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Mitigation strategies for methane emissions in ruminant livestock: a comprehensive review of current approaches and future perspectives

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Enteric methane emissions from ruminant livestock represent a major contributor to agricultural greenhouse gases and reflect an energetic inefficiency in ruminant metabolism. This review critically evaluates current mitigation strategies aimed at reducing CH₄ production in ruminants, with an emphasis on practical applicability, biological mechanisms, and integration into sustainable dairy production systems. Nutritional interventions—including tannins, saponins, essential oils, garlic compounds, seaweed (e.g., *Asparagopsis*), probiotics, and chemical inhibitors such as 3-nitrooxypropanol (3-NOP)—are discussed in the context of their effects on rumen microbiota, fermentation patterns, and animal productivity. Biological strategies such as archaeal-targeted vaccines, bacteriophage therapy, and microbiome engineering remain largely experimental but represent promising future directions. Genetic selection for low-emission phenotypes and improved manure management are also explored as complementary approaches to reduce emissions. Although some additives have achieved CH₄ reductions of 30–50% *in vivo*, results vary depending on diet, dose, delivery matrix, and duration. Notably, the long-term effects on productivity, nutrient utilization, and product quality remain underexplored. Integrated strategies combining dietary, genetic, and management interventions tailored to specific production systems are likely necessary to achieve meaningful, sustained reductions in ruminant CH₄ emissions.

KEYWORDS

methane, GHG, ruminant livestock, methanogenesis, mitigation strategies, feed additives

1 Introduction

Due to its role as a potent greenhouse gas (GHG), methane (CH₄) production in ruminants is an increasingly critical topic in scientific literature, particularly in intensive dairy farming (Króliczewska et al., 2023). Atmospheric concentrations of CH₄, a potent GHG, have risen dramatically since pre-industrial times, increasing by approximately 150% since the year 1750 (Pachauri et al., 2014). Methane is a colorless, odorless, and flammable gas that constitutes the primary component of natural gas (Candelaresi and Spazzafumo, 2021). Although it naturally occurs in the atmosphere at low concentrations, enteric CH₄—mainly produced via microbial fermentation in the gastrointestinal tract of ruminants (i.e., cattle, sheep, and goats)—represents a significant source of agricultural GHG emissions (Thacharodi et al., 2024). This biologically produced CH₄ is mostly released via eructation (belching) (Morgavi et al., 2023) and contributes both to global warming and to energy inefficiency, as it accounts for a 6–10% loss of gross dietary energy (Castelán-Ortega et al., 2014). Globally, the livestock sector contributes approximately 14.5% of total anthropogenic GHG emissions, with enteric fermentation alone accounting for nearly 40% of agricultural GHG (FAO, 2017). Among livestock-related emissions, enteric CH₄ represents the dominant source, contributing up to 88% of CH₄ emissions from the sector (Arndt et al., 2022). Since CH₄ has a significantly higher global warming potential than carbon dioxide (CO₂) (Mar et al., 2022), the livestock farming sector presents a key opportunity for reducing emissions while also improving production efficiency.

Within the rumen, a complex and diverse microbiome—including bacteria, protozoa, and fungi—ferments ingested feed to produce volatile fatty acids (VFA) such as acetate, propionate, and butyrate, which are primary energy sources for the host animal (Matthews et al., 2019). During fermentation, metabolic cofactors like NADH, NADPH, and FADH are re-oxidized, resulting in the production of molecular hydrogen (H₂). Methanogenic archaea then utilize this H₂ to reduce CO₂ to CH₄, thereby preventing the accumulation of metabolic H₂ but at the cost of significant energy

loss—energy that could otherwise contribute to productive functions such as milk synthesis (Castelán-Ortega et al., 2014). Methane production in the rumen is influenced by several factors, including feed composition, chewing behavior, salivation, and gastrointestinal motility (Snelling and John, 2017).

Microbial CH₄ emissions of anthropogenic origin are predominantly associated with three primary sources: livestock production (115 Tg CH₄ yr^{−1}), landfills and waste management (68 Tg CH₄ yr^{−1}), and rice cultivation (30 Tg CH₄ yr^{−1}). Within the livestock sector, enteric fermentation represents the principal emission pathway, contributing approximately 85% of total CH₄ emissions from this category, equivalent to 98 Tg CH₄ yr^{−1} (Sauniois et al., 2019). Cattle are the leading source of enteric CH₄ emissions globally, a consequence of their substantial global population (~1.5 billion animals), extensive rumen volume, and specific digestive physiology (Malik et al., 2021).

Estimated CH₄ emissions vary widely among livestock species and production stages (Starsmore et al., 2024b). Among dairy breeds, Holsteins generate more CH₄ than crossbreds, while heifers on fertilized pastures produce more methane (around 223 g CH₄/day) than those grazing on unfertilized pastures (around 179 g CH₄/day). Various factors, including fecal consistency, digestible material content, climate, and exposure duration, influence CH₄ emissions from manure. On dairy farms, annual CH₄ emissions from manure storage and pens can reach 120 kg per cow (Kide et al., 2017; Cezimbra et al., 2021).

Table 1 summarizes typical daily and annual CH₄ emissions for dairy cows, sheep, beef cattle, and other ruminants, highlighting differences based on physiological status, breed, and management system.

A recent study by Evangelista et al. (2024) examining trends in livestock-related methane emissions reported that cattle contribute the largest share, accounting for approximately 62% of total emissions. This is followed by buffaloes (8%), goats (4%), sheep (3%), and monogastric species such as pigs and poultry, which together account for 23% of emissions (Figure 1).

TABLE 1 Daily and annual enteric methane emissions by animal type and breed.

Animal type	Methane emission (g CH ₄ /day)	Methane emission (kg CH ₄ /animal/year)	References
Lactating Holstein cow	426 – 463	155 – 163	(Rojas De Oliveira et al., 2024b, Castillo et al.)
Crossbreed dairy cow	264	NP	(Broucek, 2014)
Dry dairy cow	269	NP	(Pedreira et al., 2009; Broucek, 2014)
Heifers	223	NP	(Broucek, 2014)
Dairy ewe	26.3	8.4	(Quail et al., 2025; Broucek, 2014)
Dairy goat	19.4	15 – 17	(Robertson et al., 2015; Quail et al., 2025)
Suffolk sheep	22 – 25	NP	(Broucek, 2014)
Beef cattle	161 – 323	NP	(Broucek, 2014)
Mature beef cow	240 – 396	NP	(Broucek, 2014)
Bison	NP	72	(Broucek, 2014)

NP, not published.

Mitigating methane production in dairy cows presents a dual opportunity: reducing environmental impact while enhancing milk production, yield, and composition. This synergistic effect underscores the importance of advancing research on effective mitigation strategies in dairy farming. The development of CH₄ mitigation strategies is crucial, considering increasing regulatory pressures to reduce agriculture's contribution to climate change (Reisinger et al., 2021).

Various strategies have been proposed, including feed additives that inhibit methane-producing microbes, breeding programs selected for low-methane cattle (Króliczewska et al., 2023), and precision monitoring systems that enable individualized intervention. Studies highlight the potential of biologically active compounds such as algae extracts, tannin preparations, and 3-Nitrooxypropanol (3-NOP) (Pepeta et al., 2024), and essential oils (EOs) in modifying the rumen microbiome and reducing enteric CH₄ production (Belanche et al., 2025).

The goal of this review is to evaluate current research findings and present viable strategies that balance enteric CH₄ reduction with economic feasibility and productive efficiency in dairy systems. Specifically, the review aims to (i) synthesize current evidence on the magnitude and variability of CH₄ emissions across dairy production contexts; (ii) assess the efficacy of leading mitigation strategies—including dietary interventions such as macroalgae (e.g., *Asparagopsis taxiformis*), tannin-rich extracts, essential oils, probiotics, and synthetic inhibitors like 3-nitrooxypropanol (3-NOP); and (iii) evaluate the potential trade-offs and co-benefits of these approaches in relation to rumen fermentation, nitrogen metabolism, animal performance, and environmental sustainability. Special emphasis is placed on the impact of these compounds on microbial activity and fermentation dynamics. Mitigation techniques are categorized based on mode of action, active

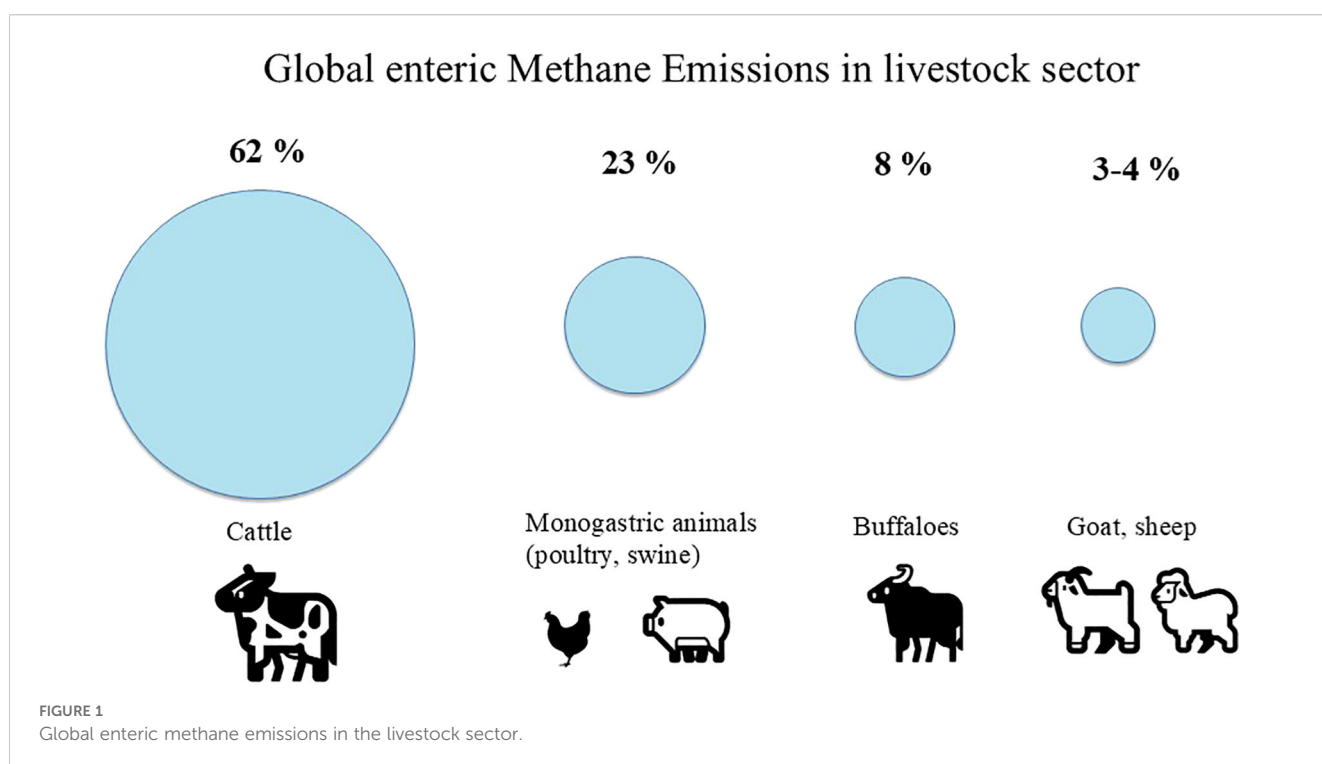
ingredient, dosage, application period, observable effects, and supporting literature. By integrating and critically appraising recent findings, this review provides a comprehensive framework to inform future research priorities, evidence-based policymaking, and practical implementation of CH₄ mitigation strategies in modern dairy production.

