likely because its conversion to propionate is stoichiometrically neutral in terms of electron balance (Avila-Stagno *et al*., 2013). Linseed inclusion in the substrate increased ammonium concentration by 2-fold, possibly due to increased digestible CP content. However, this effect was partially offset by glycerol, which may have contributed to the availability of energy for microbial protein synthesis. The authors suggested that care should be taken when supplementing oilseeds to mitigate CH_4 because N excretion can increase (Gutierrez-Gómez *et al*., 2020). A subsequent semi-continuous study with Rusitec confirmed the anti-methanogenic effect of linseed, which, however, was offset by glycerol (Vera *et al*., 2025b) (Table 2). In this study, where substrates were balanced in N, the addition of linseed did not increase ammonium concentration, whereas glycerol numerically decreased it.

In a double Latin square design study, Muñoz *et al*. (2019) compared CH_4 production, N balance, and milk fatty acid profile of early lactation dairy cows offered cottonseed, rapeseed, linseed, or an inert fat control, mixed in their total mixed rations. Cottonseed moderately decreased absolute CH_4 production and yield with respect to rapeseed and linseed, and CH_4 intensity compared to rapeseed (Table 1). However, although the diets were isonitrogenous, the effects of cottonseed supplementation on CH_4 emissions were offset by an increase in N excretion and by a greater ratio of N excreted in urine plus feces per unit of N ingested. This unfavorable result in N balance can potentially result in increased N_2O emissions from soils, partially or completely compensating the decrease in CH_4. In addition, oilseed supplementation increased the milk content of beneficial fatty acids, such as vaccenic acid and mono- and polyunsaturated fatty acids; rumenic acid in milk was increased by rapeseed, and α-linolenic acid was increased by linseed. This study showed that the effects of oilseeds and other anti-methanogenic strategies on CH_4 production should be evaluated alongside other effects on the environment and consumers' health (Muñoz *et al*., 2019).

Table 2.
Effects of various antimethanogenic strategies on *in vitro* methane production. Research conducted in Chile published in peer-reviewed papers.

Reference	Type of antimethanogenic strategy	Treatments	*In vitro* system	Total methane	Methane per gram of digested DM, OM, or total gas	Comments
Keim *et al.* (2014)	Pasture composition	Pasture composition in three different periods	Batch	0†	0	There were differences in OM and NDF digestibility and total VFA
Vera *et al.* (2018)	Tannin-containing by-product	Pine bark at various doses	Batch	0	0	Decrease in ammonium
Rivero *et al.* (2020)	Defoliation management, N fertilization, and ryegrass genotype	High sugar ryegrass combined with lower N fertilization and longer regrowth period vs. control	Batch	0	↓	High sugar ryegrass had higher total gas production and total VFA
Gutierrez-Gomez *et al.* (2020)	Oilseeds and glycerol	Linseed and glycerol	Semi-continuous	↓	↓	Glycerol did not affect methane. Linseed increased and glycerol decreased, ammonium
Vera *et al.* (2021)	Tannin-containing by-product	Pine bark extract	Batch	0	0	Decrease in ammonium
Suescun-Ospina *et al.* (2022a)	Tannin-containing by-product	Grape marc with substrates varying in forage to concentrate ratio	Batch	↓	↓	Decrease in DM digestibility, total VFA and ammonium
Vera *et al.* (2022)	Tannin-containing by-product	Pine bark and quebracho extracts	Batch	↓	0	Decreases in DM digestibility, gas production, total VFA, and ammonium
Suescun–Ospina *et al.* (2023)	Tannin-containing by-product	Different drying methods, temperatures and duration	Batch	Differences between treatments	Differences between treatments	
Vera *et al.* (2025a)	Tannin-containing by-product	Pine bark extract with high forage or high concentrate substrate	Semi-continuous	0	↓ (high forage) 0 (high concentrate)	Total VFA and ammonium decreased
Vera *et al.* (2025b)	Linseed and glycerol	Linseed with 0, 5 or 10% DM glycerol	Semi-continuous	↓	↓	Ammonium was unaffected or numerically decreased by glycerol

