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Review

Harnessing methane proxies to understand and mitigate enteric emissions from ruminant production systems

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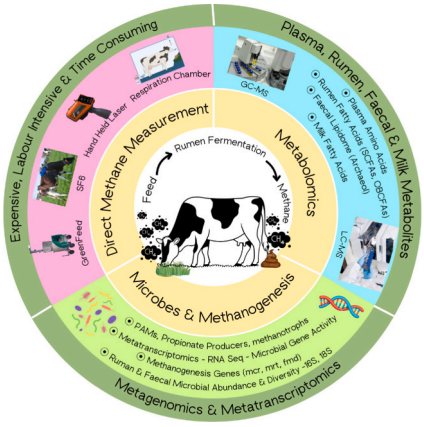
HIGHLIGHTS

- Measuring methane directly from ruminants is costly, time-consuming, and difficult.
- Methane proxies include molecules that are intermediaries in rumen methanogenesis.
- Methane proxies explain mechanisms of ruminal methane synthesis and inhibition.
- Recent 'omic' techniques enable the detection of methane proxies at the molecular level.

GRAPHICAL ABSTRACT



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ABSTRACT

Methane emissions from livestock, particularly ruminants, significantly contribute to global warming, necessitating the development of accurate methane monitoring systems. Direct methane measurement is technically complex, time-consuming, labour-intensive, and costly. Recent advances in methane inhibitors, such as 3-nitrooxy propanol and halogenated analogues, plant secondary compounds, including polyphenols and essential oils, to reduce methane emissions have necessitated the discovery of processes underlying rumen methane synthesis and inhibition. The identification of methane proxies, such as behavioural and input proxies (dry matter intake, neutral detergent fibre), microbial community proxies (rumen metagenome profiles), metabolic pathway proxies (fatty acids), molecular and genetic proxies (microbial genes), and downstream and non-invasive proxies (milk fatty acids and faecal lipidomes), is leading to more viable solutions. New developments in 'omic' techniques,

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including lipidomics, metagenomics and metatranscriptomics, have enabled the detection of proxies at the molecular level utilising rumen liquor, milk, blood, urine, and faeces. In addition to traditional methane proxies, rumen microbiota profiles, and specific genes involved in rumen methanogenesis (such as *mcr* and *mrt*, which encode methyl coenzyme reductase 1 and 2), these markers can be used to identify methane-producing pathways. Protozoa-associated methanogens (PAMs), propionate-producing bacteria, and methane-oxidising methanotrophs (*Methylocystis* sp.) are emerging as new proxies. Methane proxies provide scalable, affordable, and mechanistically insightful alternatives to conventional direct measuring techniques, which improve the understanding of rumen function and the biological causes of methane releases, enabling large-scale methane monitoring and will enable designing effective methane mitigation strategies in livestock production systems.

1. Introduction

Greenhouse gases (GHGs), including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), hold heat in the atmosphere and thus contribute to climate change (Filonchyk et al., 2024). For example, in the United States, CO_2, CH_4, and N_2O account for 79.7 %, 11.1 %, and 6.7 % of GHG emissions, respectively (EPA, 2025). Over the past century, the global warming potential (GWP), the amount of thermal energy absorbed by one tonne of gas in contrast to one tonne of CO_2, varied from 21 to 28 for CH_4 (EPA, 2025). The half-life of methane is 13.8 years, and some methane remains in the atmosphere after 60 years (Reisinger et al., 2021). Enteric fermentation and livestock manure emissions account for 25 % and 11 % of total CH_4 emissions, respectively (EPA, 2025). Therefore, reducing CH_4 output from livestock is proposed as a long-term strategy for mitigating CH_4 emissions in agriculture.

Evaluating CH_4 reduction strategies, calculating precise GHG inventories, and estimating the carbon footprint of livestock products requires accurate, cost-effective, and repeatable measuring methodologies. However, implementing these technologies on a large scale remains challenging due to their high cost and logistical complexity. For instance, genetic selection of low-methane cattle requires methane phenotyping of several individual animals in the order of 10^4–10^5 (de Haas et al., 2021). Similarly, for evaluating long-term methane mitigation strategies, such as early-life programming and foetal programming, long-term assessment of methane emissions is required (Kolathingal-Thodika et al., 2025). Also, understanding the biological processes of methane generation and mitigation requires estimation of the biological intermediaries of methanogenesis. Recently, integrating AI for the assessment of livestock emissions has generated big data on methane and related traits. These necessitate the use of proxies in addition to direct methane measurements. In recent years, several biochemical and molecular proxies have been demonstrated to evaluate traits such as heat tolerance (Elayadeth-Meethal et al., 2023a; Elayadeth-Meethal and Kolathingal-Thodika, 2024), disease tolerance (Kalaiyarasi and Elayadeth-Meethal, 2025) and growth traits (Manjutha et al., 2023). Hence, the use of proxies can be a sustainable and synergic alternative to use inexpensively, along with direct livestock methane measurements.

Methane is measured directly using different techniques. All these direct approaches have been widely utilised to measure CH_4 at the animal/farm level and to validate GHG inventories based on IPCC requirements at the regional/national/global levels (Hristov et al., 2025). However, for developing more economic and effective adoption pathways for recent methane mitigation technologies based on genetic, management and dietary interventions, methane proxies could be more viable options. Furthermore, to develop the inhibitors of methanogenesis, it is imperative to elucidate the mechanism driving CH_4 synthesis using methane proxies. As methane proxies often are the intermediaries or byproducts in the various biological pathways involved in methanogenesis, they help understand the mechanism of methanogenesis (see Fig. 1). The proxies are frequently coupled with direct measurements to better understand the mechanisms underlying CH_4 formation and the inhibitory effects of anti-methanogenic supplements. Hence, this review aims to connect the existing and emerging proxies with mechanisms associated with methane production and inhibition.

Although methane proxies have arisen as an alternative to direct methane measurement, they suffer a number of challenges. Data integration is a challenge because measurement and analysis tool methods for proxies differ significantly across production systems. As a result,

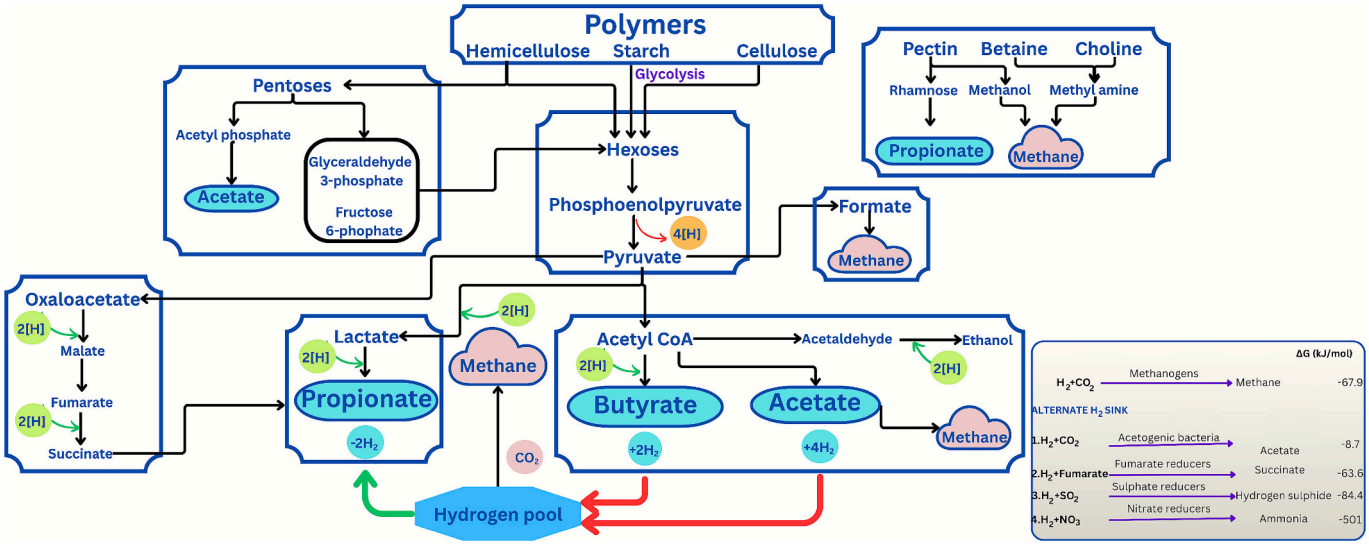

Fig. 1. Rumen fermentation pathways representing volatile fatty acids (VFAs) and CH_4 synthesis. The source of metabolic hydrogen [H] and molecular hydrogen (H_2) sinks in the rumen are also illustrated. Panel on the right: shows Gibbs energy changes (ΔG) in different hydrogen sink pathways in the rumen. Modified and adapted from: Khairunisa et al. (2023); Ungerfeld (2020).

standardisation of measuring protocols is critical for increasing the use of methane proxies. Another challenge is utilising proxies across breeds, species, and production systems. Because of variances in the digestive process, metabolism, and gut bacteria, proxies developed in one do not necessarily translate to others. Furthermore, proxy models established in a single context may require additional independent validation to tune the methane proxies to different management techniques and situations. To address these problems, context-specific proxies must be discovered and evaluated in order to expand the use of proxies.

Accordingly, this review assesses the use of various proxies for CH_4 measurement in livestock and their relationship to methanogenesis, providing a tool for validating direct CH_4 measurements and exploring the mechanism of CH_4 synthesis and its inhibition in the rumen.

2. Methane phenotypes in livestock

Methane production (MP), methane intensity (MI), and methane yield (MY) are metrics used to express methane observed directly in the breath or approximated indirectly using milk spectral data, feed intake, or faecal lipidome. The MP is quantified in litres or grammes per day per animal. The MI represents the amount of CH_4 exhaled per kilogram of milk production or daily body weight gain. MY, or the quantity of CH_4 emitted per kg of dry matter intake (DMI), is the most widely used phenotype. This is intimately related to feed intake, which is directly linked to milk output or growth. These CH_4 phenotypes are used for the selection of low methane livestock, the preparation of CH_4 inventories and evaluating CH_4 mitigation efficiency of antimethanogenic feed supplements. Targeting MY rather than overall output or intensity appears to be the most successful approach for selecting cattle with low emissions (Culbertson et al., 2025). Another significant and relatively new CH_4 metric is residual CH_4 production (RMP), which, like residual feed intake (RFI), measures the difference between observed and projected CH_4 production based on feed intake and body weight. For genetic selection for low methane, RMP is the preferred metric.

3. Direct methane measurement

The traditional and most reliable method uses respiration chambers, a high-cost and labour-intensive process, which limits its suitability for large-scale CH_4 measurements. A second method, which employs sulphur hexafluoride (SF_6, a potent greenhouse gas with a lifespan of 3200 years and an estimated GWP (global warming potential) of 23,500 times greater than that of CO_2), uses SF_6 as a tracer gas and requires invasive halters on animals, as well as laboratory testing of gases extracted. A snapshot gas sampling technique, such as the GreenFeed® system, is another commonly used sensor approach, in which exhaled gas is monitored in real-time as animals feed or drink (Hristov et al., 2015). This method, while non-invasive and suited to larger-scale assessments, has a greater risk of inter- and intra-animal variance (Hristov et al., 2015). For example, in a recent study, the animal-to-animal variation in CH_4 production measured using GreenFeed® was 13 % (Starsmore et al., 2024). GreenFeed®, as a snapshot method, extrapolates GHG measurements acquired during shorter periods (minutes or hours) to 24 h (CH_4 output, g/day/animal).

Methane concentration is also measured using a sniffer device or gas cards placed in the feeding trough in an automated milking system or concentrate feeder. Here, the exhaled CH_4 is measured using non-dispersive infrared (NDIR) or Fourier transform infrared (FTIR) spectroscopy. There is also widespread use of other snapshot techniques such as polytunnels, face masks and portable accumulation chambers (Prathap et al., 2021). An inverse dispersion model (IDM) employing open-path lasers is used for the herd-level detection of CH_4 emissions (Bai et al., 2025). While Rusitec and in vitro fermentation provide CH_4 production kinetics at the rumen level, they do not provide information on complex metabolic processes occurring at the animal level.

4. Proxies for methane measurement

Methane proxies can include microbes, molecules or compounds that provide information on rumen methanogenesis and the animal's CH_4 emissions. Unlike traditional approaches, which rely on directly detecting emissions from the animal's breath or utilising snapshot estimations, proxies utilise cutting-edge technologies to provide an improved understanding of methanogenesis at the molecular level. The ideal proxy would be phenotypically and genetically correlated with CH_4 emissions, inexpensive, and easily and routinely measured on a large scale. Proxies also help us understand the biological processes involved in inhibiting rumen methanogenesis. Methane proxies encompass a wide range of physiological, molecular, and biochemical indicators, which provide a clear indication of CH_4 emissions from ruminant animals. Broadly, these proxies may be classified into five categories (Fig. 2).

4.1. Behavioural and input proxies

These proxies include feed intake and rumination time, indicating differences in CH_4 substrate availability and fermentation dynamics, respectively.

4.1.1. Feed intake

Feed intake is responsible for most of the changes in daily CH_4 emissions and plays an essential role in CH_4 production. Enteric CH_4 is a naturally occurring byproduct of microbial fermentation that is primarily produced in the rumen (87–90 %), with a modest contribution from the animal's hindgut (10 %–13 %). The availability of substrates (feed) has a significant impact on the methanogenic pathways, which may vary depending on the feed type (fibre-rich or carbohydrate-rich) and feeding management (Meo-Filho et al., 2023). Methane synthesis and eructation occur more quickly after feeding than during rumination or rest (Watt et al., 2015). This can be easily explained since more feed reaching the rumen triggers more fermentation and offers more substrates for CH_4-producing bacteria, resulting in increased CH_4 output (Smith et al., 2021). Increasing feed intake results in more overall MP, whereas the MY decreases as feed intake increases. The MP is strongly correlated to dry matter intake (DMI), both phenotypically and genetically, with DMI accounting for up to 85–86 % of the variance in MP (Zetouni et al., 2018). However, this correlation can vary depending on the animal's breed, nutrition, composition of the feed, and the technology applied to detect emissions.

Recently, there has been increased focus on understanding the association between feed intake and CH_4 emissions. For example, Goopy et al. (2020) evaluated the link between CH_4 generation and feed intake. They found a decline in MP with decreased feeding level, roughly 1 to 0.4 of the maintenance energy requirement. However, there was an increase in MI. This is linked to rumen passage rate, as faster means less fermentation time (Arowolo et al., 2022). Previously, Hendriks et al. (2013) explored how RFI, which examines the discrepancy between an animal's actual feed intake and the estimated feed requirements based on weight and body composition, affects CH_4 output in steers and found that animals with lower RFI generated 25 % less CH_4 per day than those with greater RFI. Similarly, Johnson et al. (2022) evaluated the phenotypic and genetic association between RFI and CH_4 emissions and found that CH_4 emissions increased with feed consumption. Hence, feed intake may be a non-invasive proxy for CH_4 emissions.

Proxies related to feed intake, such as DMI and RFI, play a significant role in CH_4 production variability and can provide inexpensive markers of CH_4 outputs. Once paired with feed composition, animal behaviour, and digestibility metrics, they allow for multi-dimensional proxies that improve prediction accuracy and mechanistic knowledge of CH_4 emissions. Methane proxies connected to feed intake, their mode of action, and the possible coupled usage are summarised in Table 1.

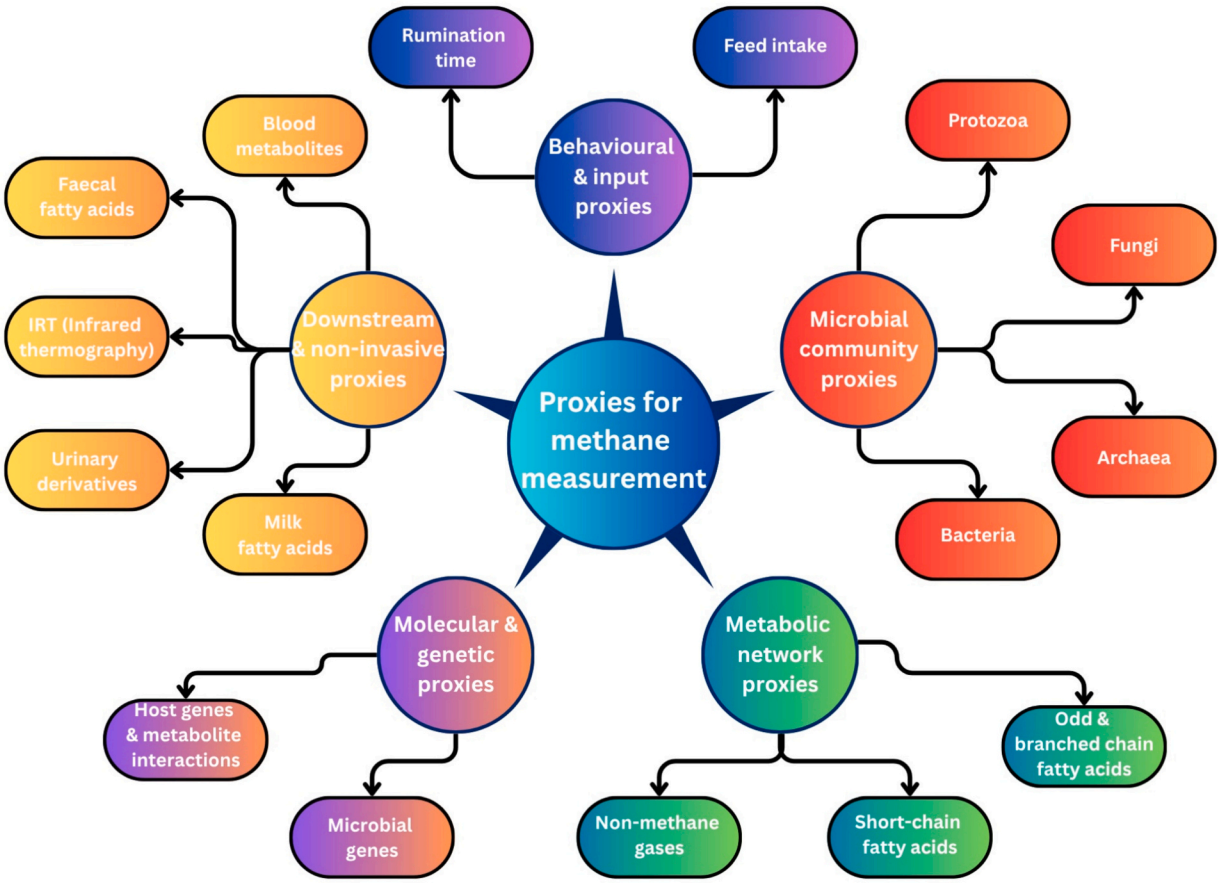

Fig. 2. Classification of CH_4 proxies used for indirect CH_4 measurements in livestock. This classification is based on physiological, molecular and biochemical processes underlying rumen CH_4 synthesis and inhibition.

4.1.2. *Rumination time*

Rumination is necessary to break down feed particles, increase surface area, and make them more accessible for fermentation by rumen microbes. This helps the feed to flow more efficiently through the reticulorumen, resulting in faster digestion and nutritional absorption. It also boosts saliva production, which maintains rumen pH stability. The duration and pattern of rumination have a major impact on feed breakdown and fermentation in the rumen. Feed digestibility, fibre intake, and pasture quality influence rumination, either positively or adversely. For example, Mikuła et al. (2021) found that cows with extended rumination durations emitted less CH_4 (MP) but had higher MY. Specifically, cows in the low rumination group (up to 412 min/day) emitted 1.8 % more CH_4 than those in the medium rumination group (412–527 min/day), and 4.2 % more than those in the high rumination group (527 min/day or more, Mikuła et al., 2021). Similarly, cows with longer rumination times digested more dry matter and produced more milk while generating less CH_4 (Castaneda et al., 2024).