2 Animal management and breeding strategies

Effective management strategies are essential for reducing GHG emissions from livestock systems. Such reductions are not only critical for improving the environmental sustainability of farming but also provide a benchmark for comparing and evaluating the relative effectiveness of different mitigation practices. By quantifying GHG reductions under alternative management strategies, researchers and policymakers can identify the most impactful interventions and prioritize their implementation at both farm and national levels (Zhang et al., 2024b). Additionally, from an economic perspective, management adjustments represent a cost-effective approach that not only mitigates direct enteric CH₄ emissions from cattle but also enhances soil quality and grassland biodiversity, thereby improving the overall CH₄ balance and sustainability of the production system (FAO, 2016).

An overview of the principal animal management and breeding strategies to mitigate enteric CH₄ emissions, together with their mechanisms, evidence maturity, and limitations, is summarized in Table 2.

Grazing management offers considerable potential. Zubieta et al. (2021) demonstrated that optimizing herbage intake and



live weight (LW) gain under light-to-moderate grazing intensities can reduce CH₄ intensity to approximately 0.2 kg CH₄/kg LW gain, representing a 55% mitigation potential for pasture-based systems. Holistic cattle management strategies, such as increasing stocking density, may replicate historic grazing patterns of large wild herbivores, thereby restoring grasslands, preventing desertification, and indirectly lowering GHG emissions (Wyffels et al., 2013; Hawkins et al., 2022).

Grasslands also act as carbon sinks. Average sequestration rates of 5 ± 30 g C/m² annually have been reported, though values vary

widely depending on soil type, grazing system, and management (Soussana et al., 2010; Bårdule et al., 2024).

Several management practices can reduce carbon losses and enhance sequestration, including: (i) minimizing soil disturbances such as tillage and grassland-to-cropland conversion, (ii) improving nutrient-poor permanent grasslands, (iii) adopting light rather than heavy grazing, (iv) extending the duration of grass leys, and (v) incorporating grass-legume mixtures or converting grass leys into permanent grasslands (Soussana, 2008). Additionally, manure management is a critical area of mitigation.

TABLE 2 Animal management and breeding strategies for reducing enteric methane production in ruminants.

Management practice/breeding option	Trait/metric used	Effect on CH ₄	Mechanism	Implementation consideration	Reference
Genetic selection: direct CH ₄ traits	Daily CH ₄ yield, intensity	Heritable ($h^2 \approx 0.16$ – 0.27); cumulative reduction	Select low emitters	Needs standardized phenotyping	(Lassen and Løvendahl, 2016) (Kamalanathan et al., 2023)
Genomic selection (MIR–predicted CH ₄)	Methane efficiency index	20–30% herd-level reduction by 2050	Proxy traits enable scalable selection	Prediction accuracy varies	(Rojas De Oliveira et al., 2024a)
Residual methane emissions (RME)	Observed – expected CH ₄	Reduction without penalizing productivity	Captures inherent animal differences	Requires validated intake and size data	(Uemoto et al., 2024)
Residual feed intake (RFI)	Feed efficiency index	$\approx 27\%$ lower CH ₄ in low–RFI animals	Improved efficiency reduces methanogenesis	System-level effects depend on feed utilization	(Da Silva Soares et al., 2025) (Dini et al., 2019)
Reproductive management	Earlier age at first calving, shorter calving	$\approx 10\%$ reduction in CH ₄ intensity	Less unproductive time and fewer replacements	Requires balanced heifer growth and fertility	(Clasen et al., 2024)
Health & welfare	Disease prevention, lameness control	≈ 4 – 8% lower GHG intensity per unit of milk/meat production	Restored intake and production	Requires monitoring and biosecurity	(Džermeikaitė et al., 2024)
Heat abatement	Cooling, shade, thermotolerance	Prevents 0.8–6.6% increase in CH ₄ intensity	Maintains intake and productivity	Resource-intensive (energy, water)	(Chen et al., 2025)
Feeding management	Forage quality, harvest timing	Lower CH ₄ yield; intensity reduced	Improves digestibility to shift fermentation	Absolute CH ₄ may rise with higher intake	(Beauchemin et al., 2022)
Advanced methane prediction models	ML-based prediction integrating empirical +mechanistic data	Improved accuracy and scalability of CH ₄ phenotyping	Combines the flexibility of empirical models with mechanistic accuracy	Requires large, diverse datasets and validation	(Ross et al., 2024)
Host genetics & rumen microbiome effects	Host heritability $\sim 21\%$; microbiability $\sim 13\%$	Dual-target strategies for CH ₄ mitigation	Genome and microbiome explain additive variance components	Requires integrated genetic and microbial datasets	(Difford et al., 2018)
Grazing management optimization	Light–moderate grazing intensity; LW gain thresholds	CH ₄ intensity reduced to ~ 0.2 kg CH ₄ /kg LW gain ($\sim 55\%$ mitigation)	Optimizes forage intake and performance per unit gain	Requires adaptive stocking and pasture monitoring	(Zubieta et al., 2021)
Holistic cattle management & grassland restoration	Increased stocking density mimicking natural grazing	Indirect CH ₄ /GHG mitigation; ecosystem restoration	Stimulates plant regrowth and carbon sequestration	Effects vary with ecosystem; requires monitoring	(Hawkins et al., 2022)
Grassland carbon sequestration practices	SOC sequestration (e.g., 129 g C/m ² in grazed systems)	Indirect GHG reduction via soil carbon sinks	Improves SOC through grazing, ley duration, and legumes	Variable across soil types; long-term benefits	(Soussana, 2008) (Soussana et al., 2010)
Manure management (anaerobic digestion, composting)	CH ₄ captured from manure; improved storage	Significant reduction in manure-derived CH ₄	Biogas recovery and reduced anaerobic methanogenesis	Requires infrastructure; potential energy offset	(Symeon et al., 2025)
Genetic selection (advanced tools)	GWAS, genomic selection for low-CH ₄ genotypes	Reduced CH ₄ per unit intake while maintaining yield	Identifies and propagates low-emission genotypes	Needs long-term monitoring and integration	(Pickering et al., 2015) (De Haas et al., 2011)

Technologies such as anaerobic digestion capture CH₄ from manure and convert it into biogas, while composting and improved storage (e.g., frequent removal and aeration) reduce CH₄ release during storage (Montes et al., 2013). Breeding and genetic selection present long-term, cumulative opportunities for CH₄ mitigation. Selecting cattle with lower residual feed intake (RFI) enhances feed efficiency and is associated with reduced CH₄ emissions per unit of feed consumed (Manzanilla-Pech et al., 2021). Studies have confirmed a strong association between RFI and methane production: efficient animals with low RFI typically consume less feed than expected for their body weight and growth rate, resulting in lower CH₄ output (Nkrumah et al., 2006; Hegarty et al., 2007).

However, in dairy cattle, early lactation physiology complicates the use of RFI because cows in negative energy balance require high feed intake to prevent metabolic and fertility problems, which may increase herd-level CH₄ intensity if not properly managed (Garnsworthy, 2004).

Evidence from quantitative genetics confirms that methane-related traits are heritable ($h^2 = 0.12\text{--}0.3$), enabling genetic improvement (Lassen and Løvendahl, 2016; Pszczola et al., 2019; Kamalanathan et al., 2023). Traditional measurement methods, such as respiration chambers, are accurate but impractical at scale. In contrast, GreenFeed systems, in-parlor sniffers, and milk mid-infrared (MIR) prediction models now enable scalable phenotyping, paving the way for genomic selection (Lassen and Løvendahl, 2016; Rojas De Oliveira et al., 2024b). For example, research on Canadian Holsteins has led to the development of a national genomic evaluation for CH₄ efficiency using MIR-predicted data, which is expected to reduce herd-level methane emissions by 20–30% by 2050 without compromising milk yield (Rojas De Oliveira et al., 2024a). In another research, the sniffer method has been reported as a reliable approach for identifying Holstein cows with lower CH₄ emissions. It can therefore serve as an indicator trait for genetic selection (Uemoto et al., 2024).

Residual methane emissions (RME), defined as the deviation between observed and expected methane output after adjusting for intake and body size, have emerged as promising breeding objectives because they capture inherent animal variation independent of productivity (Starsmore et al., 2024a). Smith et al. (2022) reported that RME is strongly associated with rumen microbiota composition, supporting its use as a robust phenotype for identifying inherently low-emission animals. Complementary host–microbiome studies indicate that both host genetics and microbial composition independently explain CH₄ variation, suggesting synergistic opportunities for genetic and microbial interventions (Wallace et al., 2002; Difford et al., 2018). These findings further emphasize the potential of manipulating the rumen microbiota as a strategy to mitigate enteric CH₄ production.

Emerging approaches include machine learning models, which integrate empirical and mechanistic data to improve CH₄ prediction and phenotyping (Ross et al., 2024). Advanced genetic tools, such as genome-wide association studies (GWAS) and genomic selection, are being applied to identify low-emission genotypes, with the potential to breed animals that maintain production while reducing CH₄ emissions (Pickering et al., 2015;

Manzanilla-Pech et al., 2021). However, the realization of genetic gain is inherently slow, often requiring decades, and possible trade-offs with other traits (e.g., fertility, robustness, or feed efficiency) must be carefully monitored to ensure long-term sustainability (De Haas et al., 2011; Pickering et al., 2015; Gatenby, 2021). Given these limitations, genetic strategies should not be viewed in isolation but rather as part of an integrated mitigation framework. While genetic improvement provides permanent, cumulative reductions in CH₄ emissions, the rate of progress is slow and dependent on long-term breeding programs. In contrast, management interventions—such as dietary modification, manure treatment, and optimized grazing—offer more immediate reductions in GHG. A combined approach, aligning rapid management-based gains with sustained genetic progress, is therefore essential to achieve both short-term emission reduction targets and long-term climate goals (Beauchemin et al., 2022).

3 Biological strategies

3.1 Bioaugmentation with homoacetogenic bacteria

One of the promising biological approaches is bioaugmentation with homoacetogenic bacteria (homoacetogens), which compete with methanogens for H₂ in the rumen, thereby reducing CH₄ emissions (Ungerfeld, 2020).

During ruminal fermentation, H₂ and CO₂ serve as the primary substrates for methanogens; methanogenesis acts as the main H₂ sink, keeping dissolved H₂ levels low (1–10 Pa), which is essential for maintaining efficient fermentation pathways (Kohn and Boston, 2000; Mackie et al., 2023; Fregulia et al., 2024).

Homoacetogens convert H₂ and CO₂ into acetate via the Wood–Ljungdahl pathway, offering an alternative electron sink to methanogenesis (Danielsson et al., 2012). However, the effectiveness of this approach depends on several factors, including rumen pH, substrate availability, and the ability of homoacetogens to establish and outcompete methanogens in the complex rumen ecosystem (Gagen et al., 2010).

According to Karekar et al. (2022) homoacetogens exhibit a versatile metabolism that is suitable for diverse substrates and can act as a carbon sink by converting CO₂ into bioproducts, potentially improving efficiency by diverting H₂ away from methanogenesis. However, their competitive advantage in mature rumen systems appears limited, as methanogens overwhelmingly dominate H₂ utilization and suppress homoacetogenic activity. Experimental approaches that integrate methanogenesis inhibition—such as the use of 2-bromoethanesulfonic acid (BES)—with microbial bioaugmentation strategies have demonstrated promising potential for mitigating enteric CH₄ production. For instance, in the study by Murali et al. (2021) BES treatment increased headspace H₂ and reduced acetate; subsequent bioaugmentation with *Acetitomaculum ruminis* and *Acetobacterium woodii* restored acetate levels by 45% and 70%, respectively. Similarly, Stefanini Lopes and Ahling (2023) demonstrated that combining a kangaroo-derived homoacetogenic

consortium with almond-shell biochar improved acetic acid production *in vitro*, albeit temporarily, highlighting transient benefits and the need for stabilization strategies.