†↑ = increase, 0 = no change, ↓ = decrease compared to control treatments

In a follow-up long-term longitudinal study, Muñoz *et al.* (2021) evaluated the supplementation of the same unprocessed oilseed cottonseed, rapeseed, and linseed compared to a control treatment supplemented with concentrate that did not contain oilseeds. By investigating the anti-methanogenic effects of oilseeds offered to grazing dairy cows for a longer experimental period (27 weeks), Muñoz *et al.* (2021) extended the few existing mid- to long-term studies evaluating oilseed supplementation as an anti-methanogenic strategy (Jordan *et al.*, 2006; Grainger *et al.*, 2010). Grazing dairy cows were offered experimental supplements during spring and summer, and oilseed supplementation was discontinued in autumn, but CH_4 and other measurements continued to investigate the possibility of residual effects. Consistent with the results of Muñoz *et al.* (2019), cottonseed effectively decreased CH_4 production moderately in spring; however, these effects were no longer sustained after 19 weeks of oilseed supplementation (Table 1). No carryover effects were observed in the autumn after oilseed supplementation ceased.

Reduced CH_4 mitigation occurs when oilseeds are offered unprocessed compared to breaking the seed hull through grinding, crushing, or rolling (Beauchemin *et al.*, 2022). Although Muñoz *et al.* (2019) anticipated that offering whole oilseeds would have a lesser effect on CH_4 decrease than offering processed oilseeds, the authors aimed to evaluate unprocessed oilseed supplementation as a potential methane-mitigation strategy that could be adopted by most dairy producers in Chile, who lack specialized mills to process oily feedstuffs. Overall, it seems that moderate decreases in CH_4 emissions in the first few months of supplementation can be obtained with unprocessed oilseeds, although at the cost of

a slight increase in the urine N output. As cottonseed supplementation did not improve milk production compared with a commercial concentrate containing no oilseeds (Muñoz *et al*., 2021), the adoption of this anti-methanogenic strategy may largely depend on its cost-effectiveness.

By-products and their extracts

Plant secondary metabolites, including saponins, polyphenols, and essential oils, can alter rumen microbiota and fermentation, and ruminant productivity and health (Calsamiglia *et al*., 2007; Tedeschi *et al*., 2021). Chile has a great diversity of ecosystems with different climates, topographies, and soil types, which harbor native plant species that could be candidates as a source of secondary compounds with the potential to modify rumen fermentation. Polyphenols, a vast family of plant secondary metabolites, are widely distributed in the plant kingdom, and can be divided into phenolic acids and flavonoids (Tedeschi *et al*., 2021). Flavonoids include flavanols, flavones, anthocyanidins and isoflavones. Polyphenols can modulate rumen fermentation by binding molecules such as proteins and carbohydrates and altering microbial populations in the rumen (Egea *et al*., 2016). These types of compounds are also present in some abundant by-products generated by the agroforestry industry worldwide. In Chile, the potential use of the polyphenol-containing by-product pine (*Pinus radiata*) bark and grape (*Vitis vinifera*) marc as enteric CH_4 mitigation ingredients in ruminant feeds has been extensively investigated at Universidad de Concepción.

Vera *et al*. (2018) found that up to 6% pine bark extract added to rumen batch cultures caused modest, numerical decreases in CH_4 production but decreased ammonium concentration extensively (Table 2), which was attributed to the binding of proteins by polyphenols contained in the bark extract. In another rumen batch culture study, Vera *et al*. (2022) compared pine bark with quebracho extract, and found a large decrease in CH_4 production and ammonium concentration with both by-products (Table 2). However, apparent DM disappearance and total volatile fatty acids (VFA) were decreased by both extracts. The long-term effects of pine bark extract were studied *in vitro* with a 70:30 and 30:70 forage to concentrate ratio substrate in a semi-continuous Rusitec system (Table 2) (Vera *et al*., 2025a). Pine bark extract decreased ammonium and VFA concentrations with both substrates, but CH_4 per gram of organic matter (OM) disappeared decreased only with high forage.