Studies showed that rumination time is an indicator of animal health, growth, production and reproduction efficiency and CH_4 emissions (Lopes et al., 2025). The rumination time is moderately heritable (heritability 0.4), and putative genomic regions affecting rumination time have been identified (Atashi et al., 2024). Lopes et al. (2024) reported comparable results when analysing the genetic relationships between RT, feed efficiency (FE), MP, and production indices. Their findings showed that RT, CH_4 emissions, energy-corrected milk, fat and protein yield had moderate heritability, while FE had low heritability. A major conclusion from their research was a negative genetic association between RT and CH_4 emissions, meaning that cows with longer RT produced less CH_4. Furthermore, they observed a substantial genetic correlation between RT and energy-corrected milk, demonstrating that extended rumination leads to enhanced milk production efficiency. In a recent study by Castaneda et al. (2025), it was found that animals with high rumination time (20 % more daily rumination time than animals with low rumination time) had 26 % less MY. However, Weller and Ezra (2024) found no significant genetic correlation between RT and MY. It could be a finite amount of CH_4 that could be generated/unit intake, thus longer RT means sigmoidal decay towards the end, i.e., the last 20 % of RT is all flat-lined (Castaneda et al., 2025).

Combining feed intake and rumination time, along with related feeding behaviour such as feeding time, feed composition and dietary source of nutrients, may serve as a better proxy for CH_4 emissions (Giagnoni et al., 2024b). Sepulveda et al. (2022) found that including eating time improved enteric CH_4 prediction accuracy. Similarly, clustering rumination time and feeding strategies improved enteric CH_4 evaluation (Ferronato et al., 2025).

4.2. *Microbial community proxies*

Through changes in community structure and operational interconnectivity, microbial population proxies, which include enteric bacteria, archaea, protozoa, and fungi, offer a direct link to CH_4 generation capability. The growing evidence suggests that individual cow CH_4 production differs based on their genotype and rumen bacterial composition. For example, Difford et al. (2018) found that bacteria and archaea accounted for 13 % of the variation in CH_4 generation, with a heritability of 21 %, and that these two components were mostly independent of one another.

Rumen microbes play a crucial role in converting otherwise unusable organic resources into protein and energy, which has a direct impact on the animal's health, performance, and feeding efficiency. While rumen

Table 1
Methane proxies linked to feed intake: mode of action and combined applications.

Proxy	Mode of action	Possible coupled usage	Reference
Dry matter intake (DMI)	Feed intake is closely associated with total CH_4 production (MP), as it improves the availability of substrate for fermentation and CH_4 formation	It can be used with nutrient composition and feeding behaviour to forecast daily CH_4 emission. It is critical for scalability over breeds and feeding methods.	Zetouni et al. (2018)
Feed conversion efficiency (FCE)	Corresponds to the ratio of feed consumed to production output (e.g., milk or weight gain). Low FCE indicates inefficient digestion and greater CH_4 loss per unit of product.	When paired with data on feed digestibility and animal productivity, it is possible to identify the biological efficiency from intake-driven impacts.	Atashi et al. (2024)
Residual feed intake (RFI)	Determine the difference between actual and anticipated feed intake for maintenance and production. Low-RFI animals produce less CH_4 due to better metabolic efficiency and lower fermentation loss	When combined with DMI and growth/milk yield data, this proxy is a useful tool for identifying genotypes with low emissions and high efficiency.	Hendriks et al. (2013); Johnson et al. (2022)
Feeding behaviour (duration, frequency, meal size)	The feeding episodes lead to fast increases in CH_4 generation through substrate input and microbial fermentation spikes.	Sensor data (e.g., accelerometers, RFID) can enhance the temporal resolution of CH_4 predictions when paired with DMI and food composition.	Watt et al. (2015)
Feed composition (fibre-to-starch ratio)	High-fibre feeds promote methanogenesis by increasing acetate and H_2 generation, whereas starch-rich diets boost propionate pathways and decrease CH_4 yield.	Integrating DMI and feed content improves the prediction of CH_4 intensity (g CH_4/kg DMI).	Meo-Filho et al. (2023)
Feeding level (relative to maintenance)	Reduced feeding rates decrease total CH_4 but increase CH_4 per unit intake due to slower passage rate and longer fermentation duration	Combining analysis with passage rate and rumen dynamics can enhance dynamic CH_4 models for different energy consumption.	Goopy et al. (2020); Arowolo et al. (2022)
Feed digestibility / energy density	Low-quality feeds enhance CH_4 per unit of energy used, while more digestible feeds result in rapid fermentation and lower CH_4 production per unit of energy consumed.	Coupling with DMI and feed type enables mechanistic modelling of CH_4 conversion rates in various diets.	Patra et al. (2024); Giagnoni et al. (2024a)

metagenomics identifies the richness of the rumen microbiome, metatranscriptomics reflects the microbiome's functional activity to fluctuations in CH_4 emissions (Li et al., 2019). Differences in CH_4 yield are primarily explained by other microbial communities and their activities, rather than by methanogens alone (Martinez-Alvaro et al., 2020). While archaea are necessary for CH_4 synthesis, their overall abundance is only weakly associated with individual animal CH_4 emissions. However, the distinct composition of the archaeal population appears to have a greater impact, with a higher proportion of *Methanobrevibacter* found among high CH_4 emitters (Auffret et al., 2018). In a recent study, combining rumination time and rumen and faecal microbiome profiles increased the accuracy of prediction. For example, low CH_4 emitters typically had high rumination times and a high abundance of *Methanosphaera stadtmanae* (methylotrophic methanogen) in the ruminal fluid. Conversely, high CH_4 emitters had low rumination time, and rumen fluid had a high abundance of *Methanobrevibacter* sp. (hydrogenotrophic methanogen), resulting in low-enteric CH_4 (Castaneda et al., 2025).

Several bacteria that utilise hydrogen (H_2) compete with methanogens for metabolic hydrogen [H] (Fig. 1). Propionate synthesis is a pathway that consumes H_2. One mole of propionate uses two moles of [H], while the synthesis of butyrate and acetate releases H_2 in the rumen. Each mole of butyrate synthesis releases two moles of [H], and acetate synthesis releases four moles of [H] (Khairunisa et al., 2023). Among the alternative H_2 sink pathways, acetate and fumarate pathways are preferred in terms of reaction kinetics (Ungerfeld, 2020). Sulfate and nitrate reduction pathways are less favourable because they can lead to the accumulation of hydrogen sulfide and ammonia or nitrite, respectively (Beauchemin et al., 2020).

Acetogens, such as *Eubacterium*, *Blautia*, and *Acetitomaculum,* convert H_2 and CO_2 into acetate. Other anaerobic bacteria that utilise H_2 are *Desulfovibrio desulfuricans* (which reduces sulphate), *Selenomonas ruminantium* and *Wolinella succinogenes* (which reduce fumarate and nitrate), and *Denitrobacterium detoxificans* (which reduces trimethylamine N-oxide). Supplementing with fumarate, sulphate, or nitrate can significantly reduce CH_4 production, plausibly by stimulating alternate H_2-use pathways (Greening et al., 2019). Low- CH_4 ruminotypes typically have fewer H_2-producing bacteria. For example, in high CH_4 emitters, carbohydrate-fermenting bacteria belonging to the phylum Firmicutes and class Clostridia (*Ruminococcus*, *Christensenellaceae*) are more abundant, providing much of the H_2 necessary for hydrogenotrophic methanogens (Kittelmann et al., 2014; Ramayo-Caldas et al., 2020). Reduced Proteobacteria and Bacteroidetes abundance was linked to increased CH_4 emissions (Tapio et al., 2017). Similarly, lower CH_4 emissions were linked to a higher abundance of ruminal *Prevotella* in Colombian buffaloes, through more efficient NH_4+ production, consuming more H_2 (Aguilar-Marin et al., 2020). *Megasphaera elsdenii* and *Coprococcus catus*, which produce propionate, have been associated with lower CH_4 emissions in dairy cows (Shabat et al., 2016). Similarly, rumen anaerobic fungi were prolific producers of H_2 and formate, and their abundance was correlated with high levels of CH_4 emissions (Bach et al., 2023). The various rumen microorganisms, their modes of action, and the effects on rumen fermentation and CH_4 synthesis are summarised in Table 2.

Methanotrophs are aerobic CH_4 -oxidising microbes utilising CH_4 as a source of energy to produce methanol and formaldehyde (Parmar et al., 2015). For example, *Methylocystis* sp. were isolated from the rumen and examined for their CH_4-reducing ability (Tseten et al., 2025). Likewise, Proteobacteria in rumen fluid and *Nitrosomonas* from rumen epithelium oxidise CH_4 in the rumen (Mitsumori et al., 2002). Auffret et al. (2018) found a negative association between the methanotrophic genus *Methylomonas* and CH_4 production. Feeding methanotroph (*Methylocystis* sp.) and methylotroph (*Methylobacterium* sp.) bacteria dramatically reduced CH_4 emissions without any implications for animal health (Tseten et al., 2025). The limitation is that they need surface area to attach, so they tend to be on the rumen wall rather than distributed through the rumen where needed. This is one of the theories why biochar may reduce methanogenesis by increasing the surface area distributed across the rumen. Methane oxidation produces formaldehyde, which is utilised by γ-proteobacteria (type I methanotrophs) through the ribulose monophosphate pathway and α-proteobacteria (type II methanotrophs) through the serine pathway (Parmar et al., 2015). Consequently, the detection of enzymes in the serine pathway,

Table 2
The various rumen microorganisms, their modes of action, and the effects on rumen fermentation and CH_4 synthesis.

Microorganisms	Description	Fermentation product	Change in abundance	Methane emission	Details	Reference
Succinovibrionaceae	Amylolytic	Acetate, propionate, succinate	Increased	Reduced CH_4 out put	Favour propionate production through succinate pathway, thus reducing the H_2 available for methanogenesis	Wallace et al. (2015)
Prevotella bryantii	Fibre degradation	Succinate, propionate	Increased	Low CH_4	Reduce fumarate into succinate which then form propionate. Also increase ammonification, consuming H+	Kittelmann et al. (2014)
Megasphaera	Lactic acid utilising bacteria	Acetate, propionate, butyric acid	Increased	Low CH_4 output	Produce propionate through alternate H_2 sink	Shabat et al. (2016); Kamke et al. (2016)
Coprococcus	Lactate utilizer	propionate	Increased	Low CH_4	Favour propionate formation through the acrylate pathway	Shabat et al. (2016)
Sharpea	Lactate producing bacteria	Lactate, propionate	Increased	Low CH_4	Favour propionate formation through the acrylate pathway	Kamke et al. (2016); Kittelmann et al. (2014)
Fibrobacter	Cellulose degrading	Succinate, formate, lactate	Increased	Low CH_4	Favour propionate formation	Kittelmann et al. (2014)
Ruminococcus	Cellulose degrading	Acetate, formate	High abundance	High CH_4	Ferment hexose sugars to acetate and butyrate, increasing H_2 and methane	Ramayo-Caldas et al. (2020); Kittelmann et al. (2014); Prathap et al. (2024)
Blautia	Acetogenic bacteria	acetate	Increased	Low CH_4	Favour acetogenesis through alternate H_2 sink	Greening et al. (2019)
Selenomonas	Amylolytic bacteria	Acetate, propionate	Increased	Low CH_4	Favour fumarate reduction, compete with methanogens for H_2	Greening et al. (2019)
Clostridium	Cellulose degrading	Propionic acid, butyrate	Increased	High CH_4	Favour nitrate reduction and provide N source for methanogens	Wang et al. (2016); Jiang et al. (2022)
Lachnospira	Pectin, cellulose degrading	Acetate, formate, lactate	Increased	High CH_4	Ferment hexose sugars to acetate and butyrate, increasing H_2 and methane.	Ramayo-Caldas et al. (2020); Kittelmann et al. (2014); Prathap et al. (2024)
Methanomicrobium	Ruminal archaea	Methane	Increased	High CH_4	Hydrogenotrophic methanogen	Jiang et al. (2022)
Methanobrevibacter	Ruminal archaea	Methane	Increased	High CH_4	Dominant methanogen	Li et al. (2024); Hook et al. (2010); Prathap et al. (2024)
Methanosphaera	Ruminal archaea	Methane	Low abundance	High CH_4	Positive interaction with Lachnospiraceae	Henderson et al. (2015); Ramayo-Caldas et al. (2020)
Epidinium	Proteolytic protozoa	Ammonium, VFA	Decreased	Low CH_4	Harbour intra and extracellular methanogenic archaea	Bach et al. (2023); Newbold et al. (2015)
Entodonium	Proteolytic protozoa	Ammonium, VFA	Increased	Low CH_4	No intracellular methanogens	Newbold et al. (2015); Bach et al. (2023)
Neocallimastix	Cellulolytic fungi	Lactate, formate, acetate, succinate, ethanol	Decreased	Low CH_4	Provide H_2 and formate for CH_4 production	Bach et al. (2023)

such as glycine hydroxyl methyl transferase, can predict CH_4 emissions (Parmar et al., 2015).

Interspecies H_2 transfer shifts a significant amount of H_2 from H_2-producing fermenters to hydrogenotrophic methanogens (Belanche et al., 2014). For example, ciliate protozoa produce substantial amounts of H_2 because of their metabolism and form a close association with methanogens. These relationships not only provide H_2 for CH_4 synthesis, but they also protect the symbiotic archaea from oxygen. Methanogens linked with protozoa make up 9 % to 25 % of the total methanogen population in the rumen and account for 9–37 % of total CH_4 emission (Belanche et al., 2014). Around 32.8 % of *Methanobrevibacter* sp., or approximately 65 % of rumen methanogens, were found in conjunction with protozoa (Dai et al., 2022). This interaction is aided by Mru_1499, an adhesion-like protein that allows *Methanobrevibacter* to bind to the surfaces of different protozoa species (Smith et al., 2022). Holotrich ciliates, such as *Dasytricha* and *Isotricha*, have a particularly significant impact on CH_4 production and are more accurate proxies in CH_4 prediction models due to their capacity to ferment soluble carbohydrates and generate VFAs and H_2 (Dai et al., 2022). As a result, defaunation often reduces CH_4 emissions by 11 % while the overall number of methanogens remains relatively constant (Newbold et al., 2015). The increase in methanogenesis was not linked to protozoal biomass, but rather to their metabolic activity and impact on the microbiome (Tapio et al., 2017). The presence of protozoa increased NH_3-N concentrations and altered the VFA profile, increasing acetate and butyrate synthesis while decreasing propionate levels (Ranilla et al., 2007). Supplementing Agolin® Ruminant, an essential oil mix, reduced *Epidinium* relative abundance (Bach et al., 2023), resulting in a 13 % reduction in daily CH_4 emissions and a 97 % decrease in protozoal count (Foggi et al., 2024). Hence, more research is needed to use rumen metagenomics as a reliable proxy for enteric CH_4 emission in livestock. Additionally, a clearer mechanistic understanding of how microbes interact with the host is needed to use rumen microbial abundance, diversity and activity alterations to accurately predict CH_4 emissions. Fig. 3 illustrates the relationship between rumen microbiome and CH_4 emissions.

4.3. Metabolic network proxies

Metabolic network proxies, such as VFAs, dissolved H_2/CO_2, and odd- and branched-chain fatty acids (OBCFAs), are intermediates of metabolism that indicate hydrogen balance and fermentation efficacy.

4.3.1. Fatty acids in rumen fluid

4.3.1.1. Short-chain fatty acids. Carbohydrate fermentation is an important metabolic activity in the rumen, generating three major VFAs: acetate, propionate, and butyrate, as well as H_2 and carbon dioxide (CO_2) (Fig. 1). The amount of hydrogen present governs the thermodynamics of fermentation processes that produce or consume [H]. High [H] levels stimulate fermentation pathways that produce less H_2, resulting in more propionate and less CH_4 (Fig. 1). When H_2 concentrations are low, fermentation pathways that produce more [H] (acetate

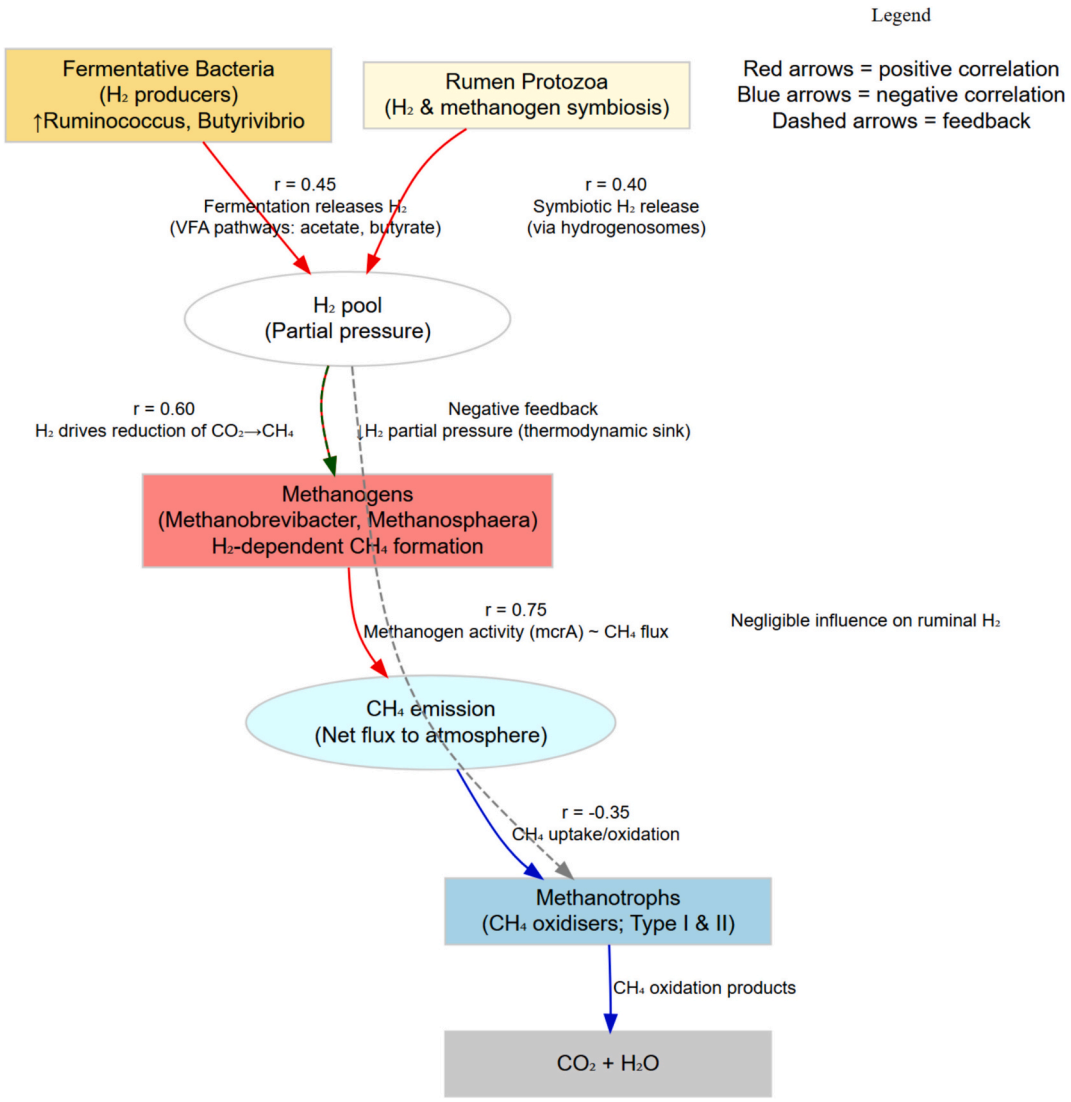

Fig. 3. Mechanistic diagram illustrating the association between CH_4 emissions and the rumen microbiome. Fermentative bacteria and protozoa generate molecular hydrogen during carbohydrate fermentation. Methanogenic archaea, predominantly *Methanobrevibacter* spp., utilise H_2 to reduce CO_2 to CH_4. Arrows represent statistically supported pathways; width corresponds to correlation strength (r). Positive effects (red) indicate CH_4-enhancing pathways, negative effects (blue) indicate CH_4-mitigating pathways. Bacteria (Firmicutes, *Prevotella*) influence H_2 production ($r = 0.45$), which fuels methanogens (*Methanobrevibacter*, *Methanosphaera*; $r = 0.60$). Methanogen abundance and activity (mcrA transcripts) drive CH_4 emission ($r = 0.39$–0.75). Some bacterial taxa reduce CH_4 via H_2-consuming pathways (propionate synthesis, $r = 0.30$). The balance between microbial H_2 production and consumption governs overall CH_4 output, with negative feedbacks stabilising ruminal H_2 partial pressure. This schematic integrates abundance, activity, and microbial composition to predict CH_4 flux. Prepared using R (R Core Team, 2024).

and butyrate synthesis) are chosen, resulting in increased CH_4 production. Acetate and butyrate formation produce H_2, which is converted into CH_4, while propionate production lowers H_2 levels (Fig. 1). Acetate is a key substrate for milk fat synthesis, whereas propionate is mostly utilised for gluconeogenesis (Fig. 4). Increasing the acetate-to-propionate ratio in the rumen can result in higher milk fat content and CH_4 release. Propionate generation provides an alternative method of disposing of H_2 and has been associated with lower CH_4 emissions. As a result, shifting H_2 out of CH_4 and to propionate could be an effective method for reducing CH_4 emissions (Wang et al., 2023). Cattle with high residual CH_4 efficiency (RME) had lower ruminal propionate levels than cattle with low RME, but higher butyrate levels than medium and low RME animals (Smith et al., 2021).