Although homoacetogenesis is energetically less favorable than methanogenesis (Conrad, 2023) its competitiveness can be enhanced through strategies such as supplementing substrates like glucose, glycerol, and xylose, along with H₂ and CO₂, to leverage its mixotrophic advantages (Tsapekos et al., 2022). To enhance the viability of homoacetogenesis, strategies such as co-supplementation with acetogenesis stimulants (e.g., fumarate, malate, or nitrate) and optimizing feeding regimens have been explored (Morgavi et al., 2010). Additionally, genetic screening of ruminant microbiomes has identified novel homoacetogenic strains with greater resilience to rumen conditions, offering potential for further development (Henderson et al., 2015).

Additional measures include the introduction of acetogenesis stimulants, such as yeast cultures, maintaining a lower ruminal pH, and identifying novel acetogen strains capable of thriving at low H₂ thresholds and increasing their densities in the rumen (Yang et al., 2015).

Propionate-producing bacteria, along with nitrate- and nitrite-reducing, and sulfate-reducing bacteria, have thermodynamic advantages over methanogens in utilizing H₂ as an electron donor (Lan and Yang, 2019). However, their low abundance or the absence of necessary substrates in the rumen limits their activity (Choudhury et al., 2022). Enhancing the propionate-producing pathway can be achieved by supplementing animals with propionate precursors such as fumarate and malate or introducing functionally complementary propionate-producing bacterial consortia as additives (Jeong et al., 2024). Given the low natural concentrations of nitrate and sulfate in the rumen, using these compounds as additives could stimulate the growth of nitrate- and sulfate-reducing bacteria. However, toxic by-products such as nitrite and hydrogen sulfide (H₂S) must be carefully managed (Latham et al., 2016). Strategies to mitigate toxicity risks include combining sulfate-reducing bacteria (SRB) with nitrate-reducing, sulfur-oxidizing bacteria or employing SRB strains capable of utilizing H₂S or nitrite (Greene et al., 2003).

Exploring microbes that compete with methanogens and redirect H₂ away from methanogenesis presents a promising strategy for reducing CH₄ emissions in the rumen (Lan and Yang, 2019). Despite its potential, bioaugmentation with homoacetogenic bacteria faces challenges, including the need for long-term microbial stability in the rumen and variations in host responses across different animal species. Large-scale field trials are necessary to evaluate the long-term feasibility and effectiveness of this approach under commercial farming conditions (Wallace, 2004). Future research should focus on strain selection, microbial adaptation strategies, and possible synergies with other methane mitigation technologies to improve implementation (Martin et al., 2010).

3.2 The use of bacteriophages

Bacteriophages (phages), traditionally applied in phage therapy to treat bacterial infections such as enteric diseases, sepsis, and

chronic infections (Lin et al., 2017), are gaining attention for broader roles, including food preservation, microbiome modulation, and even environmental applications like climate change mitigation (Elois et al., 2023). Recently, phage therapy has been proposed as a novel strategy to target methanogenic archaea in the rumen to reduce enteric CH₄ production (Lobo and Faciola, 2021). By selectively lysing methanogens, phages may suppress methane formation without significantly disturbing other rumen microbial populations (Morkhade et al., 2020).

The conceptual appeal of phage-based CH₄ mitigation lies in its specificity, ecological safety, and potential to bypass some of the limitations associated with chemical inhibitors or vaccines. However, this strategy remains in its infancy, and several critical challenges must be addressed.

To date, only a limited number of studies have investigated the isolation and characterization of archaeal phages that target rumen methanogens. For example, Ouwerkerk et al. (2011) initiated the development of a phage library specifically targeting the dominant methanogenic archaea in Australian livestock systems. However, experimental evidence on the *in vivo* efficacy of such phages remains limited. The effectiveness of archaeophage therapy mainly relies on the ability to identify highly specific phages that can infect predominant methanogenic species—such as *Methanobrevibacter ruminantium* and *Methanobacterium* spp.—without disrupting beneficial rumen microbial functions (Lobo and Faciola, 2021). Despite their potential, the identification of archaeal phages remains limited, underscoring a substantial knowledge gap in our understanding of phage-host interactions within methanogenic communities. Among fully sequenced microbial genomes, six archaeal phages have been described, including *Methanobacterium* phage psi M1, *Methanobacterium* phage psi M2 (a variant of M1), and *Methanobacterium* phage psi M100, all of which belong to the Siphoviridae phage family. These phages demonstrate the capacity to infect key rumen methanogens such as *Methanobacterium* spp., a dominant archaeal genus in the rumen. Moreover, members of the Siphoviridae family have shown infectivity toward *Methanobacterium*, *Methanobrevibacter*, and *Methanococcus* species (McAllister and Newbold, 2008). Leahy et al. (2013) presented the complete genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* M1, offering critical insights into its metabolic and cellular pathways. A prophage identified in *M. ruminantium* encodes 69 phage-related proteins, including the lytic enzyme PeiR from prophage φMru, which shows potential as a biocontrol agent against ruminal methanogens. A novel approach was proposed, utilizing viral enzyme-loaded nanoparticles that effectively lyse not only the original methanogen host strain but also a diverse range of ruminal methanogen species in pure *in vitro* cultures, resulting in significant CH₄ reductions of up to 97% (Altermann et al., 2018). However, this broad-spectrum activity raises concerns about potential disruption to the natural rumen microbial ecosystem.

Rumen phage populations are highly diverse and individualized, with concentrations ranging from 10⁷ to 10⁹ particles per milliliter (Swain et al., 1996). This high diversity, coupled with host-specific microbial interactions, raises concerns

about the stability, persistence, and consistent efficacy of introduced phages within the rumen ecosystem. To date, no study has comprehensively identified the phage taxa present in the rumen and their specific archaeal hosts, nor has it assessed their interactions with the methanogen community at a large scale. These knowledge gaps underscore a critical barrier to the development of phage-based CH₄ mitigation strategies in ruminants, highlighting the need for advanced metagenomic and host-linkage studies to inform future applications.

3.3 Use of antimethanogenic vaccines

One proposed strategy to mitigate CH₄ emissions is the development of vaccines targeting methanogenic archaea in the rumen. These vaccines aim to elicit an immune response that reduces methanogen populations, thereby lowering methane production without adversely affecting essential microbial communities in the rumen (Wedlock et al., 2013). Developing an effective methane-reducing vaccine requires identifying immunogenic proteins unique to methanogens to ensure a robust immune response while maintaining overall gut health (Baca-González et al., 2020). Research indicates that vaccines targeting key methanogen species can significantly alter rumen archaeal populations, leading to a measurable reduction in methane emissions (Williams et al., 2009). However, long-term efficacy remains a critical challenge, as the rumen microbiome is highly dynamic and capable of adapting to immune pressures over time (Wedlock et al., 2010).

In vivo (Wright et al., 2004; Zhang et al., 2015), and *in vitro* (Cook et al., 2008) studies evaluating antimethanogenic vaccines have reported variable and often time-dependent effects on enteric CH₄ production. Notably, the lack of a consistent reduction in CH₄ emissions—despite increased methanogen-specific antibody titers and observed shifts in archaeal community composition—suggests that vaccine formulations may lack broad-spectrum efficacy against the diverse rumen methanogen populations (Williams et al., 2009). Moreover, population-level differences in immune responses across species and breeds introduce high inter-animal variability, complicating the predictability and scalability of vaccine interventions (Buddle et al., 2011). One of the major limitations in the development of antimethanogenic vaccines is the challenge of identifying antigens that are both conserved and immunogenic across the diverse array of methanogenic archaea present in the rumen. Methanogens exhibit high variability in surface structures and protein epitopes (Reeve, 1992), which complicates the formulation of a broadly protective vaccine. In addition, variation in host immune response—driven by genetic background, physiological status, and rumen microbiota composition—leads to inconsistent antibody production and limited uniformity in microbial suppression. Some animals exhibit high antibody titers with negligible impact on archaeal populations or methane output, while others respond poorly to vaccination protocols. These issues have been reported in both dairy and sheep trials and represent key barriers to reliable implementation (Wedlock et al., 2013; Subharat et al., 2016). Another source of variation is animal age, as it is well-

known that young animals are more susceptible to infectious diseases than adults (Watson et al., 1994). Moreover, the durability of the immune response and the potential for microbial adaptation or vaccine escape remain unresolved. Further research is needed to identify robust antigen targets and optimize delivery systems that can consistently elicit long-term methane mitigation across diverse ruminant populations.

Despite these constraints, vaccination remains a promising and potentially cost-effective approach for mitigating methane emissions. It offers practical advantages, particularly for grazing systems with limited access to feed additives. However, successful implementation will require optimized antigen discovery, improved delivery systems (e.g., oral or slow-release formulations), and robust field trials to assess long-term impacts on CH₄ emissions, animal performance, and microbial ecology (Baca-González et al., 2020).

The advantages and challenges of biological strategies for reducing methane emissions from ruminants are presented through a SWOT analysis, which is presented in Table 3.

4 Nutritional strategies

Enteric methane (CH₄) represents both an energetic loss and a significant contributor to agricultural greenhouse gas emissions, produced predominantly via ruminal microbial fermentation and closely associated with dry matter intake (DMI) (Hornbuckle and Tennant, 1997; Dressler et al., 2024).

Nutritional strategies to mitigate CH₄ emissions primarily focus on redirecting hydrogen (H₂) toward alternative sinks and improving carbohydrate fermentability. Increasing the digestibility of non-structural carbohydrates (starch, sugars) shifts rumen fermentation toward propionate—the main competing H₂ sink—thereby lowering CH₄ yield, whereas structural carbohydrates favor acetate production and methanogenesis (Morgavi et al., 2010; Beauchemin et al., 2022). Key interventions include starch processing (e.g., steam-flaking, fine grinding), which enhances ruminal starch availability and reduces CH₄ emissions relative to whole grain; controlled use of rapidly fermentable sugars, with variable effects; and improvements in fiber digestibility through particle size reduction or exogenous fibrolytic enzymes (Johnson et al., 1994; Tavendale et al., 2005; Beauchemin and Mcginn, 2006; Mcallister and Newbold, 2008; Benchaar et al., 2014).

Forage selection also plays a critical role: replacing grass or legume silages with corn silage, which has higher non-fiber carbohydrate (NFC) content, consistently reduces CH₄ yield and intensity. Similarly, high-sugar grasses and energy-dense roughages can further mitigate emissions (Soteriades et al., 2018; Sun et al., 2022). Research by Hristov (2024) suggests that the type of roughage in the diet influences CH₄ production. When comparing corn silage with legume silage, methane emissions were either unchanged or slightly reduced with corn silage. Furthermore, replacing grass silages with corn silage resulted in a 9–16% reduction in CH₄ yield and a 6% decrease in CH₄ intensity. In total mixed rations (TMR) with a higher proportion of grass silage, methane reductions were more modest, typically reaching up

TABLE 3 SWOT analysis of biological strategies for reducing methane emissions in ruminant livestock.