Grape marc is the residue left after making wine, and represents between 20 and 30% of the original grape weight (Beres *et al*., 2017). Grape marc contains both oils and tannins and has been reported to decrease enteric CH_4 production when fed to dairy cows (Moate *et al*., 2014; Akter *et al*., 2024). In Chile, wine production exceeded one billion liters in 2020, resulting in the generation of 260,000 tons of grape marc in that year (Buzzetti Horta, 2021). Grape marc is mainly deposited in landfills, causing environmental damage. Therefore, it is of interest to promote its use in ruminant feeding (Beres *et al*., 2017). Suescun-Ospina *et al*. (2021) studied the effects of four levels of an extract obtained from grape marc ranging from 0 to 2.1% of the substrate DM on CH_4 production and fermentation in semi-continuous rumen cultures in Rusitec. Methane production and ammonium concentration decreased by 29 and 30%, respectively, without affecting gas production or DM digestibility, although total VFA concentration was not determined in that study. In another study, Suescun-Ospina *et al*. (2022a) found that the inclusion of grape marc at 0, 10, or 20% DM in batch cultures in replacement of hay in predominantly forage or concentrate substrates moderately decreased CH_4 production by up to 16%, but there was also a decrease in DM digestibility and total VFA concentration. There was also a strong decrease in the concentration of ammonium and branched-chain fatty acids, indicating diminished fermentation of amino acids (Table 2) (Suescun-Ospina *et al*., 2022a).

Further work in semi-continuous cultures found a severe decrease in the abundance of total methanogens when grape marc was included in the substrate (Suescun-Ospina *et al*., 2022b). Suescun-Ospina *et al*. (2023) reported that oven drying at 60 °C for 48 h resulted in lower *in vitro* CH_4 production than lyophilization or oven drying at 40 °C for 72 h. However, oven-drying at both temperatures resulted in lower *in vitro* digestibility and total VFA compared to lyophilization. Suescún-Ospina *et al*. (2024) evaluated combinations of grape marc extract and grape marc oil at 0, 2.5 or 5% DM in semi-continuous cultures. Both grape marc extract and oil decreased CH_4 production; however, their effects were not additive. Both grape marc and oil decreased or tended to decrease DM digestibility and ammonium concentration, and increased propionate molar percentage. Palacios *et al*. (2024) replaced grass silage with two types of grape marc (sweet or fermented) at 10, 15, 20, or 25% in batch cultures. Sweet and fermented grape marc included at 25% DM decreased CH_4 production at 24 h of incubation by 22 and 20%, respectively. However, total gas production was also decreased by 18% for both types of grape marc at the same timepoint.

Overall, the two types of polyphenol-containing by-products generated in Chile that have been evaluated to date, pine bark extract and grape marc, have shown interesting effects at decreasing ammonium concentration *in vitro*, suggesting their potential to decrease rumen protein degradation and improve N utilization efficiency when fed to ruminants. However, as decreases in digestion and fermentation have been noted in some studies, it is important to consider doses of these by-products that, while exerting favorable effects on N metabolism and CH_4 production, do not impair digestion and performance when fed to ruminants. In a subsequent *in vivo* study supplementing pine bark extract to lambs at 1 or 2% DM, no detrimental effects on performance, carcass quality, or blood circulating variables were observed. Methane production and N excretion were not measured, but pine bark improved the fatty acid profile of subcutaneous fat by increasing the content of fatty acids

beneficial to human health (Vera *et al*., 2023). Therefore, further digestion and metabolism studies, including CH_4 and N balance measurements at different doses, are of interest.

Supplementation of algae

Macroalgae contain bioactive compounds and their supplementation to ruminants is thus being investigated as an anti-methanogenic strategy (Beauchemin *et al*., 2022). Beltran *et al.* (2022) replaced in rumen batch cultures 10% of the cereal portion of a substrate composed by 80% grass pasture and 20% of a cereal-based concentrate in the DM with one of three Chilean seaweeds: the brown seaweeds *Macrocystis pyrifera* and *Lessonia spicata*, and the red seaweed *Agarophyton chilensis*, with each alga collected both in the winter and summer. On average, *M. pyrifera* produced 22 and 11% less CH_4 at 24 and 48 h of incubation, respectively, compared to the control treatment without algae. However, it produced 11 and 9% less total gas at 24 and 48 h of incubation, respectively, suggesting that the decrease in CH_4 production could be explained, at least partially, by less digestion and fermentation. Considerably larger *in vitro* screening of macroalgae for their anti-methanogenic capacity identified very few candidates, most notably the bromoform-containing red algae *Asparagopsis* (Machado *et al*., 2014; Wasson *et al*., 2023); thus, it is to an extent expected that smaller screenings do not yield promising candidates recommendable for further progress to *in vivo* research.