Plant extracts high in polyphenols and saponins reduce CH_4 production and increase total VFAs, with a significant increase in propionate and butyrate levels, accompanied by a drop in acetate and a lower acetate-to-propionate ratio (Suriyapha et al., 2024; Prathap et al., 2024). Similarly, supplementing with seaweed containing bromoform and/or high-lipid/polyphenols reduced CH_4 emissions while increasing total VFAs and propionate molar percentage (Choi et al., 2021). A comparable study on buffaloes found that a blended polyphenol-rich extract increased propionate levels while decreasing acetate content, the acetate-to-propionate ratio, and NH_3-N concentrations, while inhibiting CH_4 synthesis (Singh et al., 2022). Similarly, polyphenol extracts from *Castanea involucres* significantly decreased acetic acid levels and the acetate-to-propionate ratio, while increasing propionic acid levels and decreasing CH_4 (Wang et al., 2021). Likewise, nutmeg essential oil administration significantly reduced CH_4 production, NH_3-N levels, total VFAs, and acetate, propionate, and butyrate levels (Abdillah et al., 2024).

4.3.1.2. Odd- and branched-chain fatty acids (OBCFAs). Odd- and branched-chain fatty acids include an odd number of carbon atoms and a methyl group attached to a second (iso) or the third (anteiso) carbon

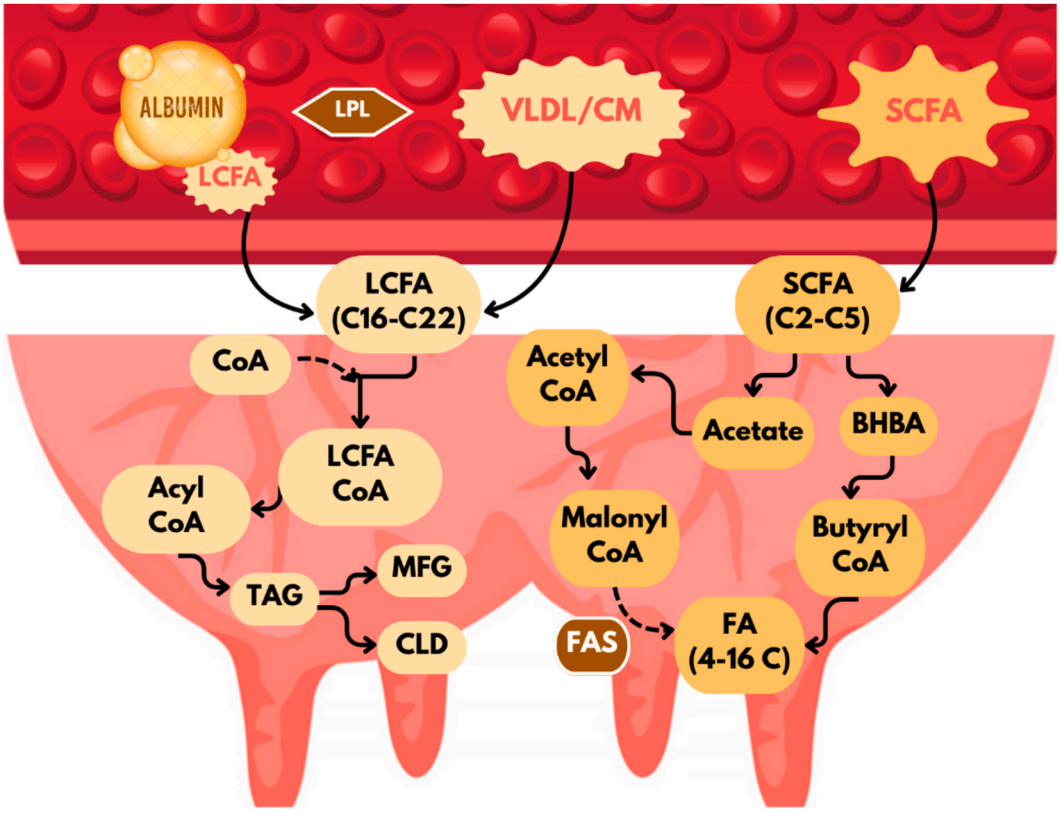

Fig. 4. De novo milk fatty acids synthesis in the udder. LCFA (long chain fatty acid), LPL (lipoprotein lipase), VLDL (very low-density lipoprotein), CM (chylomicrons), SCFA (short chain fatty acid), BHBA (beta-hydroxybutyric acid), CoA (Coenzyme A), MFG (milk fat globule), FAS (fatty acid synthase), CLD (cytoplasmic lipid droplet), TAG (triglyceride). Adapted and modified from: Kyriakaki et al. (2023).

from the methyl end (ω, Liu et al., 2019). Rumen bacteria produce OBCFAs from propionate, isobutyrate, isovalerate, and 2-methylbutyrate as precursors, with higher levels indicating more microbial productivity (Kupczynski et al., 2024). As a result, both the composition and amount of OBCFAs vary in response to variations in rumen microbial communities and fermentation procedures. The OBCFA composition is affected by the animal's energy expenditure, the concentrate-to-forage ratio, and the amount of bioactive compounds in the feed. Lipases in the rumen degrade dietary fats to produce free fatty acids. These proceed via biohydrogenation, in which specific bacteria transform unsaturated lipids into saturated fats via isomerisation and saturation (Fig. 5). Rumen bacteria hydrogenate unsaturated fatty acids using H_2, which reduces the amount of H_2 accessible to methanogens and hence lowers CH_4 emissions (Dijkstra et al., 2011). However, stoichiometric calculation shows this H_2 consumption to be rather small (Ungerfeld, 2020). Furthermore, *anteiso* C15:0 reduces H_2 availability by boosting propionate formation, limiting CH_4 production. Unsaturated fatty acids may also reduce methanogenesis by inhibiting cellulose-degrading protozoa and bacteria.

Odd- and branched-chain fatty acids are synthesised by elongating fatty acids by two carbon units with malonyl-CoA synthetase, starting with precursors such as propionate, isobutyrate, isovalerate, and 2-methylbutyrate (Kupczynski et al., 2024). For example, propionate is used to synthesise C15:0 and C17:0, isoC14:0 and isoC16:0 from isobutyrate, isoC15:0 and isoC17:0 from isovalerate, and *anteiso*-C15:0 and *ante*iso-C17:0 from 2-methylbutyrate (Kupczynski et al., 2024). Xin et al. (2021) found a negative link between acetate and both C15:0 and iso-C17:0, but propionate had a positive correlation with C15:0. In a related study, Liu et al. (2019) found that total rumen OBCFA levels correlated positively to the molar proportion of acetate, negatively linked to isobutyrate, and demonstrated a negative association between propionate and *iso*-C17:0, but a positive relationship among propionate and *anteiso*-C15:0 and butyrate.

Different bacterial species in the rumen produce distinct OBCFA profiles. Cellulolytic bacteria have stronger relationships with OBCFA levels than starch-degrading species (Zhang et al., 2020a). While starch-degrading bacteria like *Selenomonas ruminantium*, *Ruminobacter amylophilus*, and *Streptococcus bovis* have higher levels of linear odd-chain fatty acids and only trace amounts of branched-chain fatty acids, fibre-digesting bacteria like *Ruminococcus flavefaciens* and *Ruminococcus albus* have high levels of iso-fatty acids (Xin et al., 2021). *Ruminococcus flavefaciens* shows the highest connection with C15:0 levels. Notably, iso-C16:0 concentrations have been reported as possible indicators for assessing total VFAs and microbial crude protein (MCP) production in the rumen (Xin et al., 2021). Combining rumen fatty acid profiles with other non-invasive proxies, such as rumination time, can increase the accuracy of CH_4 prediction. In a recent study, Castaneda et al. (2025) found that cows with high rumination time had low CH_4 yield with higher levels of propionate in the rumen fluid, while cows with low rumination time and higher CH_4 yield had higher acetate concentrations.

4.3.2. Non-methane gases in the rumen

Anaerobic fermentation produces [H] in the rumen. The rumen produces H_2 and CO_2 in two forms: gaseous and soluble. Microbes do not consume gaseous H_2; instead, they use dissolved H_2 (dH_2). Similarly, gaseous CO_2 is expelled while the rumen organisms use dissolved CO_2 (dCO_2). A fraction of dCO_2 is absorbed into the bloodstream, and high CO_2 can result in hypercapnia (Wang et al., 2016). Thus, measuring dH_2 and dCO_2 in rumen liquor or blood may be used as a proxy for CH_4 measurement. The main H_2 sinks in the rumen are CH_4 (methanogenesis), propionate (propiogenesis), acetate (acetogenesis), hydrogen

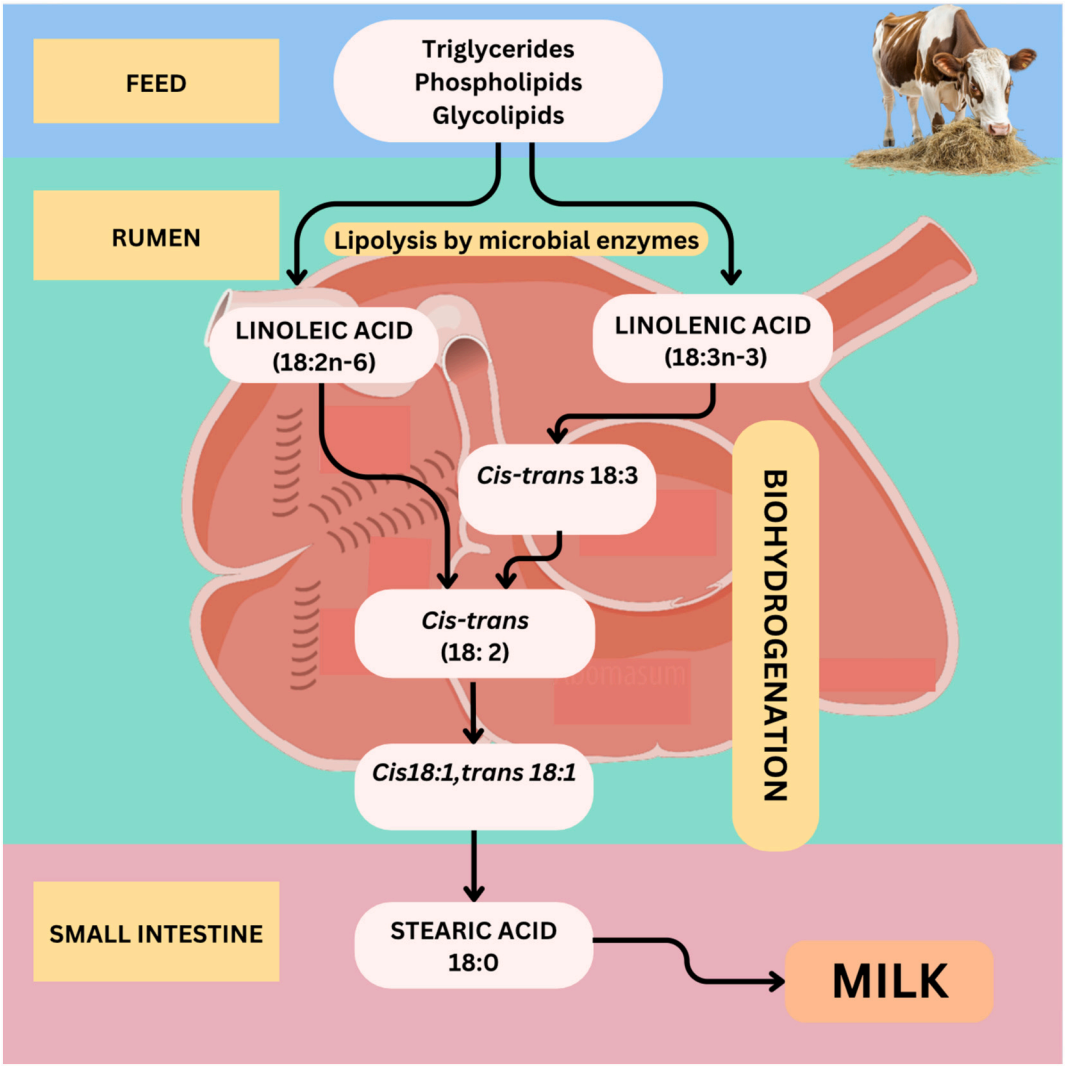

Fig. 5. Lipid metabolism and biohydrogenation pathways in the formation of long-chain fatty acids in the rumen. Modified and adapted from: Toral et al. (2024).

sulphide (H_2S, sulphate reduction), and NH_3-N (nitrate reduction) (Fig. 1). Inhibiting methanogenesis reduces total gas generation and VFAs in the rumen by inhibiting overall fermentation. For example, in beef cattle and in vitro studies, utilising seaweeds such as *Asparagopsis taxiformis* and *Colpomenia peregrina* to limit methanogenesis reduced total gas production by 10 % and 14 %, respectively (Roque et al., 2021; Wasson et al., 2023). Similarly, Martinez-Fernandez et al. (2016) observed that chloroform supplementation in Brahman steers decreased CH_4 emissions while increasing H_2 production, without altering dry matter intake. Likewise, supplementation of *Asparagopsis* spp. reduced CH_4 generation while increasing H_2 and CO_2 emissions in dairy cattle (Roque et al., 2019, 2021). In contrast, adding seaweed extracts reduced CH_4 without significantly reducing total gas production (Choi et al., 2021).

Adding essential oil-rich plant extracts, like lemongrass and dragon fruit peel pellets, enhanced total gas production while decreasing CH_4 emissions (Suriyapha et al., 2024). Wang et al. (2016) investigated the relationship between dissolved and gaseous CH_4 and hydrogen, observing that only dissolved CH_4 had a positive correlation with dH_2. Furthermore, dH_2 and gaseous H_2 were associated with higher butyrate and lower acetate levels. In another study, Roskam et al. (2025) found that feeding linseed oil to growing dairy beef bulls reduced daily CH_4 emissions by 19 % and H_2 output by 21 %. A mechanistic interaction plot illustrating non-CH_4 gases as proxies for CH_4 emissions is shown in Fig. 6.

4.4. Molecular and genetic proxies

Molecular and genetic proxies, comprising microbial genes (*mcr*, *mrt*) and host genomic signatures, provide a mechanistic understanding of fermentation control and host-microbe interplay.

4.4.1. Microbial genes

Methane synthesis in the rumen is mediated through specific microbial genes such as methyl co-enzyme M reductase (*mcr*), which reduces methyl-coenzyme M to CH_4. The kinetics of these rate-limiting enzymatic reactions determine the rate of CH_4 synthesis in the rumen (Patra and Puchala, 2023). According to Wallace et al. (2015), archaeal genes associated with CH_4 generation were 2.7 times more prevalent in high CH_4 emitters. The high emitters had a higher abundance of genes coding for coenzyme F420 hydrogenase (which is a direct electron donor in hydrogenotrophic methanogenesis) and heterodisulfide reductase, which catalyses electron bifurcation in methanogenesis (Bharanidharan et al., 2021, see Fig. 7).

Formyl-MF dehydrogenases (FMDs) convert CO_2 and methanofuran (MF) into formyl-methanofuran (formyl-MF) in the first step of methanogenesis and are encoded by the operons *fmdB*. The *ftr* genes encode formyl-transferases (FTRs), which catalyse the second step in

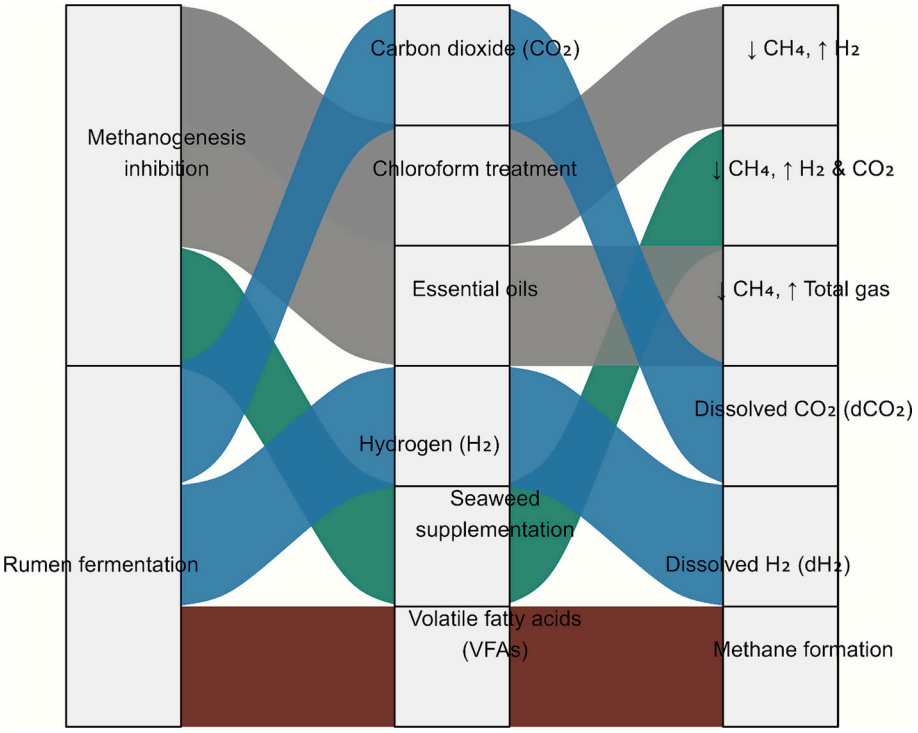

Fig. 6. Alluvial representation of non-methane gas interactions as proxies for methane emissions.