Strengths	Weaknesses
✓ Effectiveness in Methane Reduction – These strategies have shown potential in reducing CH₄ by 20-50%. ✓ Sustainability – Many biological methods are eco-friendly, utilizing natural feed additives and microbial interventions rather than chemical solutions. ✓ Animal Health Benefits – Improvement of feed efficiency by reducing energy loss from methane production; enhancing nutrient absorption and overall productivity; can enhance energy metabolism by converting hydrogen into acetate, providing an alternative energy source for the animal. ✓ Consumer and Market Demand – Increasing global pressure for sustainable agriculture creates incentives and support for adopting these strategies.	✗ Variability in Efficacy – The effectiveness of biological interventions can vary based on diet, livestock species, and environmental factors, which may limit broad application. ✗ Long-Term Sustainability Questions – Effectiveness may decline over time as methanogenic archaea evolve resistance or adapt to vaccine-induced changes. Requires regular booster doses for sustained impact, increasing logistical challenges and costs. Large-scale production and delivery systems for phages need optimization for commercial viability. ✗ Potential Impact on Productivity – strategies might inadvertently affect digestion, leading to reduced growth rates or milk yields in some cases. ✗ Cost and Adoption Barriers – Many of these strategies require investment, research, and farmer education, which can slow adoption.
Opportunities	Threats
🔬 Advancements in Biotechnology – Genetically modified microbes, precision fermentation, and genome editing may further enhance methane reduction. 💰 Policy Support and Funding – Governments and organizations are increasingly offering subsidies and incentives for sustainable livestock farming. 🌱 Carbon Markets and Sustainability Labeling – Farmers who reduce methane emissions may gain financial benefits from carbon credits or eco-labeling for climate-conscious consumers. 🔄 Integration with Holistic Farming Practices – Combining biological strategies with regenerative grazing, agroforestry, and manure management could maximize sustainability.	⚠️ Regulatory Hurdles – Some biological strategies, such as genetically modified microbes, may face strict regulatory approval processes. ⚠️ Resistance from Traditional Farmers – Adoption of new practices may be slow due to a lack of awareness, resistance to change, or cultural factors. ⚠️ Unintended Ecological Impacts – Altering the gut microbiome may have unforeseen effects on animal health and ecosystems, e.g., vaccine-induced changes can persist in manure or the environment and could impact soil microbial communities, or persistence of homoacetogenic bacteria in manure and soil may lead to altering of C and N cycles in unintended ways. ⚠️ Market Volatility and Supply Chain Issues – The availability and cost of specific feed additives (e.g., seaweed) may fluctuate, affecting long-term viability.

to 4%. These findings highlight the potential of corn silage as a viable approach for reducing CH₄ emissions in ruminant diets.

Complementary feed additives such as 3-nitrooxypropanol (3-NOP) and bromoform-rich red seaweed extracts have demonstrated enteric CH₄ reductions in the range of ~30–50%, with red seaweed (e.g., *Asparagopsis* spp.) occasionally delivering up to ~80% in experimental settings (3-NOP: ~30–45%; *Asparagopsis* average ~37%, maxima ~98%) (De Bhowmick and Hayes, 2023; Romero et al., 2023; Hristov, 2024; Meo-Filho et al., 2024). While integrated nutritional strategies, especially when combined with manure-management technologies, hold theoretical potential for aggregate reductions approaching ~60%, empirical data from combined enteric-plus-manure mitigation rarely reach this level under current commercial conditions (Hristov, 2024).

These cumulative findings underscore the critical role of diet composition and additive strategies in reducing enteric methane emissions, setting the stage for emerging approaches—such as algal supplementation—that offer targeted biochemical mechanisms and potentially greater mitigation efficacy under specific production contexts.

4.1 Algae

Algal biomass is increasingly positioned as a sustainable, circular feed ingredient with the potential to lower the carbon footprint of ruminant production. Beyond serving as a high-quality nutrient source, specific macro- and microalgal taxa contain bioactive compounds that modulate rumen microbiology and hydrogen sinks, thereby holding

high potential for enteric CH₄ mitigation. Recent reviews highlight both the promise and the practical constraints (supply, processing costs, and standardization) associated with scaling algae for livestock systems (De Bhowmick and Hayes, 2023; Wanapat et al., 2024).

The summary report of the literature analysis on the effects of supplementing ruminant diets with probiotic bacteria is presented in Table 4.

Among seaweeds, red macroalgae of the genus *Asparagopsis* remain the most potent enteric CH₄ mitigation option *in vivo*. Multiple trials in beef cattle have demonstrated substantial reductions when *A. taxiformis* is included at low dietary levels, with reported decreases often exceeding 50% and, in some cases, approaching 80%, depending on the diet composition and inclusion rate. The primary mechanism involves the inhibition of the methyl-coenzyme M reductase (MCR) pathway by halogenated methane analogs—especially bromoform (CHBr₃)—which suppresses the terminal step of methanogenesis (Thorsteinsson et al., 2023; Kelly et al., 2025).

Efficacy varies with species, dose, basal diet, and type of supplement used in the study (freeze-dried biomass vs. stabilized actives) (Alvarez-Hess et al., 2024). In a finishing-diet research study, a proprietary bromoform-containing algae product (“Alga 1.0”) fed at 69 or 103 g/d reduced methane yield by 39% and 64%, respectively, without affecting digestibility but decreasing DMI by ~10–13%. These data underscore the trade-off between mitigation and intake that may emerge at higher effective doses (Colin et al., 2024).

Safety and residue outcomes are an active area of research. Transfer of CHBr₃ to milk and urine has been detected under certain conditions in dairy cows fed *Asparagopsis*. However, tissue

TABLE 4 Summary of algal-based interventions for enteric methane mitigation in ruminants.

Algal Species/Product	Type of study	Inclusion/dose	Animal type	CH ₄ effect	Toxicity/side effects	Reference
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	0.25, 0.50% on OM basis	Beef cattle	~50–80% ↓ (diet-dependent)	No adverse impact on production and feed efficacy. No CHBr ₃ residues in the product.	(Roque et al., 2021)
<i>Asparagopsis armata</i>	<i>In vivo</i>	0.5–1.0% on OM basis	Dairy cattle	↓CH ₄ yield by 20.3 and 42.7%	↓DMI, FE, MY	(Roque et al., 2019)
Product Alga 1.0 (Alga Biosciences)	<i>In vivo</i>	69 and 103 g/d	Jersey cows	↓CH ₄ by 39 (for 69 g/d) and 64% (for 103 g/d)	↓DMI by 10.1% (for 69 g/d) and 13.3% (for 103 g/d)	(Colin et al., 2024)
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	5 g/kg on DM basis	Murciano-Granadina female goats	↓CH ₄ yield by 31.4%	NR	(Pedro et al., 2022)
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	0.15–0.3% on OM basis	Nordic Red dairy cows	↓CH ₄ at a dosage of 0.3% OM (only during the 8 weeks of the experiment)	↓DMI and ECM yield. Due to the temporary mitigating effect, further long-term studies are warranted.	(Angellotti et al., 2025)
Asparagopsis steeped in canola oil (ASP-oil)	<i>In vivo</i>	132, 267, 409, 467 mg CHBr ₃ /cow/day	Holstein-Friesian cows	↓CH ₄ production up to 38% (dose-dependent effect)	No adverse impact on FI, but presence of CHBr ₃ in milk. ↓MY with ↑CHBr ₃ in ASP-oil	(Alvarez-Hess et al., 2024)
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	0.05%, 0.10%, and 0.20% on an OM basis	Beef steers	↓CH ₄ yield by 9% (for 0.05%), 38% (for 0.10%), and 98% (for 0.20%).	No adverse impact on DMI. ↑ADG.	(Kinley et al., 2020)
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	0.25 and 0.50% on an OM basis	Holstein cows	↓CH ₄ yield by 29.4% (for 0.05%)	↓DMI, ECM, MY. ↑Iodine and bromine in milk without adverse impact on milk organoleptic traits.	(Stefenoni et al., 2021)
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	0.50% on an OM basis	Nordic Red cows	↓CH ₄ yield by 54%	↓FI. Altered milk production.	(Krizsan et al., 2023)
<i>Asparagopsis taxiformis</i> (freeze-dried)	<i>In vivo</i>	0.50, 1.00, 2.00, 3.00% on an OM basis	Merino sheep	reduce CH ₄ emissions by 50–80% over a 72-day feeding .	No adverse impact on animal health	(Li Xixi et al., 2018)
brown and green seaweeds (<i>Pelvetia canaliculata</i> , <i>Cystoseira tamariscifolia</i> , <i>Bifurcaria bifurcate</i> , <i>Fucus vesiculosus</i> , <i>Himanthalia elongata</i> , <i>Ulva intestinalis</i>)	<i>In vitro</i>	10 g/kg DM (for all seaweed species)	Rumen simulation technique system (RUSITEC)	No effect on CH ₄	no adverse effects on diet digestibility or fermentation patterns	(Roskam et al., 2022)
<i>Asparagopsis taxiformis</i>	<i>In vitro</i>	10–20 g/kg OM	<i>In vitro</i> rumen incubation	↓CH ₄	Strong dose-dependent CH ₄ mitigating effect. No adverse impact on RF profile.	(Chagas et al., 2019)
Red <i>Asparagopsis taxiformis</i> and brown <i>Zonaria farlowii</i> seaweeds	<i>In vitro</i>	5% on DM basis	<i>In vitro</i> rumen incubation (ANKOM)	↓CH ₄ by 74%	NR	(Brooke et al., 2020)
Mix of macroalgae (<i>Furcellaria</i> , <i>Laminaria</i> , and <i>Fucus</i> spp.) and Irish moss	<i>In vitro</i>	0.14, 0.28, and 0.56 g DM/day were supplemented to the donor Holstein cows	<i>In vitro</i> incubation in anaerobic CCF fermentors	↓CH ₄ by 12–16%	NR	(Kinley and Fredeen, 2015)
<i>Nannochloropsis oceanica</i> , <i>Chlorella vulgaris</i> , <i>Tetraselmis</i> sp.	<i>In vitro</i>	2.5, 5, and 10% on DM basis	<i>In vitro</i> incubation	↓CH ₄ yield with <i>N. oceanica</i> , but ↑CH ₄ yield with <i>C. vulgaris</i> (at 10% inclusion)	Efficacy and safety are species-, dose-, and basal diet-dependent; tailor inclusion rates to optimize nutritional performance while maximizing CH ₄ mitigation.	(Meehan et al., 2021)

(Continued)

TABLE 4 Continued

Algal Species/Product	Type of study	Inclusion/dose	Animal type	CH ₄ effect	Toxicity/side effects	Reference
<i>Dunaliella salina</i>	<i>In vitro</i>	3% on DM basis	<i>In vitro</i> incubation	↓CH ₄ production by 8.58–73.23%	Recommended to use in diets based on corn forage	(Elghandour et al., 2023)
<i>Chlorella vulgaris</i>	<i>In vitro</i>	25% of the total incubated DM	<i>In vitro</i> incubation	↓CH ₄ production by 34%	NR	(Sucu, 2020)
<i>Chlorella vulgaris</i>	<i>In vitro</i>	1, 2, and 3% DM basis	<i>In vitro</i> fermentation (ANKOM)	↓CH ₄ production	<i>C. vulgaris</i> at a 2 or 3% level exerted negative effects on ruminal fermentation and nutrient degradability	(Kholif et al., 2023)
Australian freshwater algal mixes (predominantly containing <i>Spirogyra maxima</i>)	<i>In vitro</i>	5%, 10%, 20%, 30% and 50% of DM	<i>In vitro</i> batch assay trials	Algal mixes containing <i>Spirogyra</i> ↓CH ₄ by >10% and had high lipid content	safe for livestock consumption at an inclusion rate of 20%	(Lester et al., 2024)

DM, dry matter; OM, organic matter; DMI, dry matter intake; FI, feed intake; ADG, average daily gain; MY, milk yield; RF, rumen fermentation; NR, not reported.

accumulation was not observed, and excretion appeared transient in that study. Additionally, some trials with *Asparagopsis armata* at 0.5–1.0% of dietary OM in dairy cows reduced CH₄ yield but also lowered DMI, highlighting the need for careful dosing and monitoring of animal performance and product quality (including iodine/halogen load) (Muizelaar et al., 2021). Similarly, in dairy cows, supplementation with *A. taxiformis* at 0.3% of dietary OM reduced enteric CH₄ emissions by ~30% during the first 8 weeks, with no sustained effect from week 9 to 12. This inclusion level also led to reductions in DMI (~7%) and ECM (~2%), shifts in VFA profiles (↓acetate; ↑propionate, butyrate, valerate), and elevated concentrations of bromine and iodine in milk (5-fold and 9-fold higher than controls, respectively), highlighting the need for long-term evaluation of efficacy, safety, and product integrity (Angellotti et al., 2025).