A follow-up study investigated the inclusion of *M. pyrifera* and *L. spicata* replacing 0, 1, 3, 6, and 8% of the concentrate portion of the substrate in 48 h *in vitro* rumen batch cultures (Stolzenbach *et al*., 2022). Again, *M. pyrifera* linearly decreased CH_4 production by 30 and 24% after 24 and 48 h of incubation, respectively. However, it also decreased asymptotic total gas production by up to 10%, suggesting that the anti-methanogenic effect might have been partially mediated by less digestion and fermentation. A separate report indicated that the inclusion of *M. pyrifera* linearly increased the molar percentage of propionate and decreased the ammonia concentration in rumen batch cultures (Ruiz-Tagle *et al*., 2023). While the authors indicated as $P > 0.05$ that organic matter and N digestibility as well as total VFA concentration were not significantly decreased by the algae, it is not possible to discern the closeness of these important response variables to significance, as treatment means and exact *P* values for digestibility and total VFA were not provided. The information provided in the conference summary by Ruiz-Tagle *et al.* (2023) precludes then concluding whether the evaluated algae might have negatively affected digestibility and fermentation. While the 0.05 threshold is considered acceptable for declaring an effect as significant, a *P* value greater than 0.05 does not confirm that a response variable is unaffected. One should be hesitant about recommending a particular additive or anti-methanogenic strategy for further research, and even more for adoption, if digestion and/or fermentation variables are numerically compromised. Researchers conducting *in vitro* studies should consider numerical decreases in key digestion and fermentation variables, such as apparent OM digestibility, total VFA concentration, or total gas production, as a warning sign (Durmic *et al*., 2025). Likewise, a numerical and non-significant ($P > 0.05$) decrease in milk production or growth in an *in vivo* experiment should not prompt researchers to recommend an additive or technology because significant detrimental effects were not found at $P < 0.05$ (Hristov *et al*., 2025).

The red algae *Asparagopsis* exerts the strongest inhibition of methanogenesis reported for any feed additive to date (Almeida *et al*., 2021). *Asparagopsis* inhibition of CH_4 production in batch culture was first reported in 2014 (Machado *et al*., 2014). Since then, numerous *in vitro* (Machado *et al*., 2015a; Vucko *et al*., 2017; Roque *et al*., 2019a; Kinley *et al*., 2021; Kinley *et al*., 2022; Romero *et al*., 2023; Terry *et al*., 2023; Thorsteinsson *et al*., 2023; Nunes *et al*., 2024) and *in vivo* (Kinley *et al*., 2016; Li *et al*., 2016; Roque *et al*., 2019b; Kinley *et al*., 2020; Muizelaar *et al*., 2021; Roque *et al*., 2021; Stefenoni *et al*., 2021; Krizsan *et al*., 2023; Indugu *et al*., 2024; Romero *et al*., 2024; Fennessy *et al*., 2025) studies with *Asparagopsis*, and more recently, various *Asparagopsis* preparations (Alvarez-Hess *et al*., 2023; Cowley *et al*., 2024; George *et al*., 2024), have consistently confirmed profound inhibition of methanogenesis with this red algae.

The inhibitory effect of *Asparagopsis* on methanogenesis is due to its haloalkane content, mainly bromoform (Machado *et al*., 2016). The *Asparagopsis* genus comprises two species, *A. taxiformis* and *A. armata*, and its use for enteric CH_4 mitigation has been patented since 2015 (Machado *et al*., 2015b) and licensed by FutureFeed (FutureFeed, 2021). Locally, Marin *et al.* (2024) evaluated *in vitro* three mixtures of undefined brown and red algae collected in Chile included at 2.0 or 5.0% of substrate DM, and reported greater than 98% inhibition of methanogenesis. Because all three mixtures of algae that they used contained a minimum of 50% DM of their red alga in the algal mix, the observed anti-methanogenic effects were ascribed to the red alga. The red alga evaluated by Marin *et al.* (2024) was collected in Antofagasta, where *A. armata* has been reported to grow as an invasive species (Ramírez *et al*., 2007; Villaseñor-Parada *et al*., 2018), and the authors speculated about the presence of halogenated compounds in the red alga. Although the specific algal species were not reported, it thus appears that the red alga evaluated by Marin *et al.* (2024) was likely *A. armata* collected in Antofagasta. The omission of an explicit identification of the algae evaluated is problematic as it affects the reproducibility of the findings of this study. It would be expected that the effects of Chilean *A. armata* on methanogenesis are consistent with previous results reported for *Asparagopsis* from other parts of the world, as Chilean *A. armata* does not constitute a separate subspecies or taxon and falls into the same genetic cluster as *A. armata* collected from places as diverse as the United Kingdom, Ireland, the Channel Islands, France, Spain, Italy, San Diego, and Australia (Chualáin *et al*., 2004).