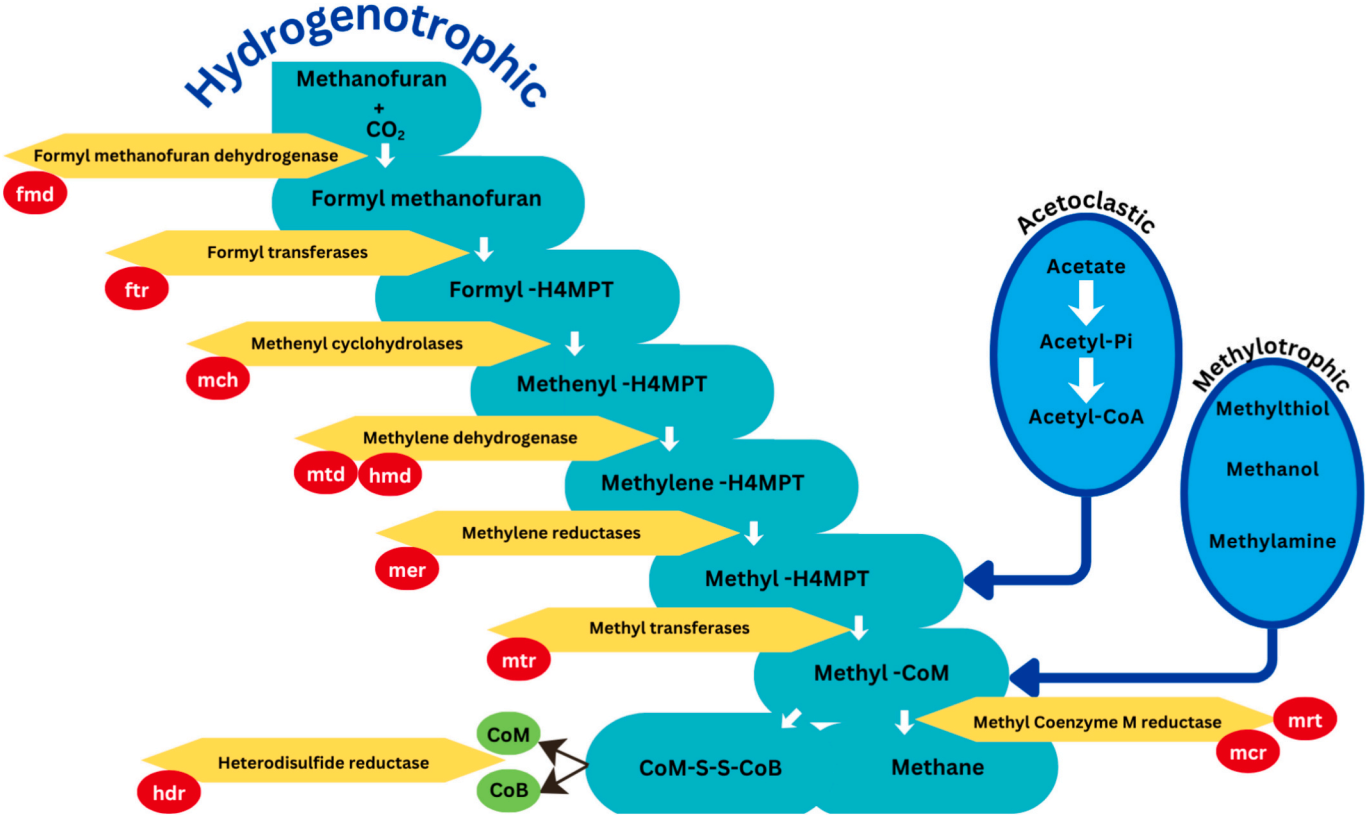

Fig. 7. Enzymes and genes associated with methanogenesis in ruminants. Modified and adapted from: Wallace et al. (2015).

methanogenesis. The third step is catalysed by methenyl cyclohydrolases (MCHs), which are encoded by the *mch* genes. In step 4, coenzyme F420-dependent methylene dehydrogenase (MTD) and H_2-forming methylene dehydrogenase (HMD) synthesise methylene-H_4MPT. These enzymes are encoded by the *mtd* and *hmd* genes. Step 5 is catalysed by coenzyme F420-dependent methylene reductases (MERs), while step 6 is carried out by methyltransferases (MTRs) encoded by the *mtr* operon. In the penultimate phase of CH_4 generation, methyl-

coenzyme M reductase (MCR) reduces the methyl group on coenzyme M, resulting in CH_4 and heterodisulfide of CoM and CoB (See Fig. 7; Wallace et al., 2015).

Microbial genes associated with non-methanogenic pathways may also be used as proxies for CH_4 emission potential. For example, genes linked with pyruvate metabolism, such as *porA* (Pyruvate ferredoxin oxidoreductase alpha subunit) and *porG* (gamma subunit), which encode enzymes that convert pyruvate to acetyl-CoA, were more common in high emitters (Bharanidharan et al., 2021). In contrast, the *ackA* (acetate kinase) gene, which converts acetate to acetyl phosphate, was more common in low-emitting Holstein steers. Similarly, butyryl-CoA dehydrogenase was upregulated when 3-NOP was administered (Pitta et al., 2022). The expression of genes involved in CH_4 generation and rumen fermentation is summarised in Table 3.

High CH_4 emitters had a 173 % greater relative abundance of *fmdB* than low emitters (Roehe et al., 2016). During methanogenesis, methyl-coenzyme M reductase (MCR) reduces the methyl group on coenzyme M, forming CH_4 and heterodisulfide of CoM and CoB (Fig. 7). MCR exists in two forms: MCR-I, encoded by the *mcr* operon, and MCR-II, encoded by the *mrt* operon (Bharanidharan et al., 2021; Khairunisa et al., 2023). Various methanogenic species have distinct *mcr* and *mrt* gene ratios, as well as structural changes in the MCR enzyme (Khairunisa et al., 2023; Leahy et al., 2010) (Fig. 8). High CH_4 emitters contain a high proportion of *mcr* genes (Roehe et al., 2016). Likewise, Shi et al. (2014) found that sheep with high CH_4 production had an increased amount of *mcr* transcripts. Similarly, Casanas et al. (2015) found a direct link between the number of *mcr* DNA copies and CH_4 emission in Holstein dairy cattle. Furthermore, Zhang et al. (2020b) found that when methanogenesis was inhibited with nitro chemicals, the expression of the *mcr* gene was more strongly influenced than the quantity of methanogens or key cofactors involved in methanogenesis, such as F420 and F430.

Table 3
Rumen fermentation changes and the expression of genes related to CH_4 production.

Reference	Gene expression	Mechanism
Denman et al. (2015)	Genes encoding transcarboxylase, malate dehydrogenase, and fumarate reductase were more abundant in goats supplemented with bromochloromethane.	Propionates randomising pathway- increasing propionate production.
Pitta et al. (2022)	Dairy cows fed with 3-NOP had lower *mcr* gene expression and higher copies of butyryl-CoA dehydrogenase. There was no increase in fumarate reductase.	Increase in butyrate pathway.
Guo et al. (2008)	In vitro, tea saponin lowered *mcrA* gene expression.	Decreases CH_4 production without affecting the methanogen number.
Wallace et al. (2015)	High emitters had higher levels of genes for coenzyme F420 hydrogenase, heterodisulfide reductase, and phosphoserine phosphatase than low emitters. Formate dehydrogenase β subunit abundance was low.	Up-regulation of acetate kinase, electron transport complex proteins (RnfC and RnfD) and glucose-6-phosphate isomerase.
Zhang et al. (2020b)	In-vitro experiments with nitro compounds such as nitroethane (NE), 2-nitroethanol (NEOH), and 2-nitro-1-propanol (NPOH) inhibit *mcrA,* coenzyme F420 and F430 levels.	Gene encoding methyl co-enzyme reductase enzyme, which catalyse the final step of methanogenesis.
Bharanidharan et al. (2021)	The abundance of *porA* and *porG* was positively associated with CH_4 generation, while *ackA*, *serB*, and *thrH* were negatively associated in steers given TMR.	*mcrA, fmdB, fdhF* formate dehydrogenase alpha subunit not identified.
Roehe et al. (2016)	Higher *mcrA* gene abundance has been identified in high-emitting beef cattle.	Gene encoding methyl co-enzyme reductase enzyme, which catalyse the final step of methanogenesis.
Auffret et al. (2018)	*mcrABG*, formate dehydrogenase, tetrahydromethanopterin *S*-methyl transferase, fmd, and hetero disulphide reductase were all abundant in high emission cattle.	All are associated with methanogenesis pathway.
Leahy et al. (2010)	Expression of *fmd, mcrBCDG*, and methyl viologen reducing hydrogenase (mvhG) increased in high-emitters. The *mtr* was increased in batch cultures.	Genes encoding the enzyme associated with methanogenesis.

4.4.2. Host genes, microbial genes and metabolite interactions

The host genome has a role in regulating methanogenesis in the rumen, with heritability ranging from 0.13 to 0.61 (Martinez-Alvaro et al., 2022a). For example, out of 1141 microbial genes discovered, 337 were functionally regulated with host genes, and 115 of these host-mediated microbial genes were associated with CH_4 emission (Martinez-Alvaro et al., 2022b). The top 30 putative microbial genes had a 17 % mitigation potential in each generation (Martinez-Alvaro et al., 2022a). Methanogenic redox cofactor F420G, *cofG*, *bicold*, *bcd*, and beta subunit of propionyl-CoA carboxylase, *pccB* (supply of substrates to archaea), ATP-binding cassette subfamily P member, ABCP (microbial communication), tissue-specific transplantation antigen P35B, and *TSTA3* (microbiome-host interaction) are some of the candidate microbial genes linked to CH_4 emissions. A more effective method for investigating the relationship between rumen microbial functions and host performance is the use of meta-transcriptomic data, as it provides a holistic view of real-time fermentation dynamics in the rumen (Li et al., 2019; Kamke et al., 2016). Consequently, CH_4 mitigation in dairy cattle was improved by microbiome-based breeding.

After assembling 4941 microbial genomes from the rumen, Stewart et al. (2019) discovered a large variety of enzymes that break down plant matter in addition to a wide spectrum of digestive proteins. In a related study, Roehe et al. (2016) identified 3970 microbial genes in the rumen using metagenomic analysis, which revealed 20 genes associated with feed conversion efficiency and 49 with CH_4 production. Notably, host genes like *TSTA3* and *FucI*, which are involved in host–microbiome interactions, were linked to feed efficiency, whereas the genes *mcrA* and *fmdB* were linked to CH_4 emissions. These findings suggest that the host animal has significant control over its microbiota, and they emphasise the potential for employing microbial gene abundance to study the genetic relationship between hosts and their microbiomes (Roehe et al., 2016). Similarly, a metagenomic investigation found that cows with high milk protein output (MPY) had a higher abundance of *Prevotella* species and a lower number of methanogens (Aguilar-Marin et al., 2020). Furthermore, these MPY-high cows had greater levels of microbial-originated metabolites such as amino, carboxylic and fatty acids, as well as VFA concentrations. Various -omic investigations found that the rumen microbial composition, function, metabolites, and serum metabolites each contributed 17.81 %, 21.56 %, 29.76 %, and 26.78 % of the host's milk protein output, respectively (Xue et al., 2022). Wallace et al. (2019) reported that a heritable core microbiome played an important role in CH_4 production, rumen and blood metabolites, and milk yield and was a viable target for rumen alteration. Similarly, characteristics such as breed, sex, and nutrition contribute to variances in the rumen microbiome among animals. A heritability estimate (h^2) of 0.15 or above suggests a considerable genetic influence. This heredity was also linked with feed efficiency and rumen VFA levels (Li et al., 2019). High-efficiency animals engage in more and stronger microbial interactions than low-efficiency species. For example, *Selenomonas* and members of the Succinivibrionaceae family showed favourable interactions with high-efficiency animals (Xue et al., 2022).

4.5. Downstream and non-invasive proxies

These include milk fatty acid profiling, faecal archaeol, blood

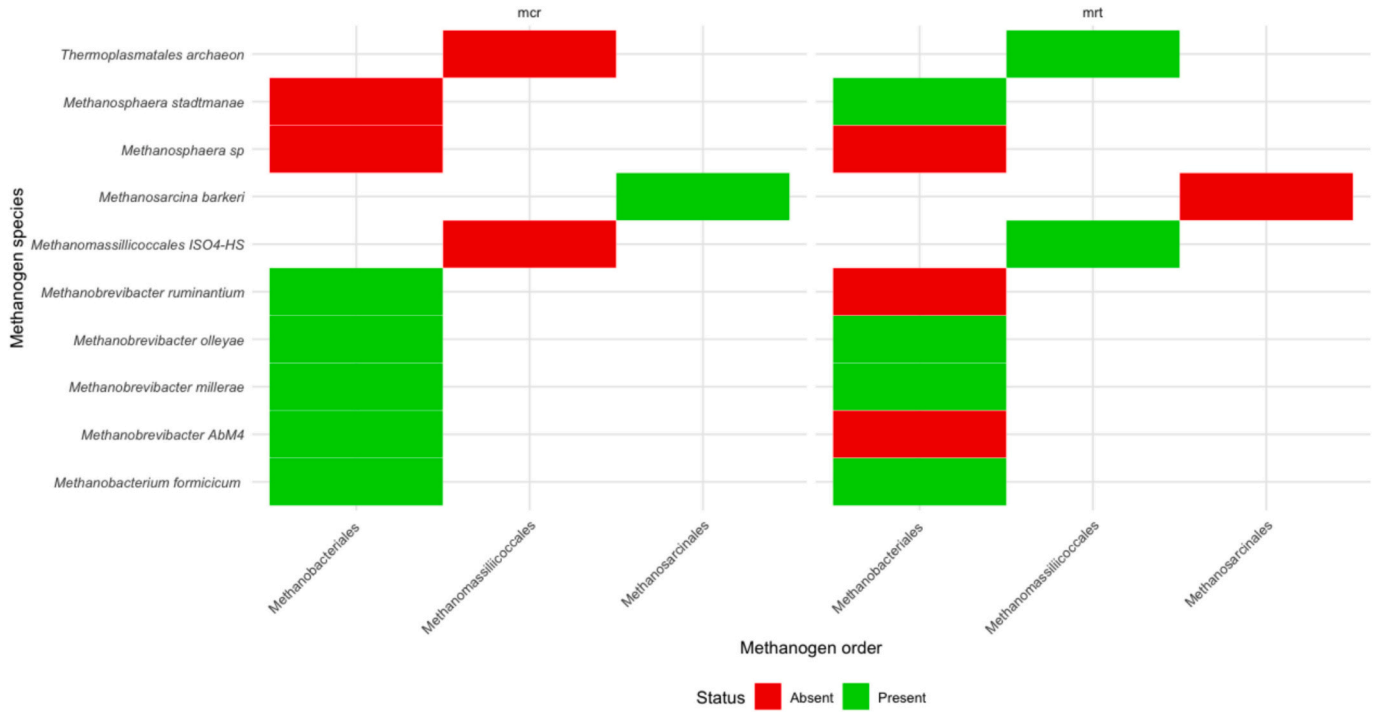

Fig. 8. Methanogenesis genes *mcr* and *mrt* (MCR1 and MCRII isozymes of methyl-CoM reductase) of prominent rumen methanogens belonging to the order Methanobacteriales, Methanomassiliicoccales and Methanosarcinales. The mcr is the primary catalysing enzyme in the order Methanobacteriales and Methanosarcinales, while the enzyme mrt predominates in the order Methanomassiliicoccales. Modified and adapted from: Khairunisa et al. (2023); Leahy et al. (2010). Prepared using R (R Core Team, 2024).

metabolites, urinary purine derivatives, and infrared thermography (IRT), which provide useful tools for large-scale profiling and farm-level CH_4 assessment.

4.5.1. Milk fatty acids

Methane emissions from dairy cows vary depending on their lactation stage (Fresco et al., 2023). Cows have a negative energy balance during early lactation and use their body stores for energy; therefore, the CH_4 produced at this time has no direct relationship to milk production (Wang et al., 2023). The cows' reliance on feed intake for energy, as well as feed intake itself, increases as lactation proceeds, and so do CH_4 emissions. Methane emissions typically rise during the first 20 weeks of lactation (as DMI increases to meet lactation demand, but do both MP and MY), and then plateau between weeks 21 and 50 (Bell et al., 2014). Similarly, CH_4 intensity increases throughout lactation, with primiparous cows emitting more CH_4 than multiparous cows (Fresco et al., 2023).

Fermentation-derived short-chain VFAs serve as the substrate for de novo fatty acid synthesis in the udder (Fig. 4). Milk fatty acids are either synthesised from acetate and 3-beta-hydroxybutyrate (BHBA) in the mammary gland or derived from the circulation (Fig. 4). Short-chain VFAs (C4, C6, and C8), most medium-chain VFAs (C10, C12, and C14), and more than 60 % of C16 FAs are generated de novo. Acetate accounts for around 85 % of this synthesis, with BHBA accounting for 10–15 % (Kyriakaki et al., 2023; Fig. 4). Long-chain fatty acids (C18 and above) are obtained from the bloodstream, either from triglycerides or VLDL, or from non-esterified fatty acids (NEFAs) bound to albumin (Fig. 4). Intermediate compounds generated during biohydrogenation are absorbed in the duodenum and utilised to produce milk fat (Fig. 5). Thus, milk fatty acids (FA) are intimately associated with microbial digestion in the rumen, making them a viable non-invasive indication of rumen fermentation, except for the initial stages of lactation.

Mid-infrared spectroscopy (MIRS) is a reliable method for forecasting milk fat composition and CH_4 emissions (Dehareng et al., 2012). Using MIRS, Dehareng et al. (2012) identified a substantial relationship between milk fatty acid profile and CH_4 emissions in dairy cows. Methane emissions had a significant association with *iso* C14:0 and *iso* C15:0 (Dijkstra et al., 2011), were negatively associated with *trans*-10,11-C18:1, and did not correlate with C15:0 or C17:0 (Dijkstra et al., 2011).

The concentration of OBCFAs in milk fat may indicate rumen fermentation activity (Vlaeminck et al., 2015). For example, propionate levels in the rumen, for example, were positively correlated with milk fat C15:0 and C17:0, whereas acetate showed a negative correlation with these fatty acids but a positive one with isoC14:0 and isoC16:0. Microbial interactions in the rumen affect changes in milk OBCFA levels. Cellulolytic bacteria had a higher connection with OBCFA concentrations than amylolytic bacteria (Zhang et al., 2020a). Cellulolytic bacteria often create more isoOBCFAs, whereas amylolytic bacteria produce more *anteiso*-OBCFAs (Melgar et al., 2020). Xin et al. (2021) found a substantial link between C13:0 levels in milk and the presence of *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Eubacterium ruminantium*. Similarly, Liu et al. (2019) found favourable associations between the amounts of C11:0, isoC15:0, *anteiso*-C15:0, C15:0, and *anteiso*-C17:0 in rumen fluid and milk fat. Furthermore, milk C15:0 was negatively correlated with the amount of isovalerate in the rumen, but total milk OBCFA level was positively correlated with acetate concentration in the rumen.

To accurately use fatty acids as a proxy for CH_4, various confounding factors such as variations due to lactation stage and energy balance need to be considered. For example, milk phenomics have been extensively used to evaluate health status, feed efficiency and negative energy balance in early lactation; thus, connecting these traits with CH_4 traits may improve prediction accuracy. In commercial herds, the lactation stage and parity need to be included in the model while using milk parameters for CH_4 prediction.

4.5.2. Faecal fatty acids and archaeol

Methanogenic archaea have distinct membrane lipids known as dialkyl glycerol diethers (DGDG) and glycerol dialkyl glycerol tetraethers (GDGT), with archaeol and caldarchaeol being the most common forms. In contrast to archaeol, caldarchaeol creates a monolayer that is less permeable to protons, shielding methanogens from ruminal pH decreases (McCartney et al., 2014a). Among these, archaeol (2,3-diphytanyl-O-sn-glycerol) has attracted a lot of attention because of its link to CH_4 synthesis. It can be determined using GC–MS following extraction and purification from the total lipid content (Elayadeth-Meethal et al., 2023b). The archaeol has been found in bovine faeces but not in other herbivores (Gill et al., 2011), and it is largely produced by foregut fermentation, with hindgut fermentation playing a minor role (Gill et al., 2010).