By contrast, brown and green seaweeds generally lack halomethanes at higher levels; their antimethanogenic potential is less consistent and often modest. For example, bromoform-free brown/green species included at 10 g/kg diet DM did not reduce CH₄ in RUSITEC tests, whereas metabolomics indicate these taxa contain phenolics (e.g., phlorotannins) and other sulfated compounds that could influence fermentation. Species, season, and geography contribute to pronounced chemical variability (Nørskov et al., 2021; Roskam et al., 2022).

Several microalgae and cyanobacteria have shown methane-mitigating potential—though, to date, none match *Asparagopsis* *in vivo*. *In vitro* study comparing *Chlorella vulgaris*, *Tetraselmis* spp., and *Nannochloropsis oceanica* found the lowest CH₄ yield with *N. oceanica* at 10% of incubated DM, likely linked to its high n-3 PUFA content (Meehan et al., 2021). Likewise, *Dunaliella salina*, when used as an additive with maize forages, lowered biogas/CH₄ kinetics without compromising fermentation characteristics (Elghandour et al., 2023).

In vivo findings are mixed and context-dependent. Some studies report that *Chlorella* can increase methanogenic archaea and protozoa in goats, whereas others (including associative feeding strategies with low-level *Chlorella*) suggest potential to improve fermentation while decreasing CH₄ (Tsiplakou et al., 2017; Kholif

et al., 2023). Cyanobacteria *Spirulina* (*Arthrospira* spp.) is widely used as a protein/antioxidant supplement. Across small-ruminant studies, *Spirulina* supplementation has been shown to modulate the rumen microbiome, but it yields inconsistent methane responses. In lactating goats, ~1% of diet DM—especially when combined with live yeast—lowered *Methanobrevibacter* prevalence and predicted CH₄, though effects were small-scale (Emara Rabee et al., 2025). In ewes, graded doses of methanogen inhibitors shifted community structure without reducing total methanogens, and *Methanobrevibacter* tended to increase at the highest inclusion rate (Christodoulou et al., 2023). In lambs, ~3% (fresh-weight basis) of the altered microbiota did not produce consistent enteric CH₄ outcomes (Wang et al., 2024b). Collectively, *Spirulina* may influence archaeal ecology at low inclusion rates, yet robust, controlled trials are needed to clarify its effects on CH₄ emissions.

Microalgal feed supplements appear to modulate rumen fermentation and H₂ disposal pathways (e.g., favoring propionate or microbial lipid sinks); however, the magnitude of CH₄ suppression is typically lower than that achieved with *Asparagopsis*.

Collectively, the literature supports algae as a diverse toolbox for enteric methane abatement. *Asparagopsis* (via bromoform) delivers the most considerable and reproducible reductions—especially in high-concentrate systems—while brown/green macroalgae and microalgae offer nutritional value and modest, formulation-dependent CH₄ mitigation. Critical research gaps include: (1) scalable, cost-efficient cultivation and processing for consistent bioactive content; (2) long-term animal health and product-quality surveillance (residues, iodine/halogens); (3) delivery formats that sustain efficacy without depressing intake; and (4) robust performance data in pasture-based and dairy systems.

4.2 Biochar supplementation for enteric methane mitigation

Biochar (BH) has garnered increasing interest as a potential CH₄ mitigation agent in ruminant nutrition due to its high surface

area, porosity, and adsorptive capacity, which may modulate rumen fermentation and microbial dynamics. Proposed mechanisms include altering microbial habitats, reducing hydrogen availability for methanogenesis, and promoting the proliferation of alternative hydrogen-utilizing microbes (Leng et al., 2013; Saenab et al., 2018). However, evidence for its effectiveness remains inconsistent across studies (Winders et al., 2019; Sperber et al., 2022).

In a recent two-phase study in beef cattle, supplementation with tailored (“fit-for-purpose”) biochars yielded modest reductions in CH₄ emissions (8.8–12.9%) under controlled pen conditions. Still, no effect was observed under grazing systems, highlighting a disconnect between controlled trials and practical field application (Martinez-Fernandez et al., 2024). Similarly, in dairy cattle, a Latin square trial revealed that neither biochar nor biochar–urea blends affected CH₄ emissions or productive performance (Terler et al., 2023), while supplementation at 1% DM in lactating Holsteins also yielded no benefits (Dittmann et al., 2024). A study in lambs found no favorable effects on CH₄ production or growth, both *in vitro* and *in vivo* (Lind et al., 2024). Additionally, mineral-enriched biochar failed to elicit any changes in CH₄ or rumen fermentation in Holstein steers (Ni et al., 2024). By contrast, an *in vivo* study in ewes reported improved feed efficiency and reduced CH₄ emissions with biochar supplementation (Bureq and Khalil, 2025), indicating that host species, diet type, and biochar formulation may all influence response. This inconsistency likely stems from differences in pyrolysis conditions, feedstock type, particle size, and chemical composition of the biochar used. Smaller particle sizes and acidic pH have been associated with greater CH₄ mitigation (Zhou et al., 2017; Osman et al., 2022), while the presence of phenolic compounds may exert additional antimicrobial effects. A recent quantitative review confirmed the modest average efficacy of biochar across studies but emphasized the substantial heterogeneity and lack of dose–response consistency, calling for standardization in biochar production and application protocols (Pepeta et al., 2024).

Overall, while biochar shows mechanistic potential as a CH₄ mitigation tool, primarily through indirect modulation of ruminal hydrogen metabolism, current *in vivo* evidence does not yet support its broad implementation in commercial livestock systems. Future work should focus on defining optimal biochar types, inclusion levels, and diet contexts, as well as the possible synergistic effects with other mitigation agents.

4.3 Garlic

Garlic (*Allium sativum*) and its organosulfur compounds—such as allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan – have attracted attention as natural feed additives for mitigating enteric CH₄ emissions in ruminants. These compounds exhibit antimicrobial activity against methanogenic archaea and rumen protozoa and have been shown to alter fermentation profiles by promoting propionate production, thereby redirecting H₂ away from methanogenesis (Shang et al., 2019; Sari et al., 2022).

However, the efficacy of garlic-based interventions appears highly variable. It is influenced by multiple factors, including the specific compound used, its concentration and stability, the delivery matrix (e.g., oil, extract, powder), and interactions with the basal diet (Kamel et al., 2008; Sari et al., 2022).

Recent *in vivo* evidence supports the methane-reducing potential of garlic-derived products under controlled and grazing conditions. In a respiration chamber study with mid-lactation dairy cows, supplementation with a garlic–citrus extract over 18 days reduced CH₄ production (–10.3%), intensity (–11.7%), and tended to lower CH₄ yield (–9.7%) without affecting dry matter intake or milk yield. Propionate concentrations increased, while *Methanobrevibacter* abundance declined. Similarly, under grazing conditions, daily supplementation of 33 g/cow of GCE over 12 weeks improved DMI and ECM yield. This led to an 8.39% reduction in milk GHG intensity, as determined by a life cycle assessment, although CH₄ was not directly quantified in the study (Khurana et al., 2024).

Meta-analyses and recent reviews have emphasized the heterogeneity in response to garlic supplementation, highlighting formulation sensitivity as a key factor influencing efficacy (Shang et al., 2019; Sari et al., 2022a; Ding et al., 2023; Martin and Chaudhry, 2024). Several studies demonstrated that garlic products provide a range of biological benefits to ruminants (Ogbuewu et al., 2019; Yang et al., 2021). While garlic-based products offer a promising natural approach to CH₄ mitigation, especially at practical inclusion levels that do not compromise intake or animal performance, their persistence and repeatability under commercial conditions remain uncertain.

In summary, garlic and its bioactive constituents have demonstrated potential for mitigating CH₄ through both direct inhibition of methanogens and fermentation shifts that favor propionate production. However, the success of such strategies depends heavily on compound selection, dosing, delivery method, and dietary context. Long-term, multi-period *in vivo* studies are needed to confirm sustained efficacy, evaluate adaptation, and guide the development of commercially viable formulations.

4.4 Tannins

Tannins—classified as condensed (CT) or hydrolyzable (HT) based on their chemical structure—are among the most widely studied plant secondary compounds for enteric CH₄ mitigation in ruminants. Their antimethanogenic effects are attributed to multiple mechanisms, including suppression of protozoa and associated methanogens, shifts in VFAs production (typically characterized by reduced acetate and increased propionate), and complexation with dietary proteins and carbohydrates, which can reduce H₂ availability for methanogenesis (Patra and Saxena, 2011; Goel and Makkar, 2012). The extent of mitigation depends heavily on the type of tannin, the botanical source, the inclusion rate, and the adaptation period.

A comprehensive meta-analysis by Jayanegara et al. (2012) covering both *in vitro* and *in vivo* data confirmed an apparent,

dose-dependent reduction in CH₄ emissions, particularly with CT sources. More recently, a systematic review by [Cardoso-Gutierrez et al. \(2021\)](#) focused on tropical forages and reported consistent CH₄ suppression across multiple studies. However, the magnitude of reduction was highly variable and linked to the specific plant species and dosage employed. [Goel and Makkar \(2012\)](#) highlighted that CT mitigates CH₄ primarily via indirect mechanisms, such as reducing fiber digestion and thus limiting H₂ availability. In contrast, HT appear to exert more direct antimethanogenic effects by inhibiting the growth and activity of methanogens and hydrogen-producing microbes. Animal-level studies further demonstrate the complex and dose-dependent impacts of tannin supplementation on CH₄ mitigation and animal productivity. In dairy goats, stepwise inclusion of quebracho-derived condensed tannins (CT; 0–6% of diet DM) elicited non-linear responses, with milk yield peaking at approximately 4% CT, beyond which diet digestibility declined and effects on methane emissions became inconsistent ([Battelli et al., 2024](#)). Similarly, dietary inclusion of hydrolyzable tannins (HT) has been associated with improvements in milk yield and udder health, further supporting their utility in dairy systems ([Ali et al., 2017](#)). In an earlier *in vivo* study, [Beauchemin et al. \(2007a\)](#) reported a 14% reduction in CH₄ emissions following dietary supplementation with Quebracho tannin extract, accompanied by a shift in VFA production toward propionate, a competitive H₂ sink. Comparable results were observed by [Grainger et al. \(2009\)](#) who supplemented condensed tannins from *Lotus pedunculatus* and reported up to 29% CH₄ reduction without adverse effects on dry matter intake or animal productivity.

In vitro investigations support the potential of forage-derived tannins. For example, purified CT extracts from *Hedysarum coronarium* (sulla) and *Lotus corniculatus* (big trefoil) decreased CH₄ production by up to ~15% at inclusion rates of 30 g/kg DM. However, gas production and fermentation efficiency were negatively affected at the highest levels ([Verma et al., 2023](#)). These findings underscore the importance of optimizing tannin inclusion levels to mitigate undesirable effects on rumen fermentation and animal productivity.

In summary, tannins represent a viable strategy for mitigating enteric CH₄ emissions in ruminants, particularly when their use is aligned with dietary context and production objectives. Low-to-moderate inclusion levels (<3–4% of diet DM) have been shown to reduce CH₄ output without adversely affecting animal performance; however, higher doses may impair nutrient digestibility and feed efficiency. Effective formulation requires careful consideration of tannin type (condensed *vs.* hydrolyzable), bioactivity, and interactions with the basal diet to ensure sustained mitigation and production efficiency.