Oyarzun *et al.* (2023) supplemented 150 g/d of an undefined red algae to dairy cows once daily at milking. The algal additive caused a 12% decrease in absolute CH_4 emissions pulse-measured at the parlor three times daily using the GreenFeed equipment (C-Lock Inc., Rapid City, SD, US). However, as DM intake and milk production and composition were not reported, it is unknown whether CH_4 production decreased at the expense of less DM intake and/or digestibility, and if animal performance was impaired by the additive. The effects of the algae evaluated by Oyarzun *et al.* (2023) on CH_4 intensity and CH_4 yield are similarly unknown. The lack of identification of the algae evaluated and of fundamental performance results that should be reported in *in vivo* trials evaluating feed additives inhibiting CH_4 production (Hristov *et al.*, 2025) makes unconvincing the authors' claim about the potential impact of this algal additive to mitigate the environmental impact of dairy production.

Inhibitors of methane production

Apart from the red alga *Asparagopsis*, supplementation with pure chemicals that inhibit rumen methanogenesis appears to be the most potent strategy to mitigate CH_4 emissions from ruminants (Almeida *et al.*, 2021; Arndt *et al.*, 2022). Garcia *et al.* (2022) hypothesized that inhibiting rumen methanogenesis with 3-nitrooxypropanol (3-NOP, Bovaer®) would stimulate the microbial synthesis of amino acids and long-chain fatty acids as an alternative electron sink to CH_4 in the rumen of dairy cows. Although an unintended dietary change precluded the authors from testing their hypothesis, the study showed that 3-NOP was highly effective in inhibiting rumen methanogenesis with diets containing both solely plant protein and urea providing part of the N (Table 1).

While confined ruminants emit a relatively minor portion of global enteric CH_4, most applied research on the inhibition of rumen methanogenesis using feed additives, whether pure chemicals or of natural origin, has been conducted with total mixed rations. However, research on the abatement of enteric CH_4 emissions from grazing ruminants has lagged behind (Ungerfeld, 2022). A search of the PubMed database for "3-nitrooxypropanol" or "3-nitroxypropanol" retrieved 93 records, 48 of which corresponded to *in vivo* experiments with ruminants fed total or partial mixed rations, 34 corresponded to literature reviews, meta-analyses, deterministic simulations and LCA, biochemistry and other studies, and only two studies with grazing ruminants, both with dairy cows (Costigan *et al.*, 2024; Muñoz *et al.*, 2024), one of which was conducted in Chile. A similar search for "*Asparagopsis* AND methane" yielded 50 records, with 15 records corresponding to original research with ruminants fed total or partial mixed rations and only one with ruminants on pastures (Meo-Filho *et al.*, 2024).