Quantifying archaeol is an alternate way for estimating methanogen abundance (McCartney et al., 2013). McCartney et al. (2014b) used archaeol to locate methanogens in the rumen. They found that the archaeol were significantly more associated with solid-associated microorganisms (SAM) than liquid-associated microbes (LAM), indicating that methanogens may face difficulty surviving in the liquid phase. Furthermore, they reported that excrement contained more archaeol than either SAM or LAM. Faecal archaeol content and CH_4 emission were found to be strongly positively correlated (McCartney et al., 2013). However, Gill et al. (2011) and Schwarm et al. (2015) found only a weak link but showed that faecal archaeol could still predict CH_4 emissions in individual animals. Sandberg et al. (2020) found that cows on a moderate starch and fat diet released less CH_4 and had significantly lower faecal archaeol contents than cows fed a low-starch, low-fat diet.

Although faecal archaeol has emerged as a reliable proxy for CH_4, both rumen (foregut) and caecum (hindgut) fermentation may need to be differentiated. Typically, enteric gas production is differentially regulated in the rumen and caecum through differential microbial fermentation, favouring methanogenesis in the rumen, and acetogenesis in the caecum, facilitated through protobiofilm formation in association with an immune-mediated process (Leng, 2018). As the archaeal composition and activity widely vary with breed and gut location, it is essential to include these factors in the prediction models. Fig. 9 depicts a mechanistic diagram showing the connection between CH_4 emissions and archaeol.

4.5.3. Insulin and other blood metabolites

Methane generation in the rumen is regarded as a loss of energy for the animal. This energy shortfall leads to less availability of blood glucose, pushing the body to draw on its energy stores. To compensate

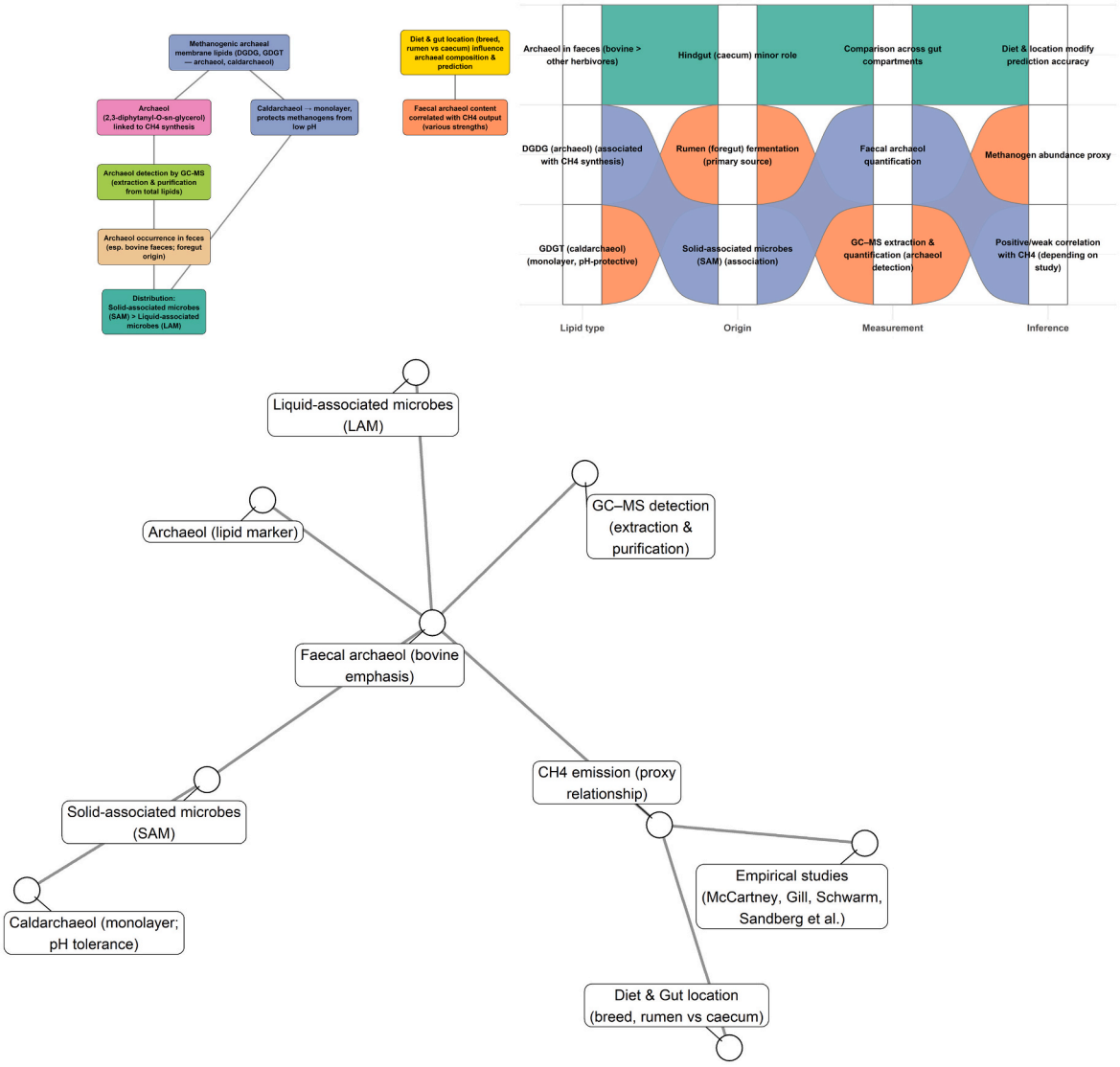

Fig. 9. Mechanistic diagram showing the connection between archaeol and CH_4 emissions. DGDG- dialkyl glycerol diethers, and GDGT-glycerol dialkyl glycerol tetraethers.

for the lost energy, the body begins breaking down amino acids from muscle and other tissues (Kim et al., 2022) (Fig. 10). In this process, insulin regulates gluconeogenesis and the uptake of amino acids. As a result, animals with lower CH_4 emissions exhibit higher glucose-to-insulin ratios and lower insulin levels. Ornelas et al. (2019) reported that, even when fed the same diet and with similar DMI, cows emitting less CH_4 had lower insulin levels than those producing more. Similarly, Kim et al. (2022) reported that insulin levels were considerably greater in high-methane-emitting calves than in low-emitting ones. Similarly, Melgar et al. (2020) found that inhibiting methanogenesis with 3-NOP reduced insulin levels.

In ruminants, the liver maintains energy balance by converting propionic acid into glucose, which is absorbed via the rumen wall (Fig. 10). The liver uses around 80–85 % of the propionate entering the portal vein for gluconeogenesis, underscoring its importance in overall energy control (Dzermeikaite et al., 2024). Given its critical involvement in metabolic processes, liver function is thought to influence both direct and indirect CH_4 generation (Dzermeikaite et al., 2024). Microbial metabolites such as dimethyl sulfone, formic acid, stachydrine (proline-betaine), sarcosine, and trimethylamine were found in significant concentrations in cows treated with an antimethanogenic agent. 3-NOP supplementation lowered arginine and citrulline levels in the blood while boosting serine and 1-methylhistidine levels (Melgar et al., 2020) and methionine levels in calves (Meale et al., 2021). Table 4 summarises the changes in plasma/serum metabolites following antimethanogenic agent administration.

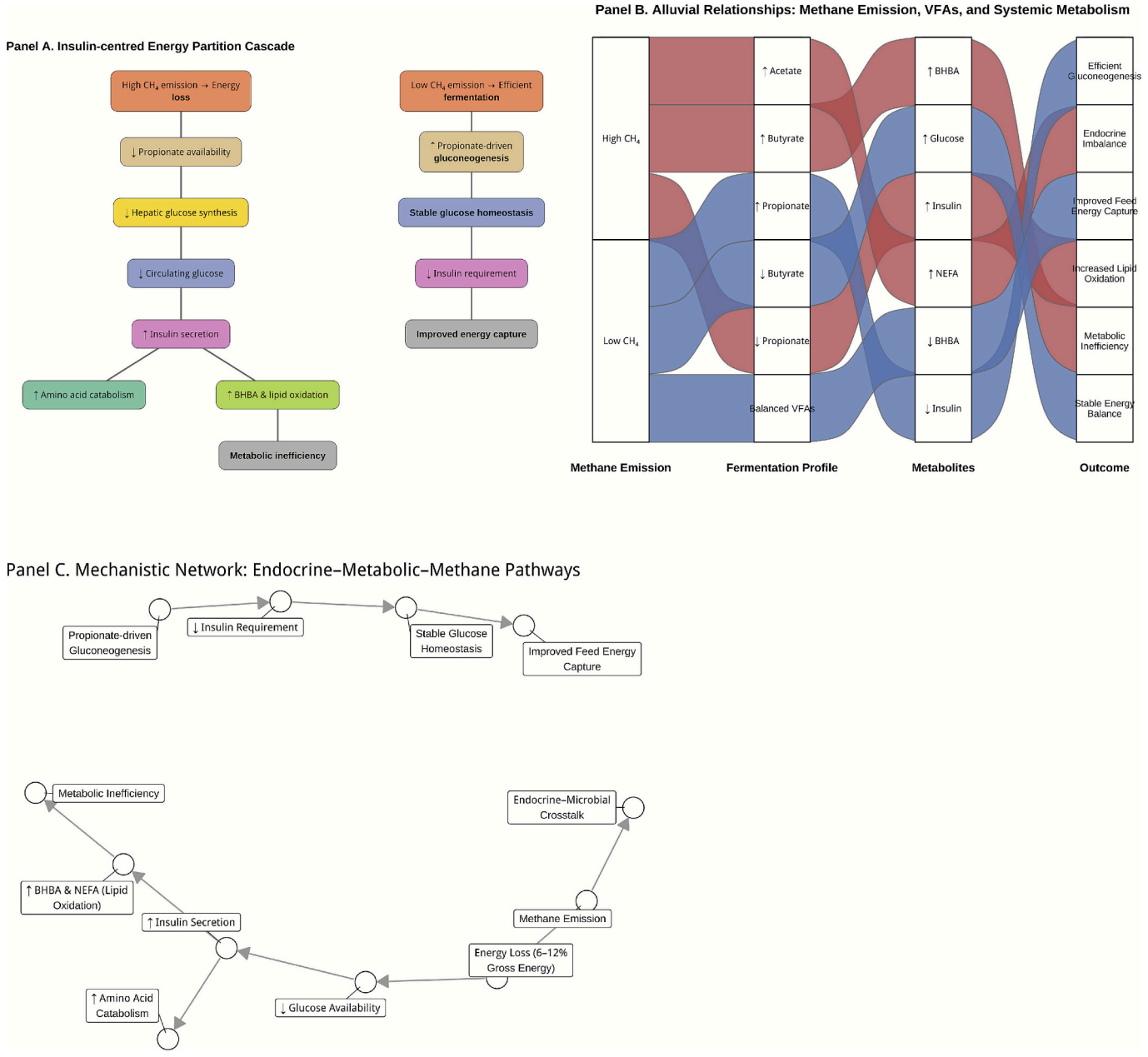

Fig. 10. Blood metabolites associated with a change in rumen methanogenesis. Panel A: Insulin-centred energy partition cascade. In high CH_4 emitters, due to the energy loss, blood glucose level is maintained through gluconeogenesis, characterised by insulin inflow in the blood. Panel B: Alluvial relationships: Methane emission, VFAs, and systemic Metabolism. When butyrate levels increase in the rumen, BHBA acts as the major energy source. Panel C: Mechanistic network: Endocrine-metabolic-methane pathways. A high propionate level in the rumen enhances gluconeogenesis, leading to low insulin requirement and better feed energy capture. On the other hand, high CH_4 emissions result in increased insulin levels, leading to metabolic inefficiency. Adapted and modified from: Kim et al. (2022).

Table 4
Change in plasma/serum metabolite after supplementation with antimethanogenic agent and its effect on rumen fermentation/ CH_4 production.

Treatment	Animal	Change in plasma/serum metabolites	Effect on rumen fermentation/ methane production	Reference
3-NOP	Dairy cow	Lysine, valine, sarcosine, 1-methyl histidine, and serine levels increased. Plasma insulin, arginine, and citrulline levels dropped. No change in urea N, glucose, NEFA, BHB, leptin, and IGF-1.	Decreased daily CH_4 emission by 26 %	Melgar et al. (2020)
Antimethanogenic agent	Dairy cow	Dimethylsulfone, formic acid, stachydrine, glycine, serine, threonine, methionine, and leucine levels have increased, as has trimethyl amine. Plasma acetate, valine, and isoleucine levels had all decreased. Glucose, urea, non-esterified fatty acids, and β-hydroxybutyrate did not change.	Methane emission reduced by 23 %	Yanibada et al. (2020)
	Japanese Black cattle.	High CH_4 emitters had elevated levels of β-hydroxybutyric acid, cysteine, and insulin. Threonine, valine, histidine, lysine, tryptophan, ALP, and aspartate aminotransferase decreased. Cortisol, IGF, and glucose were not changed.	High butyrate production in the rumen	Kim et al. (2022)
Essential oil	Goat	Thymol, *O*-acetyl carnitine, and calcium levels were elevated. Low levels of 2-hydroxyvalerate, alanine, phenylalanine, inorganic phosphate, and sarcosine. Blood urea nitrogen, creatinine, glucose, total cholesterol, total protein, albumin, alanine transaminase/serum glutamic pyruvate transaminase, and aspartate aminotransferase/serum glutamic oxaloacetic transaminase levels remained unchanged.	12.0–13.6 % reduction in CH_4 concentrations in the exhaled gas	Choi et al. (2024)
Gallic acid	Calves	Total protein, BHB, glucose, triglycerides, and catalase increased, whereas malondialdehyde (MDA) decreased. The blood urea nitrogen and cholesterol levels were unchanged.	Improved the rumen fermentation and altered the bacterial community	Xu et al. (2022)
Bacillus licheniformis and *saccharomyces cervisiae*	Lambs	Insulin, growth hormone, IgF-1, IgA, IgG, superoxide dismutase, and glutathione peroxidase levels increased. There is no change in malondialdehyde (MDA).	Low ammonia- nitrogen, Low acetate and high propionate in rumen	Jia et al. (2018)
Probiotics with native ruminal microbes(NRM)	Cattle	Insulin level decreased with no change in glucose level.	Low CH_4 in treated group	Pittaluga et al. (2023)
	Sheep	Increased level of BHB and low NEFA in high CH_4 producers. High Zn in low CH_4 emitters	Found association between metabolic indicators and CH_4 emission	Reintke et al. (2021)
3-NOP	Calves	High methionine	Persistent reduction in CH_4 production throughout the post-weening period	Meale et al. (2021)
Essential oil blend agoline ruminant	Cows	Level of plasma urea nitrogen, phosphate and glucose not affected	16.4 % reduction in CH_4	Batley et al. (2024)
Inclusion of dietary starch,	Dairy cow	Increased level of insulin but glucose not affected	Methane production high in high starch diet	Culbertson et al. (2025)
Sugarcane polyphenol extract	Beef heifer	Level of glucose and urea not affected	Increase in rumen pH, no effect on rumen fermentation or bacterial diversity	Williams et al. (2025)

4.5.4. Urinary purine derivatives

The urinary excretion of purine derivatives (PD) has been used to assess microbial protein supply in ruminants, indirectly giving an indication of CH_4 emissions. Rumen degradable protein (RDP) and rumen undegradable protein (RUP) are the two categories of dietary proteins in ruminants. In the rumen, bacterial enzymes like proteases, peptidases, and deaminases convert RDP into peptides, amino acids, and ammonia (NH_3). After that, this ammonia is converted into microbial crude protein (MCP), which makes up between 50 % and 80 % of the absorbable protein. It passes through the liquid and solid phases of the digesta and is finally absorbed in the colon as amino acids and peptides. For high-producing dairy cows, the migration of microbial protein to the duodenum is an important sign of adequate rumen metabolism. However, monitoring this flow in vivo necessitates intrusive techniques that are costly and can impact dry matter intake and production. In a recent study, it was found that legumes (*Delosperma sutherlandii* and *Gliricidia sepium*) with high degradable protein fraction had higher CH_4 production (Tunkala et al., 2023).

Measuring the urinary excretion of PD, including allantoin, uric acid, hypoxanthine, and xanthine, is a viable alternative to assess CH_4. This non-invasive method has demonstrated promise as a reliable means to estimate microbial nitrogen transport to the duodenum, providing a simpler, less intrusive alternative to monitoring rumen metabolism (da Silva Junior et al., 2021). The assumption is that most of the nucleic acids that exit the rumen come from bacteria. Once these microbial nucleic acids reach the small intestine, they are digested further, resulting in purine nucleosides and free bases that the body can absorb. Purines ingested are metabolised into substances such as hypoxanthine, xanthine, uric acid, and allantoin. In ruminants, xanthine oxidase converts hypoxanthine and xanthine to uric acid, which is then transformed to allantoin by uricase (da Silva Junior et al., 2021).

The quantity of PD excreted is proportional to the amount of purine absorbed. Purine derivative analysis indicates the effect of dietary CP and forage content on microbial protein synthesis, manure N excretion, and emissions of CH_4. Soltan et al. (2013) found that sheep fed with *Leucaena* tannins and mimosin had altered protein degradability in the rumen and reduced CH_4 emissions by 14.1 %. Soltan et al. (2021) observed that lambs fed with low tannin sorghum had higher retained N g/day, increased microbial protein uptake, and reduced CH_4 emission by 29–35 %. Increased digestibility of NDF, ADF, and CP results in lower CH_4 emissions (Pineiro-Vazquez et al., 2017). Similarly, goats given grass hay had lower total VFA and butyrate levels, higher acetate levels, increased allantoin excretion, and lower xanthine and hypoxanthine levels (Carro et al., 2012). This non-invasive technique has the possibility of detecting methane-associated rumen changes, but its proof remains restricted. Most studies were conducted under confined dietary circumstances; thus, additional testing across breeds and production systems is required. A mechanistic diagram illustrating the use of urinary purine derivatives as a proxy for the assessment of CH_4 emissions from ruminants is given in Fig. 11.