In addition, key knowledge gaps remain regarding the mechanisms by which tannins reduce methanogenesis, including their effects on nutrient utilization, direct inhibition of methanogens, suppression of protozoa, and modulation of hydrogen sinks within the rumen environment. Addressing these uncertainties through targeted *in vivo* research will be essential to optimizing tannin-based strategies for practical application.

4.5 Saponins

Saponins—diverse glycosides abundant in legumes and tropical plants—are recognized for their antiprotozoal and antimicrobial properties ([Patra and Saxena, 2009](#); [Goel and Makkar, 2012](#)). By suppressing rumen protozoa—key partners of methanogenic archaea—saponins diminish hydrogen transfer to methanogens, thereby reducing CH₄ formation. They also act directly against methanogens, shifting fermentation toward propionate production—a competitive hydrogen sink ([Hristov et al., 2013](#); [Pen et al., 2006](#); [Patra and Saxena, 2009](#); [Firkins and Mitchell, 2023](#)). Commercial saponin sources such as *Yucca schidigera* and *Quillaja saponaria* are well-characterized: QS contains ~10% triterpenoid saponins across 20+ structures, while YS offers ~4.4% steroidal saponins spanning 28 variants ([Kholif, 2023](#)). Other promising sources include *Sapindus saponaria*, which exhibits potent antiprotozoal activity ([Hu et al., 2018](#)), and fenugreek (*Trigonella foenum-graecum*), notable for its high saponin content (~4.63 g per 10 g) and potential antimethanogenic action ([Singh and Garg, 2006](#); [Visuvanathan et al., 2022](#)).

In vitro, *S. saponaria* fruit extracts (100 mg/g) significantly decreased CH₄ without impairing fermentation. At the same time, inclusion of its seed pericarp reduced protozoa and improved weight gain in sheep, though CH₄ was not measured ([Navas-Camacho et al., 2001](#); [Hess et al., 2003](#)). Fenugreek extracts also inhibited total gas and CH₄ production and shifted VFAs toward propionate *in vitro* ([Dey, 2015](#); [Niu et al., 2021](#)), while improving nitrogen utilization without affecting intake or digestibility ([Wina et al., 2005](#)).

Although saponins exhibit considerable potential to reduce enteric methane emissions across a range of inclusion levels, thereby supporting environmentally sustainable ruminant nutrition ([Ridla et al., 2021](#)). Evidence suggests that their effects may not be consistently sustained over time. Several long-term *in vitro* studies have indicated that the methane-suppressing effects of certain saponin extracts on rumen microbial fermentation may be transient rather than permanent ([Wang et al., 1998](#); [Cardozo et al., 2004](#)). This attenuation may be partly explained by microbial adaptation, as rumen microbes can adjust to repeated exposure to bioactive compounds such as saponins ([Makkar and Becker, 1997](#); [Wallace et al., 2002](#)).

However, *in vivo* responses to saponin supplementation remain inconsistent. For instance, supplementation of whole-plant *Yucca schidigera* or *Quillaja saponaria* at 10 g/kg DM failed to reduce CH₄ emissions in lactating dairy cows ([Holtshausen et al., 2009](#)), while lower-dose inclusion in sheep yielded only numerical reductions ([Pen et al., 2007](#)). Similarly, in dairy goats, supplementation with fenugreek seeds at 0.1 kg/d had no significant impact on milk yield or health status ([El-Tarabany et al., 2018](#); [Akbağ et al., 2022](#)). By contrast, substantial CH₄ reductions of 28%, 35.8%, and 47.9% were observed in sheep supplemented with tea seed saponins at 5, 10, and 20 g/kg DM, respectively ([Zhang et al., 2021](#)), highlighting the role of the botanical source and dose in determining efficacy. Beyond ruminant systems, low-level inclusion of fenugreek (0.04%) has demonstrated benefits in aquaculture species—improving growth,

antioxidant capacity, and immune function (Yu et al., 2019; Abdel-Wareth et al., 2021; Yang et al., 2022; Paneru et al., 2022), indicating the broader applicability of saponins across animal production systems. A recent meta-analysis encompassing 66 *in vivo* treatments (up to 40 g/kg DM) revealed no adverse effects on feed intake; however, the effects on productivity and fermentation were highly variable and dependent on the plant source, animal species, and dietary context (Yanza et al., 2024).

These findings underscore the need for additional long-term, species-specific studies to better understand the persistence of saponin-induced CH₄ mitigation and to refine supplementation strategies for practical livestock systems.

The summary report of the analysis of literature data on the effects of supplementation of ruminant diets with garlic, tannins, or saponins is shown in Table 5.

4.6 Essential oils as natural methane mitigation agents

Essential oils (EOs) are plant-derived volatile compounds with antimicrobial properties that have been explored as natural feed additives to mitigate enteric CH₄ emissions in ruminants. Their effects are attributed to the modulation of rumen microbial communities, the inhibition of methanogens and protozoa, and alterations in fermentation profiles (Castillejos et al., 2005; Calsamiglia et al., 2007; Patra and Yu, 2012). Compounds such as thymol, eugenol, carvacrol, cinnamaldehyde, and flavonoids (e.g., naringin, hesperidin) have demonstrated methane-reducing potential in both *in vitro* and *in vivo* systems (Busquet et al., 2005a; Patra and Yu, 2015; Yu et al., 2024).

In vitro studies report CH₄ reductions ranging from 10% to 91%, depending on EO type, dose, and microbial sensitivity (Busquet et al., 2006; Cobellis et al., 2016). For example, garlic oil constituents—diallyl disulfide and allyl mercaptan—reduced CH₄ production by up to 74% in batch cultures (Busquet et al., 2005a), high-carvacrol oregano oil reduced methane by 22% at 1000 mg/L, although with concurrent suppression of VFA production and feed digestion (Benchaar and Hassanat, 2024).

Similarly, citrus flavonoids (naringin and hesperidin, each at 10 g/kg DM) or citrus flavonoid extract (20 g/kg DM) significantly reduced CH₄ and ammonia concentrations *in vitro*, alongside declines in archaea *Methanobrevibacter* spp. and protozoa *Isotricha* spp. populations (Yu et al., 2024). The authors suggest that flavonoids may possess synergistic effects in mitigating ruminal CH₄ and have the potential to enhance N utilization. Using the rumen simulation technique (RUSITEC), Soliva et al. (2011) reported a 91% reduction in daily CH₄ emissions, accompanied by a decrease in protozoal counts and an increase in total bacterial populations, highlighting the strong methane-mitigating potential of the garlic oil under controlled *in vitro* conditions. In another *in vitro* study, five essential oils—clove, eucalyptus, garlic, oregano, and peppermint—reduced CH₄ production by 34.4%, 17.6%, 42.3%,

87.0%, and 25.7%, respectively, at 1.0 g/L, with oregano oil showing the most significant CH₄ inhibition (Patra and Yu, 2012).

In vivo, results have been inconsistent. Agolin[®] Ruminant (a commercial EOs blend) reduced CH₄ emissions by 8.8%, improved milk yield by 4.1%, and enhanced feed efficiency by 4.4% in lactating dairy cows (Belanche et al., 2020). A carbon footprint modelling study confirmed a 6% reduction in GHG emissions across several feeding strategies (Becker et al., 2023). However, Benchaar and Hassanat (2025) found no effect of the same blend (1 g/day) on lactational performance or CH₄ output in dairy cows. Castro-Montoya et al. (2015) reported a 15% CH₄ reduction after 6 weeks of supplementation with 0.2 g/d of Agolin[®] Ruminant in dairy cows. Interestingly, no significant changes were seen in beef heifers supplemented with the same dose.

Conversely, several studies have reported inconsistent or non-significant effects of essential oil supplementation on CH₄ mitigation and animal performance. For example, an EOs blend of cresols, thymol, limonene, vanillin, eugenol, and salicylates (1.2 g/day) did not confer any measurable benefits in mid-lactation Holstein dairy cows in terms of CH₄ mitigation, lactational performance, or rumen fermentation parameters (Joch et al., 2019). Likewise, Jiménez-Ocampo et al. (2021) demonstrated CH₄ reductions with 1.5 g/kg DMI of naringin and chitosan in *in vivo* trials. However, *in situ* tests using the same doses (1.5–3.0 g/kg DMI) showed no significant changes in CH₄ or nutrient use. Supplementation with eucalyptus and anise oils at 0.5 g/animal/day in sheep had no significant effect on methane production (Wang et al., 2018). An *in vitro* experiment using rumen inoculum from Daragh ewes demonstrated that sage, pine, and clove EOs at 300–900 mg/L led to dose-dependent CH₄ suppression and improved the ruminal fatty acid profile (Bokharaeian et al., 2023).

These contrasting findings underscore the complexity of host-additive interactions and suggest that the delivery method, dosage, and microbial adaptation may have a significant influence on experimental results.

Recommended effective doses for CH₄ mitigation typically range from 20 to 1000 mg/L *in vitro* and 500 to 1000 mg/day *in vivo*. However, high doses may impair fibre digestion and reduce feed intake (Cobellis et al., 2016; Joch et al., 2019). Long-term exposure to EOs may induce microbial adaptation, reducing their effectiveness over time.

Thus, EOs supplementation should be approached with caution—strategies such as encapsulation, rotational use, or combination with other phytochemicals are recommended to sustain efficacy while minimizing adverse effects (Benchaar and Greathead, 2011; Patra and Yu, 2015).

4.7 Probiotics

Ezema (2013) described probiotics as live, non-pathogenic, and non-toxic microorganisms that, when administered in appropriate

TABLE 5 Observations from different articles reporting effects of garlic, tannins, and saponins on enteric CH₄ mitigation.