In a 2 × 2 factorial arrangement study, Muñoz *et al.* (2024) evaluated the supplementation of 3-NOP mixed with a concentrate with or without whole cottonseeds. Supplementation of 3-NOP moderately decreased CH_4 emissions by 13% with the non-cottonseed-containing concentrate and had no effect when it was offered with cottonseed (Table 1). This mild CH_4 mitigation with grazing dairy cows is in sharp contrast to an average 33% CH_4 decrease obtained in a meta-analysis of 14 experiments quantifying the effects of 3-NOP offered to dairy cows on total mixed rations (Kebreab *et al.*, 2023). Muñoz *et al.* (2024) concluded that supplementing 3-NOP mixed with concentrates offered at milking and before grazing introduced a time gap between the peak of 3-NOP concentration in the rumen and grass ingestion, which considerably decreased the effectiveness of 3-NOP. The study by Costigan *et al.* (2024) in Ireland, who also evaluated the supplementation of 3-NOP to grazing dairy cows, was published shortly after the Muñoz *et al.* (2024) study. Costigan *et al.* (2024) also found a mild decrease of about 5% in daily CH_4 production in grazing dairy cows supplemented 3-NOP. In contrast, Meo-Filho *et al.* (2024) reported a 38% decrease in CH_4 production in grazing steers fed pellets containing *Asparagopsis*. Meo-Filho *et al.* (2024) estimated an average total DMI of about 8.5 kg/d, of which about 0.5 kg corresponded to the *Asparagopsis*-containing pellet. Thus, it seems that the considerable CH_4 mitigation achieved in the Meo-Filho *et al.* (2024) study was obtained with grass constituting most of the DMI. Meo-Filho *et al.* (2024) offered *Asparagopsis*-containing pellets in GreenFeed units three times daily, with at least 6 h between each offering, whereas Muñoz *et al.* (2024) and Costigan *et al.* (2024) supplemented 3-NOP twice daily.

Frequent supplementation of anti-methanogenic additives seems therefore important to maximize their effectiveness in pasture-based systems, although this appears difficult in extensive ruminant production systems. In addition, while bromoform is rapidly metabolized by *in vitro* rumen cultures, its reduction product, dibromomethane, would prolong the effect of *Asparagopsis* (Glasson *et al.*, 2022; Romero *et al.*, 2023). This said, 3-NOP is metabolized in the rumen to 1, 3-propanediol and nitrite, with nitrite also inhibiting methanogens (Duin *et al.*, 2016).

Studies evaluating the supplementation of grazing ruminants with anti-methanogenic feed additives are scarce, which contrasts with the global importance of pasture-based systems in enteric CH_4 emissions (FAO, 2022). Solutions for pasture-based systems, including slow-release feed additives and optimal grazing and supplementation management to enhance their effectiveness, are urgently required. Unfortunately, developing feed additives that inhibit CH_4 production in pasture systems has not been a priority for the industry developing feed additives (Hegarty *et al.*, 2021).

Khurana *et al.* (2024) supplemented a garlic and citrus extract (Mootral Ltd., Abertillery, UK) to grazing dairy cows in a commercial dairy in the south of Chile. They measured milk production and composition, estimated DMI based on an equation derived for Brazilian tropical conditions (Souza *et al.*, 2014), and estimated the total output of CO_2e based on the Global Livestock Environmental Assessment Model-interactive tool (GLEAM-i) (FAO, 2025). They concluded that garlic and citrus extracts increased energy-corrected

milk production and decreased CO_2e emission intensity. Whilst GLEAM-i can be used to estimate CO_2e emissions of commercial herds for carbon accounting purposes (e.g., national inventories), using GLEAM-i in original research intended to demonstrate the efficacy of an anti-methanogenic feed additive is grossly inadequate. Demonstrating mitigation of total CO_2e output at the farm level requires, among other aspects, direct measurements of enteric CH_4 production (Hristov *et al*., 2025), mathematical models derived from various trials demonstrating the efficacy of feed additives at decreasing CH_4 production (Dijkstra *et al*., 2025), and an LCA tailored for a specific farm (del Prado *et al*., 2025). The response in milk production reported by Khurana *et al.* (2024) is of much interest, but their claim of a lower CO_2e intensity is dismissed because of lack of CH_4 measurements demonstrating the effects of the feed additive and because of lack of a farm-specific LCA.

Future perspectives

As the role of CH_4 in global warming has gained attention worldwide, the volume of research on enteric CH_4 mitigation conducted in Chile has been steadily increasing. A total of 18 peer-reviewed papers (10 *in vitro* and 8 *in vivo*, with 50% of the *in vitro* and 88% of the *in vivo* reports published in top impact factor quartile (Q1) journals) have been published in this period, apart from numerous conference presentations. However, as evidenced in this review, many results are only available as conference papers, often with incomplete information, and only available at the national level. The limited number of scientific publications and the time lag between study completion and publication in a scientific journal appear to be a common challenge for Chilean research groups working in this field. This is of concern as it implies that a significant portion of the research has not yet been evaluated under rigorous scientific standards, potentially limiting its reach and influence. In addition, all *in vivo* research in which CH_4 production was measured has been conducted with dairy cows. This is also a limitation, and future research also needs to address enteric CH_4 mitigation in beef cattle, sheep, and goats.