4.5.5. Infrared thermography (IRT)

The generation of CH_4 from acetate is endothermic, whereas the reduction of CO_2 to CH_4 is exothermic (Gabbi et al., 2022). Standardised to concentrations of 1 M, pH of 0, gas pressure of 1 bar, adjusted to 312 K, and the average stoichiometric number χ, the Gibbs energy change for

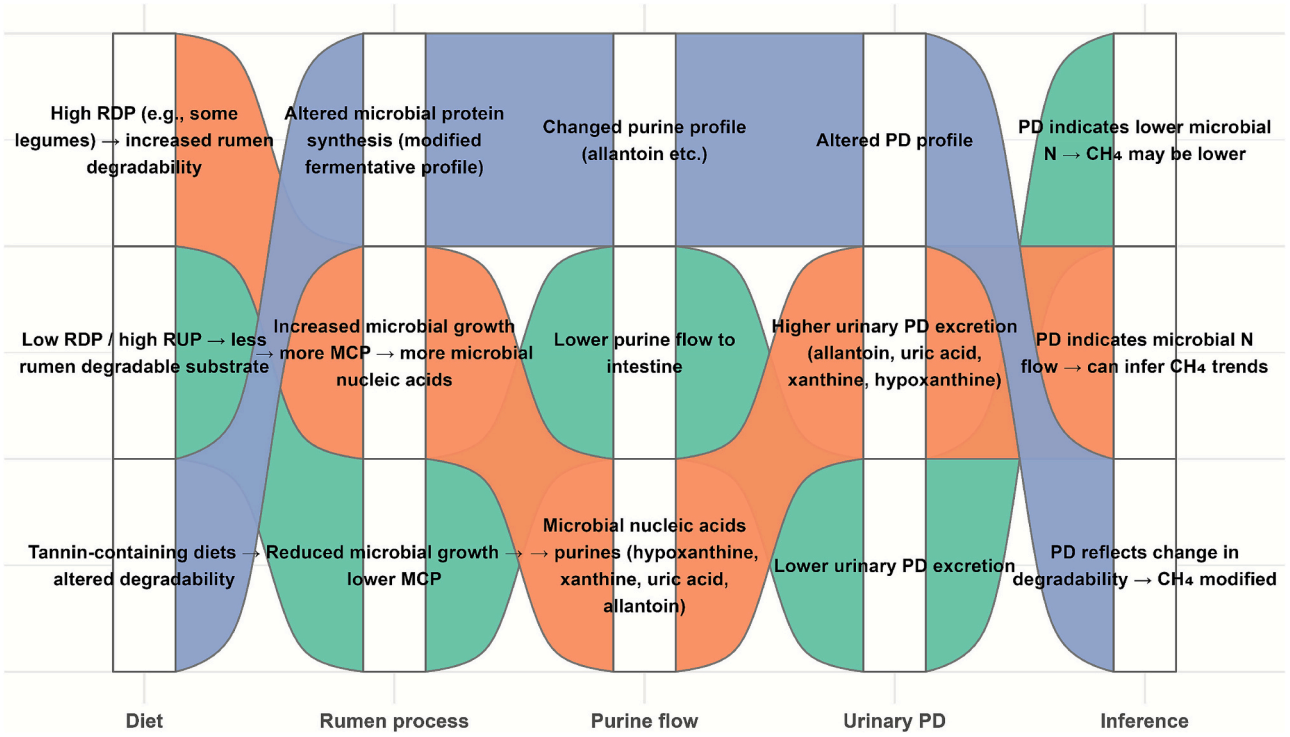

Fig. 11. Mechanistic graphic illustrates the use of urinary purine derivatives as a proxy for estimating ruminant methane emissions. RDP-Rumen degradable protein, RUP-Rumen undegradable protein, MCP-Microbial crude protein, PD-Purine derivatives.

CH_4 generation in the rumen is (−)172 kJ/mol (Van Lingen et al., 2016). The difference in rumen temperature affects body temperature, notably the surface temperature in the flank region. As a result, the difference in temperature between the left (rumen side) and right flanks is utilised to estimate rumen CH_4 production (Gabbi et al., 2022). IRT can be used to measure the heat generated within an animal's body. Montanholi et al.

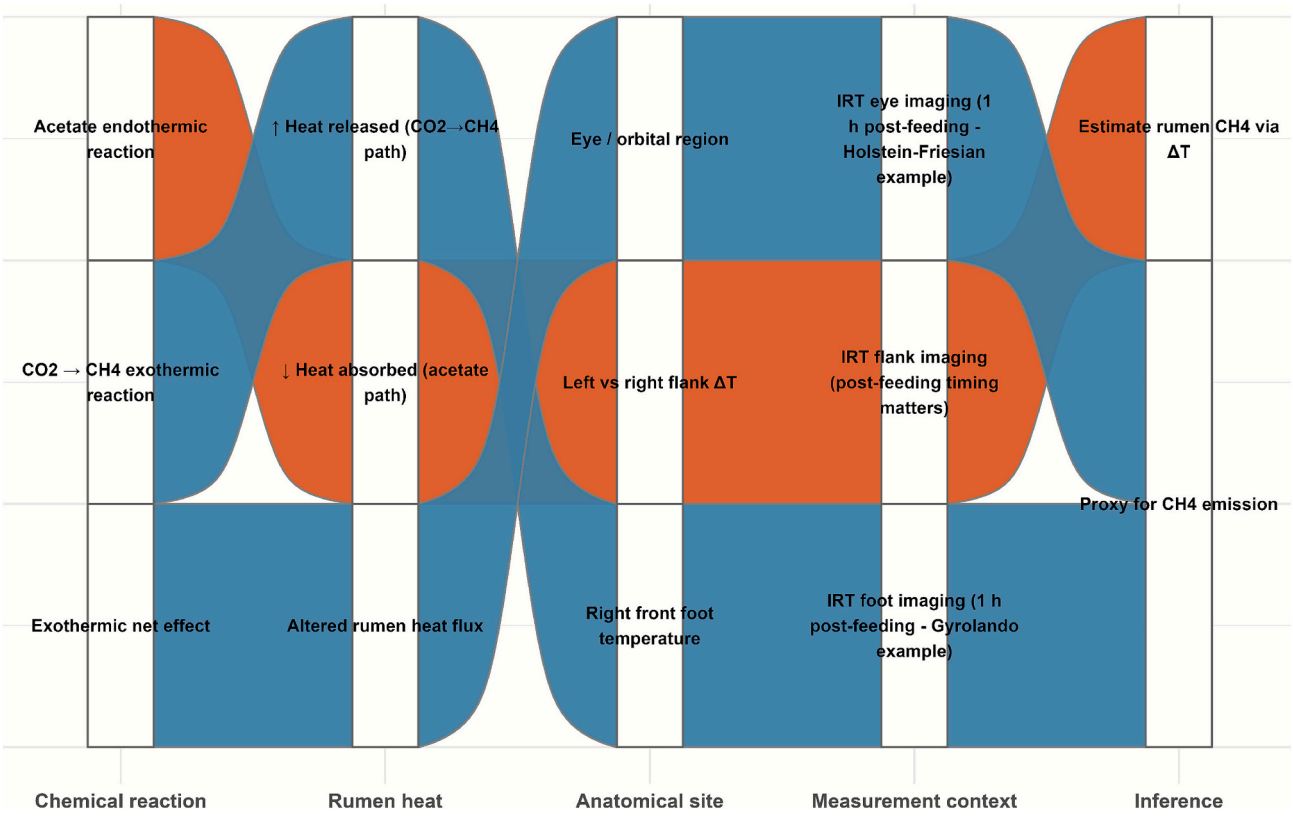

Fig. 12. Mechanistic diagram illustrating the use of infra-red thermography (IRT) as an indirect proxy for assessment of methane emissions from ruminants.

(2008) reported that IRT can identify heat production and CH_4 release by detecting foot temperature and temperature differences between the left and right flanks, respectively. Guadagnin et al. (2023) found that IR was positively related to CH_4 generation in Holstein-Friesian and Gyrolando lactating cows based on anatomical locations measured and IR imaging time. One hour after feeding, right front foot IR imaging in Gyrolando and eye imaging in Holstein-Friesian revealed a high correlation with intestinal CH_4 emission. Similarly, in hairy sheep, IRT correctly predicted CH_4 emission (Crisostomo et al., 2025). Though IRT is a prospective emerging CH_4 proxy, validation remains in the early stages. The majority of positive outcomes so far have come from restricted testing environments and a small number of breeds. Standardised imaging procedures and extensive on-farm research are required before IRT can be deemed a reliable CH_4 forecast tool.

A mechanistic diagram illustrating the use of infra-red thermography (IRT) as an indirect proxy for assessment of CH_4 emissions from ruminants is given in Fig. 12.

The detailed network diagram, showing the direct and indirect CH_4 measurement strategies, mitigation measures, outcomes, and potential proxies, is summarised in Fig. 13.

5. Conclusions and prospects

Methane is a potent greenhouse gas produced as a by-product of rumen fermentation. Developing adaptation and mitigation plans, establishing sector-specific and regional GHG inventories, and assessing the effectiveness of antimethanogenic feed additives all depend on precise CH_4 measurement from animals. However, direct CH_4 monitoring at the individual animal and farm levels is challenging due to the high expense, labour intensity, and technical complexity. Methane proxies include molecules or microorganisms that participate in the CH_4 synthesis pathway, providing a clear indication of CH_4 production in the rumen. Conventional CH_4 measurements are based on static and mechanistic models of methanogenesis to explain only one conceivable scenario. Proxies, on the other hand, can use empirical modelling of dynamic and stochastic processes based on numerous pathways to explain a wide range of methanogenic events. These proxies can be quantified utilising high-throughput omics approaches, which are appropriate for large-scale and long-term CH_4 evaluations across a variety of agricultural systems. Metabolomic investigations have identified compounds involved in methanogenesis. Rumen metagenomics and metatranscriptomics provide information about microbiome diversity and functional state. The transcriptome and epigenetic profiles reveal a host-microbe relationship that leads to alterations in CH_4 emissions. The expression monitoring and assessment of *mcr* and *mrt* genes involved in the latter stages of CH_4 synthesis has also been utilised as a proxy for CH_4 emissions. Several candidate microbes, including archaea, bacteria, and protozoa, have been identified as proxies for various methane-generating pathways in the rumen, such as hydrogenotrophic, methylotrophic, and acetoclastic, as well as alternate H_2 sinks such as propionate, nitrate and sulphate synthesis pathways. Methane proxies, including those derived using 'omics' techniques paired with machine learning, artificial intelligence, and traditional features, can be a viable CH_4 phenotyping tool for large-scale and long-term assessment across multiple livestock production systems in the future. However, several hurdles must be overcome before they can be widely deployed. We need better standardisation of proxy measures across testing facilities and production systems, improved methods for integrating diverse omics information, and widespread evaluation of proxies, particularly novel ones like urine PD and IRT, across several farms and breeds. Resolving these challenges will make it easier to use CH_4 proxies in national inventories, breeding schemes, and precision agriculture.

CRediT authorship contribution statement

Naseema Kolathingal-Thodika: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis,

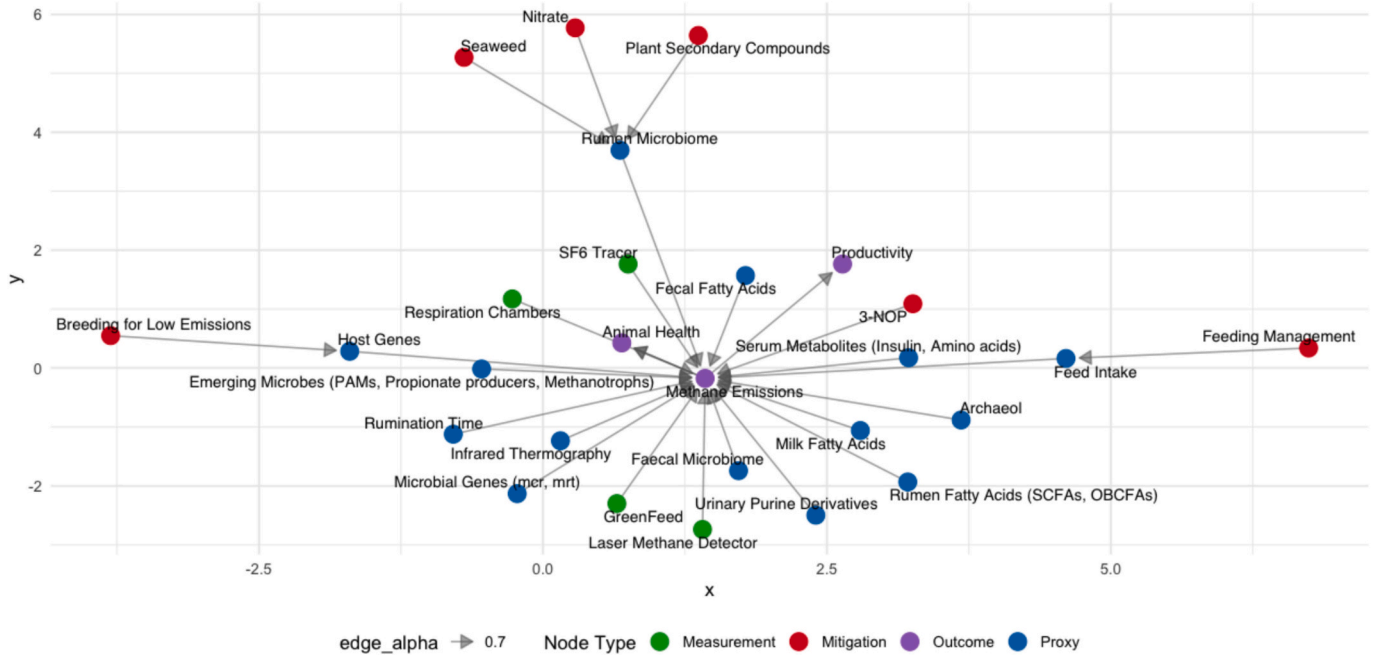

Fig. 13. Force-directed network diagram showing how methane measuring techniques (green), abatement measures (red), intervention outcomes (violet), and possible proxies (blue) relate to one another. The graph illustrates the relationships between several methane proxies, mitigation actions, and the results of direct and indirect measuring techniques. Strong conceptual or methodological ties are reflected in edge thickness, and the degree of relatedness is indicated by node closeness: those that are strongly linked are represented by closely packed nodes, and nodes that are weakly connected are represented by distant nodes. Edge transparency ($\alpha = 0.7$) reflects relative connection intensity. The graphic illustrates how methane proxies function as integrated elements that interconnect the measurement, mitigation, and outcome domains, offering new perspectives on potential emission reduction strategies. R was used to build the network layout algorithmically using Fruchterman-Reingold optimisation (R Core Team, 2024).

Data curation, Conceptualization. **Muhammed Elayadeth-Meethal:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Frank R. Dunshea:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Richard Eckard:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Data curation, Conceptualization. **Matthew Flavel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Conceptualization. **Surinder.S. Chauhan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that we have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Abdillah, A.E., Sarah, D., Ardian, A.A., Anas, M.A., Aprianto, M.A., Hanim, C., Kurniawati, A., Muhlisin, Yusiati, L.M., 2024. Effect of nutmeg essential oil (*Myristica fragrans* Houtt.) on methane production, rumen fermentation, and nutrient digestibility *in vitro*. Sci. Rep. 14, 3554. https://doi.org/10.1038/s41598-024-52532-3.

Aguilar-Marin, S.B., Betancur-Murillo, C.L., Isaza, G.A., Mesa, H., Jovel, J., 2020. Lower methane emissions were associated with higher abundance of ruminal *Prevotella* in a cohort of Colombian buffalos. BMC Microbiol. 20, 1–13. https://doi.org/10.1186/s12866-020-02037-6.

Arowolo, M.A., Zhang, X.M., Wang, M., Wang, R., Wen, J.N., Hao, L.Z., He, J.H., Shen, W.J., Ma, Z.Y., Tan, Z.L., 2022. Proper motility enhances rumen fermentation and microbial protein synthesis with decreased saturation of dissolved gases in rumen simulation technique. J. Dairy Sci. 105, 231–241. https://doi.org/10.3168/jds.2021-20165.

Atashi, H., Lemal, P., Tran, M.N., Gengler, N., 2024. Estimation of genetic parameters and single-step genome-wide association studies for eating time and rumination time in Holstein dairy cows. J. Dairy Sci. 107, 3006–3019. https://doi.org/10.3168/jds.2023-23790.

Auffret, M.D., Stewart, R., Dewhurst, R.J., Duthie, C.A., Rooke, J.A., Wallace, R.J., Freeman, T.C., Snelling, T.J., Watson, M., Roehe, R., 2018. Identification, comparison, and validation of robust rumen microbial biomarkers for methane emissions using diverse *Bos taurus* breeds and basal diets. Front. Microbiol. 8, 2642. https://doi.org/10.3389/fmicb.2017.02642.

Bach, A., Elcoso, G., Escartín, M., Spengler, K., Jouve, A., 2023. Modulation of milking performance, methane emissions, and rumen microbiome on dairy cows by dietary supplementation of a blend of essential oils. Animal 17, 100825. https://doi.org/10.1016/j.animal.2023.100825.

Bai, M., Prathap, P., Elayadeth-Meethal, M., Flavel, M., Eckard, R., Dunshea, F.R., Osei-Amponsah, R., Ashar, M.J., Chen, D., Chauhan, S., 2025. Polyphenol-containing feed additive Polygain™ reduces methane production and intensity from grazing dairy cows measured using an inverse-dispersion technique. Animals 15, 926. https://doi.org/10.3390/ani15070926.

Batley, R.J., Romanzini, E.P., da Silva, K.D., de Souza, W.L., Quigley, S.P., Harper, K.J., Trotter, M.G., Bernardes, P.A., Naiker, M., Costa, D.F., 2024. The essential oil blend Agolin ruminant L reduces methane production in vitro and in vivo when included in the drinking water of cattle. J. Anim. Sci. 102, skae315. https://doi.org/10.1093/jas/skae315.

Beauchemin, K.A., Ungerfeld, E.M., Eckard, R.J., Wang, M., 2020. Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. Animal 14, 2–16. https://doi.org/10.1017/S1751731119003100.

Belanche, A., de la Fuente, G., Newbold, C.J., 2014. Study of methanogen communities associated with different rumen protozoal populations. FEMS Microbiol. Ecol. 90, 663–677. https://doi.org/10.1111/1574-6941.12423.

Bell, M.J., Potterton, S.L., Craigon, J., Saunders, N., Wilcox, R.H., Hunter, M., Goodman, J.R., Garnsworthy, P.C., 2014. Variation in enteric methane emissions among cows on commercial dairy farms. Animal 8, 1540–1546. https://doi.org/10.1017/S1751731114001530.

Bharanidharan, R., Lee, C.H., Thirugnanasambantham, K., Ibidhi, R., Woo, Y.W., Lee, H. G., Kim, J.G., Kim, K.H., 2021. Feeding systems and host breeds influence ruminal fermentation, methane production, microbial diversity and metagenomic gene abundance. Front. Microbiol. 12, 701081. https://doi.org/10.3389/fmicb.2021.701081.

Carro, M.D., Cantalapiedra-Hijar, G., Ranilla, M.J., Molina-Alcaide, E., 2012. Urinary excretion of purine derivatives, microbial protein synthesis, nitrogen use, and ruminal fermentation in sheep and goats fed diets of different quality. J. Anim. Sci. 90, 3963–3972. https://doi.org/10.2527/jas.2011-4577.

Casanas, M.A., Rangkasenee, N., Krattenmacher, N., Thaller, G., Metges, C.C., Kuhla, B., 2015. Methyl-coenzyme M reductase A as an indicator to estimate methane production from dairy cows. J. Dairy Sci. 98, 4074–4083. https://doi.org/10.3168/jds.2015-9310.

Castaneda, A., Indugu, N., Lenker, K., Narayan, K., Rassler, S., Bender, J., Baker, L., Purandare, O., Chai, D., Webb, T., Zhao, X., 2024. Investigating rumination and eating time as proxies for identifying dairy cows with low methane-emitting potential. JDS Commun. 6, 186–191. https://doi.org/10.3168/jdsc.2024-0611.

Castaneda, A., Indugu, N., Lenker, K., Narayan, K., Rassler, S., Bender, J., Baker, L., Purandare, O., Chai, D., Zhao, X., Pitta, D., 2025. Host-specific microbiome-rumination interactions shape methane-yield phenotypes in dairy cattle. mSphere 00090, 25. https://doi.org/10.1128/msphere.00090-25.

Choi, Y., Lee, S.J., Kim, H.S., Eom, J.S., Jo, S.U., Guan, L.L., Seo, J., Kim, H., Lee, S.S., Lee, S.S., 2021. Effects of seaweed extracts on in vitro rumen fermentation characteristics, methane production, and microbial abundance. Sci. Rep. 11, 24092. https://doi.org/10.1038/s41598-021-03356-y.

Choi, Y., Lee, S.J., Kim, H.S., Eom, J.S., Jo, S.U., Guan, L.L., Lee, S.S., 2024. Metataxonomic and metabolomic profiling revealed *Pinus koraiensis* cone essential oil reduced methane emission through affecting ruminal microbial interactions and host-microbial metabolism. Anim. Microbiome 6, 37. https://doi.org/10.1186/s42523-024-00325-4.