Additive/source	Type of study	Inclusion/dose	Animal type	CH ₄ effect	Toxicity/side effects	Reference
Garlic + citrus extract	<i>In vivo</i>	44 g/cow/d	mid-lactation Nordic Red cows	↓CH ₄ 10.3% g/d; 11.7% intensity; trend 9.7% CH ₄ yield	ND	(Khurana et al., 2023)
Garlic + citrus extract	<i>In vivo</i>	33 g/cow/d	Irish Holstein-Friesian dairy cows	Not measured	↑MY, DMI, FE.	(Khurana et al., 2024)
GAR, DAS, DAD, ALL, and ALM	<i>In vitro</i>	3–3000 mg/L (medium)	<i>In vitro</i> batch incubation	↓CH ₄ with GAR	Shift VFA: ↓acetate, ↑propionate & butyrate	(Busquet et al., 2005b)
Garlic powder	<i>In vitro/in vivo</i>	2, 4, and 6% of DMI (incubation <i>in vitro</i>), 2% of DMI (<i>in vivo</i>)	lactating Murrah buffaloes	↓CH ₄ by 34%	ND	(Zafarian and Manafi, 2013)
Quebracho CT	<i>In vivo</i>	2%, 4%, and 6% on DM basis	lactating goats	no influence on CH ₄	Reduced the milk efficiency	(Battelli et al., 2024)
Chestnut tannin extract	<i>In vivo</i>	40 and 80 g/day	mid-lactation Holstein cows	↓CH ₄ yield by 34%24%	ND	(Prodanović et al., 2025)
Quebracho–chestnut tannin extract	<i>In vivo</i>	Dosages tested: 10, 20, and 30 g/d. Dosage recommended: 30 g/d.	Early-lactating Holstein cows	Not measured	ND	(Wang et al., 2024a)
Extracted tannins from birdsfoot trefoil, big trefoil, salad burnet, and sulla	<i>In vitro</i>	10, 20, and 30 g/kg DM	<i>In vitro</i> incubation	↓CH ₄ by up to 12%	ND	(Verma et al., 2024)
Quebracho CT	<i>In vivo</i>	1 and 2% of DM	Angus heifers and Angus steers	No impact on CH ₄	ND	(Beauchemin et al., 2007b)
<i>Mimosa tenuiflora</i> CT	<i>In vivo</i>	30 g/kg DM	male sheep	No impact on CH ₄	ND	(Lima et al., 2019)
<i>Acacia mearnsii</i> CT	<i>In vivo</i>	163 and 326 g/d/cow	Holstein Friesian cows	↓CH ₄ by 14–29%	↓MY	(Grainger et al., 2009)
Acacia and quebracho CT, chestnut and valonia HT	<i>In vitro</i>	20, 50, 100, 150, and 200 g kg DM	<i>In vitro</i> incubation	Chestnut HT, acacia CT (at 50 g/kg DM) ↓CH ₄	↓VFA and ruminal protein degradation	(Hassanat and Benchaar, 2013)
Blend of tannin and saponins	<i>In vivo</i>	0.7 g/kg DM	Nellore bulls	↓CH ₄ by 17.3% or 88.76 kg of CO ₂ equivalent	ND	(Magnani et al., 2023)
<i>Sapindus saponaria</i> (SS), <i>Enterolobium cyclocarpum</i> (EC), <i>Pithecellobium saman</i> (PS)	<i>In vitro</i>	100 mg/g (SS), 200 mg/g (EC), 200 mg/g (PS)	Rumen simulation technique (Rusitec)	↓CH ₄ by 14–29%	ND	(Hess et al., 2003)
Fenugreek forage	<i>In vitro</i>	Not specified	48-h <i>in vitro</i> batch culture incubations	↓CH ₄ ; ↑propionate	ND	(Niu et al., 2021)
Tea saponins	<i>In vitro/in vivo</i>	5, 10, 20 g/kg DM (<i>in vitro</i> , <i>in vivo</i>)	Han ×Dorper male castrated sheep	↓CH ₄	ND	(Zhang et al., 2021)
<i>Yucca schidigera</i> or <i>Quillaja saponaria</i>	<i>In vivo</i>	10 g/kg of DM	Dairy cows	No effect on CH ₄	ND	(Holtshausen et al., 2009)
<i>Yucca schidigera</i>	<i>In vivo</i>	0.12 and 0.15 g/kg DM	cannulated Cheviot sheep	↓CH ₄	ND	(Santoso et al., 2004)

ND, not detected. MY, milk yield; DM, dry matter; DMI, dry matter intake; FE, feed efficiency; GAR, garlic oil; DAS, diallyl sulfide; DAD, diallyl disulfide; ALL, allicin; ALM, allyl mercaptan; CT, condensed tannin; HT, hydrolysable tannin.

amounts, confer beneficial effects on the host animal. Their mechanism of action includes improving feed digestibility, enhancing beneficial microbial populations, competing with methanogens for substrates (e.g., hydrogen), and modulating ruminal fermentation pathways (Uyeno et al., 2015). In ruminant nutrition, commonly used probiotics—also referred to as direct-fed microbials—include yeast species such as *Saccharomyces cerevisiae*, as well as bacterial genera including *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Propionibacterium*, *Megasphaera elsdenii*, and *Prevotella bryantii* (Seo et al., 2010).

The summary report of the literature analysis on the effects of supplementing ruminant diets with probiotic bacteria is presented in Table 6.

Bacterial probiotics have been shown to improve rumen function, enhance dry matter intake, feed efficiency, and weight gain in ruminants (Elghandour et al., 2015). They may also inhibit pathogenic microbes, modulate gut microbiota, and stimulate the immune system via bacteriocin production (Khan et al., 2016). Additionally, their supplementation has been associated with increased milk yield, fat-corrected milk, and milk fat content (Elghandour et al., 2015; Khan et al., 2016).

Studies of *Bacillus subtilis* supplementation in cattle have reported improvements in digestibility, performance, milk production, reductions in somatic cell counts, reductions in CH₄ emissions, and stimulation of proteolytic and amylolytic bacterial growth (Sun et al., 2013; Jia et al., 2022). The inclusion of *B. subtilis* under *in vitro* conditions has demonstrated potential for reducing ruminal methane production when supplemented in mid-lactation dairy cow diets, suggesting its promise as a methane mitigation additive (Sarmikasoglou et al., 2024). In young Holstein calves, dietary supplementation with a probiotic mixture (*L. plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *B. subtilis*) has been shown to enhance health status and decrease the need for medicinal treatments (Wang et al., 2022).

M. elsdenii, a lactic acid-utilizing bacterium, has also been investigated for its probiotic potential. Its capacity to metabolize lactate into VFAs such as butyrate and propionate supports pH stability and reduces lactate accumulation, which can limit methanogenic activity (Carberry et al., 2012; Cabral and Weimer, 2024). A recent meta-analysis by Susanto et al. (2023) integrating 32 studies (136 data points) found that *M. elsdenii* inclusion significantly reduced CH₄ emissions ($p < 0.05$), while simultaneously improving fermentation profiles (e.g., increased propionate, butyrate, isobutyrate, valerate; decreased lactic acid and acetate proportion) and enhancing livestock performance (e.g., average daily gain, body condition score, carcass traits).

Yeast-based probiotics have emerged as a potential strategy for mitigating enteric CH₄ emissions in ruminants. Although supplementation with live yeast, particularly *Saccharomyces cerevisiae*, is known to stimulate cellulolytic bacterial populations, potentially increasing H₂ production—a key substrate for methanogenesis—it may also simultaneously enhance the

proliferation of alternative H₂-utilizing microorganisms. This dual microbial modulation may lead to a net reduction in CH₄ production by diverting metabolic H₂ flux away from methanogens and toward competing fermentation pathways, such as propionate or acetogenesis. Such mechanisms suggest that yeast probiotics could play a supportive role in reducing CH₄ emissions while improving overall rumen function and fermentation efficiency (Newbold and Rode, 2006; Chaucheyras-Durand et al., 2008; Newbold et al., 1996; Fonty and Chaucheyras-Durand, 2006). In several *in vitro* studies, the addition of *S. cerevisiae* has been shown to decrease CH₄ production (Bayat et al., 2015; Kamal et al., 2025).

While direct anti-methanogenic effects of yeast are less pronounced, their supportive role in maintaining rumen health and competitive microbial dynamics can indirectly contribute to CH₄ mitigation. Additionally, *S. cerevisiae* can improve feed intake, nutrient digestibility, rumen ecology, and growth performance (Khalouei et al., 2020; Phesatcha et al., 2021), and milk production in dairy cows (Majdoub-Mathlouthi et al., 2009; Moallem et al., 2009; Maamouri et al., 2014; Bayat et al., 2015; Rossow et al., 2018; Perdomo et al., 2020; Cattaneo et al., 2023). It can also reduce oxidative stress and improve dairy cattle performance under heat-stress conditions (Perdomo et al., 2020; Benedetti et al., 2024). Despite promising results, the application of probiotics in ruminants for CH₄ mitigation remains limited compared to chemical inhibitors or feed formulation strategies. In addition, the effectiveness of probiotics is often inconsistent due to variations in strain specificity, dosage, delivery method, dietary context, and host microbiome composition. Long-term, large-scale *in vivo* studies under commercial conditions are necessary to validate their efficacy in CH₄ reduction and assess potential interactions with other mitigation strategies.

Nonetheless, probiotics—particularly when used in synergistic combinations or in conjunction with complementary additives—represent a sustainable and biologically integrated strategy for mitigating methane. In addition to their environmental benefits, probiotics contribute to enhanced rumen health, improved nutrient utilization, and increased overall animal productivity.

5 Chemical compounds

Chemical compounds have emerged as effective feed additives to mitigate enteric CH₄ emissions in ruminants. These compounds typically function by inhibiting methanogenic archaea, redirecting H₂ utilization to alternative pathways, or modifying rumen fermentation profiles. Among the most extensively studied are 3-nitrooxypropanol (3-NOP), nitrate salts, and organic acids like fumarate and malate. Each exhibits unique mechanisms of action and variable efficacy depending on diet composition, animal species, and dosage.

Recommended dosages and toxicity of chemical compounds reducing enteric methane are represented in Table 7.

TABLE 6 Observations from different articles reporting effects of bacterial and yeast probiotics on enteric CH₄ and rumen functions.

Probiotic Type/ Strain	Study type	Methane reduction	Other effects	Recommended/effective dosage	Reference
<i>Megasphaera elsdenii</i>	Meta-analysis	↓CH ₄	↑propionate, butyrate, isobutyrate, and valerate. ↓Lactate, total bacteria. Production performance and health improvement (↑ADG, carcass quality, and gain).	up to 13.30 log 10 CFU	(Susanto et al., 2023)
Methanotroph-based probiotics (<i>Methylocystis</i> sp., <i>Methylobacterium</i> sp.)	<i>In vitro</i> and <i>In vivo</i> (Hanwoo steers, n=12, 30 days supplementation)	↓CH ₄ 40% and 50% after 12 and 24 h (<i>in vitro</i>); ↓CH ₄	No effect on RF profile and production <i>in vivo</i>	5.1 × 10 ⁷ CFUs/ml (<i>in vitro</i>); low and high dosage (3 × 10 ⁷ , 3 × 10 ⁸ CFUs/ml) were effective in CH ₄ reduction.	(Tseten et al., 2025)
<i>Bacillus subtilis</i>	<i>In vitro</i> (24 and 48 h after inoculation)	Mixed effect on CH ₄	↓acetate, propionate; ↑butyrate, valerate.	NR. Potential reduction of CH ₄ in mid lactation.	(Sarmikasoglou et al., 2024)
<i>Bacillus subtilis</i> , <i>Saccharomyces cerevisiae</i>	<i>In vitro</i> fermentation in the rumen of Hu sheep	↓CH ₄	<i>S. cerevisiae</i> ↑TGP, microbial protein, ↑NH ₃	<i>S. cerevisiae</i> and <i>B. subtilis</i> at doses of 8 and 20 × 10 ⁶ CFU g/l	(Kamal et al., 2025)
<i>Enterococcus faecium</i> + <i>Saccharomyces cerevisiae</i> , <i>Bacillus licheniformis</i> + <i>Bacillus subtilis</i>	<i>In vitro</i> (donors of rumen fluid → three Nellore steers)	↓CH ₄ (dose and diet dependent effect)	Improved RF profile, ↓CH ₄ , and CO ₂ .	<i>E. faecium</i> (5 × 10 ⁹ CFU/g) + <i>S. cerevisiae</i> (5 × 10 ⁹ CFU/g) and <i>B. licheniformis</i> + <i>B. subtilis</i> (3.2 × 10 ⁹ CFU/g).	(Silva et al., 2024)
<i>Bacillus subtilis</i> (BS) and <i>Macleaya cordata</i> extract (MCE)	<i>In vivo</i> (Holstein cows, n = 60)	↓CH ₄ yield (g/kg DMI) and intensity (g/kg ECM, FCM).	↑MY, milk fat and protein yields; ↑DMI, and nutrient digestibility.	50 g/head/d (BS) or 450 mg/head/d (MCE).	(Jia et al., 2022)
Active dry Yeast (<i>Saccharomyces cerevisiae</i> , strain Sc47), 1 × 10 ¹⁰ CFU/g	<i>In vivo</i> (high-production dairy cows, n = 50)	No effect on CH ₄	↑FE, MY	NR	(Garnsworthy et al., 2025)
Active dry Yeast (<i>Saccharomyces cerevisiae</i> , strain Sc47), 2 × 10 ⁹ CFU/g	<i>In vivo</i> (early lactating Holstein cows, n = 60)	No effect on CH ₄	Improved lactation performance and nutrient digestibility.	The optimal dose is 20 g/g/animal/day.	(Li et al., 2021)
Active dry Yeast (<i>Saccharomyces cerevisiae</i> , strain Sc47), 2 × 10 ¹⁰ CFU/g	<i>In vivo</i> (lactating dairy cows)	No effect on total CH ₄ . ↑CH ₄ per unit of FI.	No impact on MY, FI	NR	(Muñoz et al., 2016)
Active dry Yeast (<i>Saccharomyces cerevisiae</i> , strain Sc47), 1 × 10 ¹⁰ CFU/g	<i>In vivo</i> (transition ., Holstein dairy cows, n = 20)	Not measured	Improved RF profile. ↑ DMI, MY; ↓ risk of metabolic diseases.	NR	(Cattaneo et al., 2023)

NR, no recommendation; ADG, average daily gain; CFU, colony-forming unit; RF, rumen fermentation; FE, feed efficiency; MY, milk yield; FI, feed intake; DMI, dry matter intake; TGP, total gas production; ECM, energy corrected milk; FCM, fat corrected milk.