It is highly important that research on enteric CH_4 mitigation in Chile accelerates, not only because of the existing urgency to mitigate global warming, but also because most of the applied research on enteric CH_4 mitigation conducted elsewhere took place with total mixed rations and may often not be applicable to Chile's (and vast areas of the world) pasture-based conditions, as illustrated by the Muñoz *et al*. (2024) study supplementing 3-NOP to grazing dairy cows. Even with supplementation, diets in predominantly pasture-based systems are imbalanced, as the amount and composition of supplements cannot be adjusted quickly enough to balance for changes in the composition and digestibility of pastures. In addition, depending on the season and pasture, DMI may be constrained by grass availability. These are inherent characteristics of the diets ingested by grazing ruminants in comparison with ruminants fed total mixed rations. Therefore, it is important to consider whether these conditions inherent to pasture-based systems might interact with the treatments being evaluated for DMI, performance, and CH_4 production. This highlights the need for Chilean research to develop and adapt solutions feasible for implementation by Chilean producers, considering biological, economic, and societal aspects and constraints.

Moving forward, it is important to develop and establish reliable verification methods that can be applied by producers to demonstrate their efforts to mitigate enteric CH_4 emissions. This, in turn, should be reflected in the national greenhouse gas inventory to support Chile's commitment to reducing greenhouse gas emissions. Most importantly, any credible mitigation claims must be backed by scientific literature. Addressing these challenges is crucial for ensuring the accountability of the ruminant production industry to the national climate goals.

It is challenging to find solutions for enteric CH_4 mitigation that are both technically and economically feasible, safe to use, and not compensated for by increased emissions of other greenhouse gases. Research on enteric CH_4 mitigation in Chile has primarily focused on evaluating nutritional strategies, including supplementation with concentrates, use of more digestible forages, inclusion of oils via oilseeds, presence of secondary compounds in by-products from the national agricultural industry, and introduction of anti-methanogenic additives, both chemical and contained in algae. Solutions based on the intensification of ruminant production and improvement of production efficiency are considered straightforward and economically attractive ways to decrease CH_4 intensity. Production intensification strategies to decrease CH_4 production evaluated *in vivo* in Chile include supplementation with concentrates and improvement of forage quality. Of these, only the improvement of forage quality was effective in moderately decreasing CH_4 intensity, but at the same time likely increased N voided to the environment. Increasing the supplementation of concentrates to dairy cows did not decrease CH_4 intensity and increased total CH_4 emissions. A specific antimethanogenic strategy, supplementation with cottonseeds, moderately decreased CH_4 intensity and total CH_4 emissions, but also increased N voided to the environment in urine. The latter aspect highlights the need to conduct LCA of CH_4 mitigation strategies proven effective in assessing their potential environmental impacts in terms of total greenhouse gas emissions. To date, no LCA of agricultural greenhouse gases from ruminants conducted in Chile have been published in peer-reviewed journals.

Currently, only 3-NOP can be recommended for practical application in Chile, and solely for confined cattle. This limits its potential contribution to reducing national CH_4 emissions, as only 1.22% of beef and dairy cattle are managed in confined systems in Chile with controlled diets that allow precise mixing and feeding of the additive (ODEPA, 2019). Research on optimizing the supplementation of feed additives that inhibit CH_4 production in the rumens of grazing ruminants is urgently required. Other anti-methanogenic strate-