Crisostomo, C., Bernardi, R.F., Gurgeira, D.N., Silveira, R.M., Vicentini, R.R., Marquez, S. P., Abdalla, A.L., de Paz, C.C.P., Ferreira, J., da Costa, R.L.D., 2025. Relationship between body temperature measured by infrared thermography and performance, feed efficiency and enteric gas emission of hair lambs. J. Therm. Biol. 127, 104070. https://doi.org/10.1016/j.jtherbio.2025.104070.

Culbertson, R.L., Gutierrez-Oviedo, F.A., Uzun, P., Seneviratne, N., Fontoura, A.B., Yau, B.K., Judge, J.L., Davis, A.N., Reyes, D.C., McFadden, J.W., 2025. Effects of dietary starch concentration on milk production, nutrient digestibility, and methane emissions in mid-lactation dairy cows. Agriculture 15, 211. https://doi.org/10.3390/agriculture15020211.

Dai, X., Kalscheur, K.F., Huhtanen, P., Faciola, A.P., 2022. Effects of ruminal protozoa on methane emissions in ruminants—a meta-analysis. J. Dairy Sci. 105, 7482–7491. https://doi.org/10.3168/jds.2021-21139.

Dehareng, F., Delfosse, C., Froidmont, E., Soyeurt, H., Martin, C., Gengler, N., Vanlierde, A., Dardenne, P., 2012. Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows. Animal 6, 1694–1701. https://doi.org/10.1017/S1751731112000456.

Denman, S.E., Martinez Fernandez, G., Shinkai, T., Mitsumori, M., McSweeney, C.S., 2015. Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analogue. Front. Microbiol. 6, 1087. https://doi.org/10.3389/fmicb.2015.01087.

Difford, G.F., Plichta, D.R., Lovendahl, P., Lassen, J., Noel, S.J., Hojberg, O., Wright, A.D. G., Zhu, Z., Kristensen, L., Nielsen, H.B., Guldbrandtsen, B., 2018. Host genetics and the rumen microbiome jointly associate with methane emissions in dairy cows. PLoS Genet. 14, 1007580. https://doi.org/10.1371/journal.pgen.1007580.

Dijkstra, J., Van Zijderveld, S.M., Apajalahti, J.A., Bannink, A., Gerrits, W.J.J., Newbold, J.R., Perdok, H.B., Berends, H., 2011. Relationships between methane production and milk fatty acid profiles in dairy cattle. Anim. Feed Sci. Technol. 166–167, 590–595. https://doi.org/10.1016/j.anifeedsci.2011.04.042.

Dzermeikaite, K., Kristolaityte, J., Antanaitis, R., 2024. Relationship between dairy cow health and intensity of greenhouse gas emissions. Animals 14, 829. https://doi.org/10.3390/ani14060829.

Elayadeth-Meethal, M., Kolathingal-Thodika, N., 2024. Comparative physiological and biochemical assessment of the heat tolerance of dwarf Vechur, Kasaragod, and standard-size crossbred cattle under humid, hot conditions. Int. J. Biometeorol. 1–15. https://doi.org/10.1007/s00484-024-02845-9.

Elayadeth-Meethal, M., Tiambo, C.K., Naseef, P.P., Kuruniyan, M.S., Maloney, S.K., 2023a. The profile of HSPA1A gene expression and its association with heat tolerance in crossbred cattle and the tropically adapted dwarf Vechur and Kasaragod. J. Thermal Biol. 111, 103426. https://doi.org/10.1016/j.jtherbio.2022.103426.

Elayadeth-Meethal, M., Rivero, M.J., Mead, A., Lee, M.R.F., Misselbrook, T.H., 2023b. Comparison of lipid profiles in the faeces of beef cattle fed three common temperate grass silage diets and their relevance to dietary composition. J. Anim. Feed Sci. 32, 427–437. https://doi.org/10.22358/jafs/166079/2023.

EPA (2025), United States Environmental Protection Agency, https://www.epa.gov/ghgemissions/overview-greenhouse-gases. Accessed on 17th January 2025.
Ferronato, G., Tobanelli, N., Simonetto, A., Gilioli, G., Formigoni, A., 2025. Clustering of feeding strategies to improve the evaluation of enteric and slurry methane emissions in dairy cows: an observational study based on Italian dairy farms. Ital. J. Anim. Sci. 24, 1390–1403. https://doi.org/10.1080/1828051X.2025.2517229.
Filonchyk, M., Peterson, M.P., Zhang, L., Hurynovich, V., He, Y., 2024. Greenhouse gases emissions and global climate change: examining the influence of CO_2, CH_4, and N_2O. Sci. Total Environ. 935, 173359. https://doi.org/10.1016/j.scitotenv.2024.173359.
Foggi, G., Terranova, M., Daghio, M., Amelchanka, S.L., Conte, G., Ineichen, S., Agnolucci, M., Viti, C., Mantino, A., Buccioni, A., Kreuzer, M., 2024. Evaluation of ruminal methane and ammonia formation and microbiota composition as affected by supplements based on mixtures of tannins and essential oils using Rusitec. J Anim. Sci. Biotechnol. 15, 48. https://doi.org/10.1186/s40104-024-01005-8.
Fresco, S., Boichard, D., Fritz, S., Lefebvre, R., Barbey, S., Gaborit, M., Martin, P., 2023. Comparison of methane production, intensity, and yield throughout lactation in Holstein cows. J. Dairy Sci. 106, 4147–4157. https://doi.org/10.3168/jds.2022-22855.
Gabbi, A.M., Kolling, G.J., Fischer, V., Pereira, L.G.R., Tomich, T.R., Machado, F.S., Campos, M.M., Silva, M.V.B.D., Cunha, C.S., Santos, M.K., McManus Pimentel, C.M., 2022. Use of infrared thermography to estimate enteric methane production in dairy heifers. Quant. InfraRed Thermogr. J. 19, 187–195. https://doi.org/10.1080/17686733.2021.1882075.
Giagnoni, G., Lund, P., Johansen, M., Hellwing, A.L.F., Noel, S.J., Thomsen, J.P., Poulsen, N.A., Weisbjerg, M.R., 2024a. Effect of carbohydrate type in silages and concentrates on feed intake, enteric methane, and milk yield from dairy cows. J. Dairy Sci. 107, 7851–7866. https://doi.org/10.1016/j.aninu.2023.10.002.
Giagnoni, G., Friggens, N.C., Johansen, M., Maigaard, M., Wang, W., Lund, P., Weisbjerg, M.R., 2024b. How much can performance measures explain of the between-cow variation in enteric methane? J. Dairy Sci. 107, 4658–4669. https://doi.org/10.3168/jds.2023-24094.
Gill, F.L., Dewhurst, R.J., Dungait, J.A., Evershed, R.P., Ives, L., Li, C.S., Pancost, R.D., Sullivan, M., Bera, S., Bull, I.D., 2010. Archaeol–a biomarker for foregut fermentation in modern and ancient herbivorous mammals? Org. Geochem. 41, 467–472. https://doi.org/10.1016/j.orggeochem.2010.02.001.
Gill, F.L., Dewhurst, R.J., Evershed, R.P., McGeough, E., O'Kiely, P., Pancost, R.D., Bull, I.D., 2011. Analysis of archaeal ether lipids in bovine faeces. Anim. Feed Sci. Technol. 166, 87–92. https://doi.org/10.1016/j.anifeedsci.2011.04.006.
Goopy, J.P., Korir, D., Pelster, D., Ali, A.I.M., Wassie, S.E., Schlecht, E., Dickhoefer, U., Merbold, L., Butterbach-Bahl, K., 2020. Severe below-maintenance feed intake increases methane yield from enteric fermentation in cattle. Br. J. Nutr. 123, 1239–1246. https://doi.org/10.1017/S0007114519003350.
Greening, C., Geier, R., Wang, C., Woods, L.C., Morales, S.E., McDonald, M.J., Rushton-Green, R., Morgan, X.C., Koike, S., Leahy, S.C., Kelly, W.J., 2019. Diverse hydrogen production and consumption pathways influence methane production in ruminants. ISME J. 13, 2617–2632. https://doi.org/10.1038/s41396-019-0464-2.
Guadagnin, A.R., Matiello, J.P., Ribeiro, R.S., Pereira, L.G., Machado, F.S., Tomich, T.R., Campos, M.M., Heisler, G., Fischer, V., 2023. Assessment of heat production and methane emission using infrared thermography in lactating Holstein and gyrolando-F1 (½ Holstein ½ Gyr) crossbreed cows. J. Therm. Biol. 115, 103628. https://doi.org/10.1016/j.jtherbio.2023.103628.
Guo, Y.Q., Liu, J.X., Lu, Y., Zhu, W.Y., Denman, S.E., McSweeney, C.S., 2008. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms. Lett. Appl. Microbiol. 47, 421–426. https://doi.org/10.1111/j.1472-765X.2008.02459.x.
de Haas, Y., Veerkamp, R.F., De Jong, G., Aldridge, M.N., 2021. Selective breeding as a mitigation tool for methane emissions from dairy cattle. Animal 15, 100294. https://doi.org/10.1016/j.animal.2021.100294.
Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Janssen, P.H., 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep. 5, 14567. https://doi.org/10.1038/srep14567.
Hendriks, J., Scholtz, M.M., Neser, F.W.C., 2013. Possible reasons for differences in residual feed intake: an overview. S. Afr. J. Anim. Sci. 43, 107–110. https://doi.org/10.4314/sajas.v43i5.19.
Hook, S.E., Wright, A.D.G., McBride, B.W., 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, 945785. https://doi.org/10.1155/2010/945785.
Hristov, A.N., Oh, J., Giallongo, F., Frederick, T., Weeks, H., Zimmerman, P.R., Harper, M.T., Hristova, R.A., Zimmerman, R.S., Branco, A.F., 2015. The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. J. Vis. Exp. 103, 52904. https://doi.org/10.3791/52904.
Hristov, A.N., Bannink, A., Battelli, M., Belanche, A., Sanz, M.C.C., Fernandez-Turren, G., Garcia, F., Jonker, A., Kenny, D.A., Lind, V., Meale, S.J., 2025. Feed additives for methane mitigation: recommendations for testing enteric methane-mitigating feed additives in ruminant studies. J. Dairy Sci. 108, 322–355. https://doi.org/10.3168/jds.2024-25050.
Jia, P., Cui, K., Ma, T., Wan, F., Wang, W., Yang, D., Wang, Y., Guo, B., Zhao, L., Diao, Q., 2018. Influence of dietary supplementation with Bacillus licheniformis and Saccharomyces cerevisiae as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs. Sci. Rep. 8, 16712. https://doi.org/10.1038/s41598-018-35081-4.
Jiang, L., Dang, Q., Zhao, X., Zhang, C., Tan, X., Yan, Q., 2022. Mechanism of microbial involvement in nitrogen conversion affecting methane production in dry anaerobic digestion. J. Clean. Prod. 369, 133324. https://doi.org/10.1016/j.jclepro.2022.133324.
Johnson, P.L., Hickey, S., Knowler, K., Wing, J., Bryson, B., Hall, M., Jonker, A., Janssen, P.H., Dodds, K.G., McEwan, J.C., Rowe, S.J., 2022. Genetic parameters for residual feed intake, methane emissions, and body composition in New Zealand maternal sheep. Front. Genet. 13, 911639. https://doi.org/10.3389/fgene.2022.911639.
Kalaiyarasi, K., Elayadeth-Meethal, M., 2025. Characterization and identification of novel polymorphisms in the *OR51H1* gene associated with resistance/tolerance to natural *Theileria* infection in Vechur (*Bos indicus*) and crossbred (*B. taurus*× *B. indicus*) cattle in a *Theileria*-endemic region. Vet. Parasitol. Reg. Stud. Reports, 101242. https://doi.org/10.1016/j.vprsr.2025.101242.
Kamke, J., Kittelmann, S., Soni, P., Li, Y., Tavendale, M., Ganesh, S., Janssen, P.H., Shi, W., Froula, J., Rubin, E.M., Attwood, G.T., 2016. Rumen metagenome and metatranscriptome analyses of low methane yield sheep reveals a *Sharpea*-enriched microbiome characterised by lactic acid formation and utilisation. Microbiome 4, 1–16. https://doi.org/10.1186/s40168-016-0201-2.
Khairunisa, B.H., Heryakusuma, C., Ike, K., Mukhopadhyay, B., Susanti, D., 2023. Evolving understanding of rumen methanogen ecophysiology. Front. Microbiol. 14, 1296008. https://doi.org/10.3389/fmicb.2023.1296008.
Kim, M., Masaki, T., Ikuta, K., Iwamoto, E., Nishihara, K., Hirai, M., Uemoto, Y., Terada, F., Roh, S., 2022. Physiological responses and adaptations to high methane production in Japanese black cattle. Sci. Rep. 12, 11154. https://doi.org/10.1038/s41598-022-15146-1.
Kittelmann, S., Pinares-Patino, C.S., Seedorf, H., Kirk, M.R., Ganesh, S., McEwan, J.C., Janssen, P.H., 2014. Two different bacterial community types are linked with the low-methane emission trait in sheep. PLoS One 9, 103171. https://doi.org/10.1371/journal.pone.0103171.
Kolathingal-Thodika, N., Elayadeth-Meethal, M., Dunshea, F.R., Eckard, R., Flavel, M., Chauhan, S.S., 2025. Is early life programming a promising strategy for methane mitigation and sustainable intensification in ruminants? Sci. Total Environ. 982, 179654. https://doi.org/10.1016/j.scitotenv.2025.179654.
Kupczynski, R., Pacyga, K., Lewandowska, K., Bednarski, M., Szumny, A., 2024. Milk odd-and branched-chain fatty acids as biomarkers of rumen fermentation. Animals 14, 1706. https://doi.org/10.3390/ani14111706.
Kyriakaki, P., Zisis, F., Pappas, A.C., Mavrommatis, A., Tsiplakou, E., 2023. Effects of PUFA-rich dietary strategies on ruminants' mammary gland gene network: a nutrigenomics review. Metabolites 13, 44. https://doi.org/10.3390/metabo13010044.
Leahy, S.C., Kelly, W.J., Altermann, E., Ronimus, R.S., Yeoman, C.J., Pacheco, D.M., Li, D., Kong, Z., McTavish, S., Sang, C., Lambie, S.C., 2010. The genome sequence of the rumen methanogen Methanobrevibacter ruminantium reveals new possibilities for controlling ruminant methane emissions. PLoS One 5, 8926. https://doi.org/10.1371/journal.pone.0008926.
Leng, R.A., 2018. Unravelling methanogenesis in ruminants, horses and kangaroos: the links between gut anatomy, microbial biofilms and host immunity. Anim. Prod. Sci. 58, 1175–1191.
Li, F., Hitch, T.C., Chen, Y., Creevey, C.J., Guan, L.L., 2019. Comparative metagenomic and metatranscriptomic analyses reveal the breed effect on the rumen microbiome and its associations with feed efficiency in beef cattle. Microbiome 7, 1–21. https://doi.org/10.1186/s40168-019-0618-5.
Li, Q., Ma, Z., Huo, J., Zhang, X., Wang, R., Zhang, S., Jiao, J., Dong, X., Janssen, P.H., Ungerfeld, E.M., Greening, C., 2024. Distinct microbial hydrogen and reductant disposal pathways explain interbreed variations in ruminant methane yield. ISME J. 18, 016. https://doi.org/10.1093/ismejo/wrad016.
Liu, K., Li, Y., Luo, G., Xin, H., Zhang, Y., Li, G., 2019. Relations of ruminal fermentation parameters and microbial matters to odd-and branched-chain fatty acids in rumen fluid of dairy cows at different milk stages. Animals 9, 1019. https://doi.org/10.3390/ani9121019.
Lopes, L.S.F., Schenkel, F.S., Houlahan, K., Rochus, C.M., Oliveira Jr., G.A., Oliveira, H. R., Miglior, F., Alcantara, L.M., Tulpan, D., Baes, C.F., 2024. Estimates of genetic parameters for rumination time, feed efficiency, and methane production traits in first lactation Holstein cows. J. Dairy Sci. 107, 4704–4713. https://doi.org/10.3168/jds.2023-23751.
Lopes, L.S.F., Fonseca, P.A.S., Makanjuola, B.O., Miglior, F., Tulpan, D., Baes, C.F., Schenkel, F.S., 2025. A genome-wide association study on rumination time in first-lactation dairy cattle. J. Dairy Sci. 108, 7297–7309. https://doi.org/10.3168/jds.2024-26054.
Manjutha, S., Elayadeth-Meethal, M., Liz Abraham, B., Asaf, M., Senthil Murugan, S., Radhika, G., 2023. Screening of InDel variants in PRDM6, myostatin and IGF2BP1 genes and association analysis with body measurement traits in Malabari and Attappadi black goats. Anim. Biotechnol. 34, 4760–4774. https://doi.org/10.1080/10495398.2023.2189916.
Martinez-Alvaro, M., Auffret, M.D., Stewart, R.D., Dewhurst, R.J., Duthie, C.A., Rooke, J. A., Wallace, R.J., Shih, B., Freeman, T.C., Watson, M., Roehe, R., 2020. Identification of complex rumen microbiome interaction within diverse functional niches as mechanisms affecting the variation of methane emissions in bovine. Front. Microbiol. 11, 659. https://doi.org/10.3389/fmicb.2020.00659.
Martinez-Alvaro, M., Auffret, M.D., Duthie, C.A., Dewhurst, R.J., Cleveland, M.A., Watson, M., Roehe, R., 2022a. Bovine host genome acts on rumen microbiome function linked to methane emissions. Commun. Biol. 5, 350. https://doi.org/10.1038/s42003-022-03293-0.
Martinez-Alvaro, M., Mattock, J., Auffret, M., Weng, Z., Duthie, C.A., Dewhurst, R.J., Cleveland, M.A., Watson, M., Roehe, R., 2022b. Microbiome-driven breeding strategy potentially improves beef fatty acid profile benefiting human health and