3-NOP is widely recognized for its ability to selectively inhibit methyl-coenzyme M reductase (MCR), a key enzyme in the methanogenesis process. This compound shares structural similarity with methyl-coenzyme M. The practical use of 3-NOP remains under evaluation, primarily due to safety considerations (Yu et al., 2021; Pitta et al., 2022; Hristov et al., 2015; Yu et al., 2021b).

In both dairy and beef cattle, 3-NOP has consistently demonstrated CH₄ reductions ranging from 20% to 40% without adversely affecting feed intake, nutrient digestibility, or animal productivity (Dijkstra et al., 2018; Romero-Perez et al., 2014; Kebreab et al., 2023). While productivity effects are generally modest, they tend to be favorable—several studies have reported improvements in milk composition, particularly in fat and protein

content, in dairy cattle, as well as enhanced feed conversion efficiency in beef cattle (Melgar et al., 2020; Yu et al., 2021).

Commercially available as Bovaer[®], 3-NOP has received regulatory approval in over 65 countries, including the EU, US, and Brazil (Elanco, 2024). The European Food Safety Authority (EFSA) recommends a maximum dose of 100 mg/kg DM or 88 mg of 3-NOP per kilogram of complete feed (Bampidis et al., 2021). However, several studies report on enhanced CH₄ mitigation at higher doses. For instance, a recent study demonstrated that supplementing dairy cattle with 3-NOP at an average dose of 123 mg/kg DM resulted in a significant mean reduction in enteric methane emissions of 39.0 ± 5.4% (Dijkstra et al., 2018). Similarly, Alemu et al. (2021) observed that supplementing corn-

TABLE 7 Recommended dosages and toxicity of chemical methane mitigation additives.

Compound	Mechanism of action	Recommended dosage	CH ₄ reduction (%)	Toxicity/limitations
3-NOP	Inhibits methyl-coenzyme M reductase, the final step in methanogenesis	Dairy: 60–150 mg/kg DM	20–40%	Generally safe at recommended levels; Did not have an adverse impact on animal production or rumen fermentation.
Nitrate (e.g., calcium or potassium nitrate)	Acts as an alternative H ₂ sink; reduces CH ₄ by redirecting H ₂ to ammonia synthesis	1–2% of dietary DM	10–30%	Risk of nitrite toxicity; requires adaptation . and precise control
Fumarate/Malate	Serves as an alternative electron acceptor; promotes propionate formation and reduces H ₂ availability	50–100 g/day	<10%	Efficacy is more pronounced in high-concentrate diets; cost-effectiveness varies.

based finishing diets with 3-NOP at 100, 125, and 150 mg/kg DM significantly reduced CH₄ yield in a commercial feedlot setting, with the 125 mg/kg DM dose yielding a 76% reduction, highlighting its efficacy as a methane mitigation strategy in beef production systems. A recent meta-analysis by [Kebreab et al. \(2023\)](#) further confirmed a dose-dependent response, with significantly greater methane reductions achieved at inclusion rates exceeding 100 mg/kg DM. It is essential to note that while current regulatory recommendations are specific to dairy cattle, the application of 3-NOP in other ruminant species, such as beef cattle, requires further research to validate efficacy, optimal dosage, and safety. [Dijkstra](#)

[et al. \(2018\)](#) reported that 3-Nitrooxypropanol has more substantial antimethanogenic effects in dairy cattle than in beef cattle. The nutrient composition of the diet significantly influences the efficacy of 3-NOP diet ([Almeida et al., 2023](#)). Diets with higher concentrations of neutral detergent fiber (NDF) and crude fat tend to reduce their methane-mitigating potential. In contrast, increased starch content enhances their effectiveness in lowering CH₄ yield and intensity ([Kebreab et al., 2023](#); [Zhang et al., 2024a](#)). A short-term study in lactating dairy cows by [Van Gastelen et al. \(2022\)](#) confirmed that both 3-NOP dose and diet composition are critical determinants of efficacy. Cows receiving 60 or 80 mg

TABLE 8 SWOT analysis of nutritional strategies for reducing methane emissions in ruminant livestock.

Strengths	Weaknesses
✓ Effective reduction in enteric methane emissions through feed manipulation and additives. • 3-NOP: 20-40% • Tannins: 10-29% • Saponins: 10-25% • EOs: 10-30% • Garlic compounds: 20-74% • Algae: 30-98% • Nitrate salts: 10-30% • Nitrate salts: 10-30% • Fumarate/malate: < 10% ✓ Enhances feed efficiency by reducing energy loss as methane. ✓ Can improve animal productivity (milk yield, growth rate) when optimized. ✓ Natural compounds (e.g., tannins, saponins, essential oils) align with consumer preferences for clean, sustainable agriculture.	✗ Variable efficacy depending on animal species, diet composition, and environmental factors. ✗ Lack of long-term <i>in vivo</i> data. Inconsistent results are common between <i>in vitro</i> and <i>in vivo</i> studies. ✗ Some additives (e.g., high levels of tannins, saponins, nitrates) may reduce feed intake, fiber digestibility, or nutrient absorption. ✗ <u>Complexity in Practical Implementation</u>. Requires precise dosing, consistent feed formulation, and farmer training for practical application at scale. ✗ <u>Cost and Adoption Barriers</u> – Premium additives like 3-NOP, algae-based, or encapsulated EOs can be expensive. Limited access in low- and middle-income countries due to distribution or production barriers. ✗ The rumen microbial population may adapt over time, reducing the long-term efficacy of certain additives (e.g., EOs).
Opportunities	Threats
🚀 Development of novel feed additives with multi-functional benefits (e.g., antimethanogenic, immunomodulatory). 🚀 Integration into precision feeding and low-carbon farming programs. 🚀 Expansion of regulatory approvals (e.g., 3-NOP, seaweed-based products). 🚀 Use of local feed resources (e.g., tannin-rich tropical forages) to reduce costs and promote sustainability.	⚠️ <u>Regulatory Hurdles</u> – Regulatory limitations and approval processes for new feed additives. ⚠️ Risk of adverse effects on rumen microbiota or animal health if not properly dosed. ⚠️ Potential environmental risks (e.g., bromoform from seaweed additives). ⚠️ <u>Market Volatility and Supply Chain Issues</u> – The availability and cost of specific feed additives (e.g., seaweed) may fluctuate, affecting long-term viability.

3-NOP/kg DM across three different diets exhibited significantly greater CH₄ mitigation when fed a corn silage-based diet compared to a grass silage-based one. Importantly, 3-NOP had no adverse effects on dry matter intake, milk yield, milk composition, or feed efficiency. Similar findings were reported in another study by Van Gastelen et al. (2020), which found that supplementation with 60 mg 3-NOP/kg DM did not affect production or intake parameters.

In contrast, results from a longer-term study by Van Gastelen et al. (2024) suggested that diet composition may have an even greater effect on the efficacy of 3-NOP than the duration of supplementation following its initial introduction. Schilde et al. (2021) reported a synergistic reduction in CH₄ emissions when 3-NOP was combined with a high-concentrate, low-fiber (CFP) diet. At the same time, the mitigating effect of 3-NOP declined over time when added to a high-forage ration. These findings underscore the need for further long-term research to clarify the persistent impact of 3-NOP on CH₄ emissions and to better understand how dietary variability influences its mitigation potential.

Another class of methane-reducing compounds includes nitrate salts, such as calcium nitrate or potassium nitrate (Yang et al., 2016). Nitrate serves as an alternative H₂ sink in the rumen, competing with carbon dioxide for hydrogen and by redirecting the reductive potential toward ammonia synthesis (Datta et al., 2017). While nitrate can reduce CH₄ emissions by 10–30%, its application is limited by the potential risk of nitrite accumulation and toxicity, requiring careful management of dosage and adaptation periods (Yang et al., 2016). To mitigate the risk of nitrite toxicity associated with nitrate supplementation, several strategies have been proposed, including the use of sulfur-based additives, inoculation with nitrite-reducing bacteria (Latham et al., 2019; Zhao and Zhao, 2022), and gradual acclimation of animals to dietary nitrate (Lee and Beauchemin, 2014). These approaches aim to enhance the safety of nitrate application while preserving its potential for mitigating methane.

Fumarate and malate, organic acids involved in the tricarboxylic acid (TCA) cycle, have also been evaluated for their ability to reduce CH₄. These compounds function as alternative electron acceptors, promoting propionate formation over acetate and butyrate, thereby reducing hydrogen availability for methanogenesis (Asanuma et al., 1999). However, the efficacy of fumarate and malate appears to be dose-dependent and is often more pronounced in high-concentrate diets, with CH₄ reductions typically below 10% (Morgavi et al., 2010).

Despite their demonstrated efficacy in controlled trials, the large-scale application of chemical compounds in methane mitigation must consider factors such as cost, safety, consumer acceptance, and regulatory approval. Nonetheless, these compounds—particularly 3-NOP—represent important tools in the development of low-emission livestock systems.

The advantages and challenges of nutritional strategies for reducing methane emissions from ruminants are presented through a SWOT analysis, as shown in Table 8.

6 Conclusions

Reducing enteric methane emissions in ruminants requires the strategic application of validated nutritional, botanical, and management interventions. Among currently available tools, 3-nitrooxypropanol (3-NOP) offers the most consistent and repeatable reductions in CH₄ emissions under both research and commercial conditions. Products derived from *Asparagopsis* spp. can achieve greater absolute mitigation—often exceeding 50%—but require careful management of inclusion rates, potential impacts on dry matter intake and milk composition, and regulatory concerns related to bromoform and iodine residues.

Botanical additives such as garlic, tannins, and saponins hold additional promise by modulating the rumen microbiota and suppressing methanogens and protozoa. However, their efficacy is highly dependent on the delivery matrix, dose, ruminant species, and background diet. Notably, higher inclusion levels—particularly of condensed tannins—can impair fiber digestibility and animal performance, necessitating diet-specific optimization and formulation limits to avoid negative trade-offs.

In parallel, management-based strategies such as improving forage quality, selecting silages with higher non-fiber carbohydrate (NFC) content, and refining grazing intensity offer additional avenues for reducing CH₄ yield and intensity. These approaches can enhance overall nutrient use efficiency and complement additive-based interventions at the farm level.

Collectively, these findings underscore the importance of integrating proven feed additives with targeted dietary formulation and forage management to achieve sustained, cost-effective methane mitigation in ruminant systems.

Author contributions

SM: Investigation, Writing – review & editing, Formal Analysis, Writing – original draft, Conceptualization, Data curation, Methodology, Software. SH: Conceptualization, Methodology, Writing – review & editing, Writing – original draft. PH: Project administration, Methodology, Writing – review & editing, Writing – original draft, Formal Analysis.

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Conflict of interest

Author SM was employed by company Agrovyzkum Rapotin Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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