gies cannot yet be endorsed due to insufficient evidence of effectiveness, potential increases in the emissions of other greenhouse gases (which decrease the net impact of enteric CH_4 mitigation on total emissions of CO_2e), or negative externalities on production, such as reduced feed intake or product yield, as well as concerns about the quality and safety of the final product; all of these aspects still need to be addressed by local research. Some anti-methanogenic strategies that are potentially applicable to pasture-based systems and have not been investigated in Chile include the genetic selection of ruminants producing less CH_4 and vaccination against methanogens. Selecting ruminants based on residual CH_4 can allow moderate (~10%) decreases in CH_4 output per animal. This requires measuring CH_4 production in large numbers of animals or discovering reliable markers that are easy to measure and can accurately predict CH_4 production. Progress has been made in basic research on the development of vaccines against methanogens, but so far, there are no preparations effective at decreasing CH_4 production *in vivo* (Beauchemin *et al*., 2022). While these research lines are of great interest because of their potential to be applied in pasture-based systems, even in extensive ones, beginning long-term research in these areas in Chile might mean diluting already scarce human and financial resources. It is important to consider that there are several constraints that limit research productivity in Chile: lack of funding from government and the private sector (0.36 % of GDP investment in R&D, which is considerably lower than the 2.7% average of OCDE countries), limited availability of facilities and equipment, limited access to trained personnel, low appreciation of peer-reviewed research outside of the academic community, among others.

Importantly, the lack of solutions for enteric CH_4 mitigation that are ready for implementation by producers is a global problem. Even in intensive production systems, solutions that are technically feasible face economic constraints and require the adoption of financial incentives. An example are the subsidies in Denmark for producers who use 3-NOP or incorporate fat in dairy cows' diets (Danish Dairy Board, 2025). Pilot projects on the incorporation of 3-NOP into the diet of confined cows have been privately financed in Chile (Vargas García, 2024). Possible policies to be implemented to favor the adoption of anti-methanogenic strategies should be discussed by scientists in conjunction with the government, industry, consumers, and other decision makers.

CONCLUSIONS

Considerable research on enteric CH_4 mitigation, reviewed in this paper, has been conducted in Chile in the last 10 years; however, much of this research remains as conference summaries and still needs to be published in peer-reviewed journals. There was ample variation in CH_4 production among ryegrass genotypes *in vitro*. However, given that the least methanogenic genotypes were the most digestible, it is possible that these more digestible genotypes allow greater DMI *in vivo*, compensating for the lower CH_4 per gram of organic matter disappeared, and result in no differences in total CH_4, as found by one of the *in vivo* studies conducted in Chile evaluating forage quality. Forage rape and chicory did not decrease total CH_4 but increased milk production and decreased N excretion.

There is interest in further *in vivo* evaluation of pine bark extract and grape marc at different doses to assess whether they can decrease CH_4 production and urine N excretion without impairing performance and digestibility. Supplementation with cottonseed moderately decreased CH_4 production in the majority, but not in all studies. An increase in N excretion highlights the need to conduct LCA to estimate the effects of anti-methanogenic strategies on CO_2e emissions. The *in vitro* and *in vivo* evaluation of Chilean algae showed no promise at decreasing CH_4 production without compromising digestion and fermentation. In other instances, the information provided in conference summaries was insufficient to conclude about the effects of the algae evaluated on digestion, fermentation, and performance, or the algal species was not informed, casting doubt about the novelty of the results.

The chemical inhibitor 3-NOP was highly effective at inhibiting CH_4 production in dairy cows fed a total mixed ration; however, the inhibition was mild in grazing dairy cows. At present, there is no strategy to mitigate enteric CH_4 emissions proven consistently effective under pasture-based conditions in Chile, demonstrated to have no negative effects on performance, economically attractive, and not offset by greater emissions of other greenhouse gases. Future research should focus on optimizing the supplementation of anti-methanogenic feed additives to maximize their effectiveness, as well as evaluating *in vivo* anti-methanogenic by-products containing secondary compounds at different doses, seeking to lower CH_4 emissions and N excretion without negatively affecting performance and health. Other lines of research, such as tannin-containing forages and genetic selection of low CH_4-producing animals may be considered. Life cycle assessments and research on ruminants other than dairy cows are needed. Despite the many challenges in conducting research in Chile, after one decade of progress, research on enteric CH_4 mitigation in Chile has reached a stage of maturity.

DECLARATIONS

Competing Interests Statement

The authors declare that they have no competing interests.

Author contributions

Conceptualization: EMU, NLU, CM; Visualization: EMU, NLU, CM; Writing – Original Draft: EMU, NLU, CM, JA, JPK; Writing – Review & Editing: EMU, NLU, CM, JA, JPK.

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