reduces methane emissions. Microbiome 10, 166. https://doi.org/10.1186/s40168-022-01352-6.
Martinez-Fernandez, G., Denman, S.E., Yang, C., Cheung, J., Mitsumori, M., McSweeney, C.S., 2016. Methane inhibition alters the microbial community, hydrogen flow, and fermentation response in the rumen of cattle. Front. Microbiol. 7, 1122. https://doi.org/10.3389/fmicb.2016.01122.
McCartney, C.A., Bull, I.D., Yan, T., Dewhurst, R.J., 2013. Assessment of archaeol as a molecular proxy for methane production in cattle. J. Dairy Sci. 96, 1211–1217. https://doi.org/10.3168/jds.2012-6042.
McCartney, C.A., Dewhurst, R.J., Bull, I.D., 2014a. Changes in the ratio of tetraether to diether lipids in cattle faeces in response to altered dietary ratio of grass silage and concentrates. J. Anim. Sci. 92, 4095–4098. https://doi.org/10.2527/jas.2014-7929.
McCartney, C.A., Bull, I.D., Dewhurst, R.J., 2014b. Using archaeol to investigate the location of methanogens in the ruminant digestive tract. Livest. Sci. 164, 39–45. https://doi.org/10.1016/j.livsci.2014.02.020.
Meale, S.J., Popova, M., Saro, C., Martin, C., Bernard, A., Lagree, M., Yanez-Ruiz, D.R., Boudra, H., Duval, S., Morgavi, D.P., 2021. Early life dietary intervention in dairy calves results in a long-term reduction in methane emissions. Sci. Rep. 11, 3003. https://doi.org/10.1038/s41598-021-82084-9.
Melgar, A., Harper, M.T., Oh, J., Giallongo, F., Young, M.E., Ott, T.L., Duval, S., Hristov, A.N., 2020. Effects of 3-nitrooxypropanol on rumen fermentation, lactational performance, and resumption of ovarian cyclicity in dairy cows. J. Dairy Sci. 103, 410–432. https://doi.org/10.3168/jds.2019-17085.
Meo-Filho, P., Hood, J., Lee, M.R.F., Fleming, H., Meethal, M.E., Misselbrook, T., 2023. Performance and enteric methane emissions from housed beef cattle fed silage produced on pastures with different forage profiles. Animal, 100726. https://doi.org/10.1016/j.animal.2023.100726.
Mikuła, R., Pszczola, M., Rzewuska, K., Mucha, S., Nowak, W., Strabel, T., 2021. The effect of rumination time on milk performance and methane emission of dairy cows fed partial mixed ration based on maize silage. Animals 12, 50. https://doi.org/10.3390/ani12010050.
Mitsumori, M., Ajisaka, N., Tajima, K., Kajikawa, H., Kurihara, M., 2002. Detection of Proteobacteria from the rumen by PCR using methanotroph-specific primers. Lett. Appl. Microbiol. 35, 251–255. https://doi.org/10.1046/j.1472-765x.2002.01172.x.
Montanholi, Y.R., Odongo, N.E., Swanson, K.C., Schenkel, F.S., McBride, B.W., Miller, S. P., 2008. Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (*Bos taurus*). J. Therm. Biol. 33, 468–475. https://doi.org/10.1016/j.jtherbio.2008.09.001.
Newbold, C.J., De La Fuente, G., Belanche, A., Ramos-Morales, E., McEwan, N.R., 2015. The role of ciliate protozoa in the rumen. Front. Microbiol. 6, 1313. https://doi.org/10.3389/fmicb.2015.01313.
Ornelas, L.T.C., Silva, D.C., Tomich, T.R., Campos, M.M., Machado, F.S., Ferreira, A.L., Mauricio, R.M., Pereira, L.G.R., 2019. Differences in methane production, yield and intensity and its effects on metabolism of dairy heifers. Sci. Total Environ. 689, 1133–1140. https://doi.org/10.1016/j.scitotenv.2019.06.489.
Parmar, N.R., Nirmal Kumar, J.I., Joshi, C.G., 2015. Exploring diet-dependent shifts in methanogen and methanotroph diversity in the rumen of Mehsani buffalo by a metagenomics approach. Front. Life Sci. 8, 371–378. https://doi.org/10.1080/21553769.2015.1063550.
Patra, A.K., Puchala, R., 2023. Methane mitigation in ruminants with structural analogues and other chemical compounds targeting archaeal methanogenesis pathways. Biotechnol. Adv. 69, 108268. https://doi.org/10.1016/j.biotechadv.2023.108268.
Patra, A.K., dos Santos Ribeiro, L.P., Yirga, H., Sonibare, A.O., Askar, A.R., Hussein, A.H., Puchala, R., Goetsch, A.L., 2024. Effects of the concentration and nature of total dissolved solids in drinking water on feed intake, nutrient digestion, energy balance, methane emission, ruminal fermentation, and blood constituents in different breeds of young goats and hair sheep. Anim. Nutr. 16, 84–95. https://doi.org/10.1016/j.aninu.2023.10.002.
Pineiro-Vazquez, A.T., Jimenez-Ferrer, G.O., Chay-Canul, A.J., Casanova-Lugo, F., Diaz-Echeverria, V.F., Ayala-Burgos, A.J., Solorio-Sanchez, F.J., Aguilar-Perez, C.F., Ku-Vera, J.C., 2017. Intake, digestibility, nitrogen balance and energy utilization in heifers fed low-quality forage and Leucaena leucocephala. Anim. Feed Sci. Technol. 228, 194–201. https://doi.org/10.1016/j.anifeedsci.2017.04.009.
Pitta, D.W., Indugu, N., Melgar, A., Hristov, A., Challa, K., Vecchiarelli, B., Hennessy, M., Narayan, K., Duval, S., Kindermann, M., Walker, N., 2022. The effect of 3-nitrooxypropanol, a potent methane inhibitor, on ruminal microbial gene expression profiles in dairy cows. Microbiome 10, 146. https://doi.org/10.1186/s40168-022-01341-9.
Pittaluga, A.M., Yang, F., Gaffney, J.R., Embree, M., Relling, A.E., 2023. Effect of supplementation with ruminal probiotics on growth performance, carcass characteristics, plasma metabolites, methane emissions, and the associated rumen microbiome changes in beef cattle. J. Anim. Sci. 101, 308. https://doi.org/10.1093/jas/skac308.
Prathap, P., Chauhan, S.S., Leury, B.J., Cottrell, J.J., Dunshea, F.R., 2021. Towards sustainable livestock production: estimation of methane emissions and dietary interventions for mitigation. Sustainability 13, 6081. https://doi.org/10.3390/su13116081.
Prathap, P., Chauhan, S.S., Flavel, M., Mitchell, S., Cottrell, J.J., Leury, B.J., Dunshea, F. R., 2024. Effects of sugarcane-derived polyphenol supplementation on methane production and rumen microbial diversity of second-cross lambs. Animals 14, 905. https://doi.org/10.3390/ani14060905.
R Core Team, 2024. R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Ramayo-Caldas, Y., Zingaretti, L., Popova, M., Estelle, J., Bernard, A., Pons, N., Bellot, P., Mach, N., Rau, A., Roume, H., Perez-Enciso, M., 2020. Identification of rumen microbial biomarkers linked to methane emission in Holstein dairy cows. J. Anim. Breed. Genet. 137, 49–59. https://doi.org/10.1111/jbg.12427.
Ranilla, M.J., Jouany, J.P., Morgavi, D.P., 2007. Methane production and substrate degradation by rumen microbial communities containing single protozoal species in vitro. Lett. Appl. Microbiol. 45, 675–680. https://doi.org/10.1111/j.1472-765X.2007.02251.x.
Reintke, J., Brugemann, K., Yin, T., Wagner, H., Wehrend, A., Muller, A., Konig, S., 2021. Associations between minerals and metabolic indicators in maternal blood pre-and postpartum with ewe body condition, methane emissions, and lamb body weight development. Animal 15, 100034. https://doi.org/10.1016/j.animal.2020.100034.
Reisinger, A., Clark, H., Cowie, A.L., Emmet-Booth, J., Gonzalez Fischer, C., Herrero, M., Howden, M., Leahy, S., 2021. How necessary and feasible are reductions of methane emissions from livestock to support stringent temperature goals? Phil. Trans. R. Soc. A 379, 20200452. https://doi.org/10.1098/rsta.2020.0452.
Roehe, R., Dewhurst, R.J., Duthie, C.A., Rooke, J.A., McKain, N., Ross, D.W., Hyslop, J.J., Waterhouse, A., Freeman, T.C., Watson, M., Wallace, R.J., 2016. Bovine host genetic variation influences rumen microbial methane production, with best selection criterion for low methane-emitting and efficiently feed-converting hosts based on metagenomic gene abundance. PLoS Genet. 12, 1005846. https://doi.org/10.1371/journal.pgen.1005846.
Roque, B.M., Salwen, J.K., Kinley, R., Kebreab, E., 2019. Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 per cent. J. Clean. Prod. 234, 132–138. https://doi.org/10.1016/j.jclepro.2019.06.193.
Roque, B.M., Venegas, M., Kinley, R.D., de Nys, R., Duarte, T.L., Yang, X., Kebreab, E., 2021. Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 per cent in beef steers. PLoS One 16, 0247820. https://doi.org/10.1371/journal.pone.0247820.
Roskam, E., Kenny, D.A., Kelly, A.K., Hayes, M., Palevich, N., Maclean, P.H., O'Flaherty, V., Biswas, A., Waters, S.M., 2025. Effects of dietary supplementation with linseed oil, *Ascophyllum nodosum* or treated a. nodosum on animal performance, gaseous emissions, ruminal fermentation and microbiota, and meat quality in growing dairy beef bulls. J. Anim. Sci. 32. https://doi.org/10.1093/jas/skaf032.
Sandberg, L.M., Thaller, G., Gors, S., Kuhla, B., Metges, C.C., Krattenmacher, N., 2020. The relationship between methane emission and daytime-dependent faecal archaeol concentration in lactating dairy cows fed two different diets. Arch. Anim. Breed 63, 211–218. https://doi.org/10.5194/aab-63-211-2020.
Schwarm, A., Schweigel-Rontgen, M., Kreuzer, M., Ortmann, S., Gill, F., Kuhla, B., Meyer, U., Loholter, M., Derno, M., 2015. Methane emission, digestive characteristics and faecal archaeol in heifers fed diets based on silage from brown midrib maize as compared to conventional maize. Arch. Anim. Nutr. 69, 159–176. https://doi.org/10.1080/1745039X.2015.1043211.
Sepulveda, B.J., Muir, S.K., Bolormaa, S., Knight, M.I., Behrendt, R., MacLeod, I.M., Pryce, J.E., Daetwyler, H.D., 2022. Eating time as a genetic indicator of methane emissions and feed efficiency in Australian maternal composite sheep. Front. Genet. 13, 883520. https://doi.org/10.3389/fgene.2022.883520.
Shabat, S.K.B., Sasson, G., Doron-Faigenboim, A., Durman, T., Yaacoby, S., Berg Miller, M.E., White, B.A., Shterzer, N., Mizrahi, I., 2016. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J. 10, 2958–2972. https://doi.org/10.1038/ismej.2016.62.
Shi, W., Moon, C.D., Leahy, S.C., Kang, D., Froula, J., Kittelmann, S., Fan, C., Deutsch, S., Gagic, D., Seedorf, H., Kelly, W.J., 2014. Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. Genome Res. 24, 1517–1525. https://doi.org/10.1101/gr.168245.113.
da Silva Junior, J.M., Rodrigues, J.P.P., Valadares Filho, S.D.C., Detmann, E., Paulino, M. F., Renno, L.N., 2021. Estimating purine derivatives and nitrogen compound excretion using total urine collection or spot urine samples in grazing heifers. J. Anim. Physiol. Anim. Nutr. 105, 861–873. https://doi.org/10.1111/jpn.13525.
Singh, S., Hundal, J.S., Patra, A.K., Sethi, R.S., Sharma, A., 2022. A composite polyphenol-rich extract improved growth performance, ruminal fermentation and immunity, while decreasing methanogenesis and excretion of nitrogen and phosphorus in growing buffaloes. Environ. Sci. Pollut. Res. Int. 1–17. https://doi.org/10.1007/s11356-021-17674-1.
Smith, P.E., Waters, S.M., Kenny, D.A., Kirwan, S.F., Conroy, S., Kelly, A.K., 2021. Effect of divergence in residual methane emissions on feed intake and efficiency, growth and carcass performance, and indices of rumen fermentation and methane emissions in finishing beef cattle. J. Anim. Sci. 99, 275. https://doi.org/10.1093/jas/skab275.
Smith, P.E., Kelly, A.K., Kenny, D.A., Waters, S.M., 2022. Enteric methane research and mitigation strategies for pastoral-based beef cattle production systems. Front. Vet Sci. 9, 958340. https://doi.org/10.3389/fvets.2022.958340.
Soltan, Y.A., Morsy, A.S., Sallam, S.M., Lucas, R.C., Louvandini, H., Kreuzer, M., Abdalla, A.L., 2013. Contribution of condensed tannins and mimosine to the methane mitigation caused by feeding *Leucaena leucocephala*. Arch. Anim. Nutr. 67, 169–184. https://doi.org/10.1080/1745039X.2013.801139.
Soltan, Y., Abdalla Filho, A., Abdalla, A., Berenchtein, B., Schiavinatto, P., Costa, C., 2021. Replacing maize with low tannin sorghum grains: lamb growth performance, microbial protein synthesis and enteric methane production. Anim. Prod. Sci. 61, 1348–1355. https://doi.org/10.1071/AN20605.
Starsmore, K., Lopez-Villalobos, N., Shalloo, L., Egan, M., Burke, J., Lahart, B., 2024. Animal factors that affect enteric methane production measured using the GreenFeed monitoring system in grazing dairy cows. J. Dairy Sci. 107, 2930–2940. https://doi.org/10.3168/jds.2023-23915.
Stewart, R.D., Auffret, M.D., Warr, A., Walker, A.W., Roehe, R., Watson, M., 2019. Compendium of 4,941 rumen metagenome-assembled genomes for rumen

microbiome biology and enzyme discovery. Nat. Biotechnol. 37, 953–961. https://doi.org/10.1038/s41587-019-0202-3.

Suriyapha, C., Phupaboon, S., Dagaew, G., Sommai, S., Matra, M., Prachumchai, R., Haitook, T., Wanapat, M., 2024. In vitro fermentation end-products and rumen microbiome as influenced by microencapsulated phytonutrient pellets (LEDRAGON) supplementation. Sci. Rep. 14, 14425. https://doi.org/10.1038/s41598-024-59697-.

Tapio, I., Snelling, T.J., Strozzi, F., Wallace, R.J., 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. J. Anim. Sci. Biotechnol. 8, 1–11. https://doi.org/10.1186/s40104-017-0141-0.

Toral, P.G., Hervas, G., Frutos, P., 2024. Invited review: research on ruminal biohydrogenation: achievements, gaps in knowledge, and future approaches from the perspective of dairy science. J. Dairy Sci. 107, 10115–10140. https://doi.org/10.3168/jds.2023-24591.

Tseten, T., Sanjorjo, R.A., Son, J.W., Baik, K.S., Berdos, J.I., Kim, S.H., Yoon, S.H., Kang, M.K., Kwon, M., Lee, S.S., Kim, S.W., 2025. Reduction of enteric methane emission using methanotroph-based probiotics in Hanwoo steers. Anim. Microbiome 7, 19. https://doi.org/10.1186/s42523-025-00385-0.

Tunkala, B.Z., DiGiacomo, K., Hess, P.S.A., Gardiner, C.P., Suleria, H., Leury, B.J., Dunshea, F.R., 2023. Evaluation of legumes for fermentability and protein fractions using in vitro rumen fermentation. Anim. Feed Sci. Technol. 305, 115777. https://doi.org/10.1016/j.anifeedsci.2023.115777.

Ungerfeld, E.M., 2020. Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. Front. Microbiol. 11, 589. https://doi.org/10.3389/fmicb.2020.00589.

Van Lingen, H.J., Plugge, C.M., Fadel, J.G., Kebreab, E., Bannink, A., Dijkstra, J., 2016. Thermodynamic driving force of hydrogen on rumen microbial metabolism: a theoretical investigation. PLoS One 11, 0161362. https://doi.org/10.1371/journal.pone.0161362.

Vlaeminck, B., Gervais, R., Rahman, M.M., Gadeyne, F., Gorniak, M., Doreau, M., Fievez, V., 2015. Postruminal synthesis modifies the odd- and branched-chain fatty acid profile from the duodenum to milk. J. Dairy Sci. 98, 4829–4840. https://doi.org/10.3168/jds.2014-9207.

Wallace, R.J., Rooke, J.A., McKain, N., Duthie, C.-A., Hyslop, J.J., Ross, D.W., 2015. The rumen microbial metagenome associated with high methane production in cattle. BMC Genomics 16, 839. https://doi.org/10.1186/s12864-015-2032-0.

Wallace, R.J., Sasson, G., Garnsworthy, P.C., Tapio, I., Gregson, E., Bani, P., Huhtanen, P., Bayat, A.R., Strozzi, F., Biscarini, F., Snelling, T.J., 2019. A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. Sci. Adv. 5, 8391. https://doi.org/10.1126/sciadv.aav8391.

Wang, M., Ungerfeld, E.M., Wang, R., Zhou, C.S., Basang, Z.Z., Ao, S.M., Tan, Z.L., 2016. Supersaturation of dissolved hydrogen and methane in rumen of Tibetan sheep. Front. Microbiol. 7, 850. https://doi.org/10.3389/fmicb.2016.00850.

Wang, Y., Yu, S., Li, Y., Zhang, S., Qi, X., Guo, K., Guo, Y., Fortina, R., 2021. Pilot study of the effects of polyphenols from chestnut involucre on methane production, volatile fatty acids, and ammonia concentration during in vitro rumen fermentation. Animals 11, 108. https://doi.org/10.3390/ani11010108.

Wang, K., Xiong, B., Zhao, X., 2023. Could propionate formation be used to reduce enteric methane emission in ruminants? Sci. Total Environ. 855, 158867. https://doi.org/10.1016/j.scitotenv.2022.158867.

Wasson, D.E., Stefenoni, H., Cueva, S.F., Lage, C., Raisanen, S.E., Melgar, A., Fetter, M., Hennessy, M., Narayan, K., Indugu, N., Pitta, D., 2023. Screening macroalgae for mitigation of enteric methane in vitro. Sci. Rep. 13, 9835. https://doi.org/10.1038/s41598-023-36359-y.

Watt, L.J., Clark, C.E.F., Krebs, G.L., Petzel, C.E., Nielsen, S., Utsumi, S.A., 2015. Differential rumination, intake, and enteric methane production of dairy cows in a pasture-based automatic milking system. J. Dairy Sci. 98, 7248–7263. https://doi.org/10.3168/jds.2015-9463.

Weller, J.I., Ezra, E., 2024. Genetic analysis of rumination time based on analysis of 77,697 Israeli dairy cows. J. Dairy Sci. https://doi.org/10.3168/jds.2023-24095.

Williams, M.S., O'Hara, E., Coates, T., McAllister, T.A., Gruninger, R.J., Terry, S.A., 2025. The effect of a sugarcane polyphenol extract on intake, apparent total tract digestibility, rumen fermentation, pH, methane emissions, and rumen microbiome of beef heifers fed a high forage diet. Can. J. Anim. Sci. https://doi.org/10.1139/cjas-2024-0124.

Xin, H., Khan, N.A., Liu, X., Jiang, X., Sun, F., Zhang, S., Sun, Y., Zhang, Y., Li, X., 2021. Profiles of odd-and branched-chain fatty acids and their correlations with rumen fermentation parameters, microbial protein synthesis, and bacterial populations based on pure carbohydrate incubation in vitro. Front. Nutr. 8, 733352. https://doi.org/10.3389/fnut.2021.733352.

Xu, H.J., Zhang, Q.Y., Wang, L.H., Zhang, C.R., Li, Y., Zhang, Y.G., 2022. Growth performance, digestibility, blood metabolites, ruminal fermentation, and bacterial communities in response to the inclusion of gallic acid in the starter feed of preweaning dairy calves. J. Dairy Sci. 105, 3078–3089. https://doi.org/10.3168/jds.2021-20838.

Xue, M.Y., Xie, Y.Y., Zhong, Y., Ma, X.J., Sun, H.Z., Liu, J.X., 2022. Integrated meta-omics reveals new ruminal microbial features associated with feed efficiency in dairy cattle. Microbiome 10, 32. https://doi.org/10.1186/s40168-022-01228-9.

Yanibada, B., Hohenester, U., Petera, M., Canlet, C., Durand, S., Jourdan, F., Boccard, J., Martin, C., Eugène, M., Morgavi, D.P., Boudra, H., 2020. Inhibition of enteric methanogenesis in dairy cows induces changes in plasma metabolome highlighting metabolic shifts and potential markers of emission. Sci. Rep. 10, 15591. https://doi.org/10.1038/s41598-020-72145-w.

Zetouni, L., Difford, G.F., Lassen, J., Byskov, M.V., Norberg, E., Lovendahl, P., 2018. Is rumination time an indicator of methane production in dairy cows? J. Dairy Sci. 101, 11074–11085. https://doi.org/10.3168/jds.2017-14280.

Zhang, Z., Niu, X., Li, F., Li, F., Guo, L., 2020a. Ruminal cellulolytic bacteria abundance leads to the variation in fatty acids in the rumen digesta and meat of fattening lambs. J. Anim. Sci. 98, 228. https://doi.org/10.1093/jas/skaa228.

Zhang, Z., Wang, Y., Si, X., Cao, Z., Li, S., Yang, H., 2020b. Rumen methanogenesis, rumen fermentation, and microbial community response to nitroethane, 2-nitroethanol, and 2-nitro-1-propanol: an in vitro study. Animals 10, 479. https://doi.org/10.3390/ani10030479.