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Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants—Approaches and recommendations

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ABSTRACT

Over the past decade, there has been considerable attention on mitigating enteric methane (CH_4) emissions from ruminants through the utilization of antimethanogenic feed additives (AMFA). Administered in small quantities, these additives demonstrate potential for substantial reductions of methanogenesis. Mathematical models play a crucial role in comprehending and predicting the quantitative impact of AMFA on enteric CH_4 emissions across diverse diets and production systems. This study provides a comprehensive overview of methodologies for modeling the impact of AMFA on enteric CH_4 emissions in ruminants, culminating in a set of recommendations for modeling approaches to quantify the impact of AMFA on CH_4 emissions. Key considerations encompass the type of models employed (i.e., empirical models including meta-analyses, machine learning models, and mechanistic models), the modeling objectives, data availability, modeling synergies and trade-offs associated with using AMFA, and model applications for enhanced understanding, prediction, and integration into higher levels of aggregation. Based on an evaluation of these critical aspects, a set of recommendations is presented concerning modeling approaches for quantifying the impact of AMFA on CH_4 emissions and in support of farm-level, national, regional, and global inventories for accounting greenhouse gas emissions in ruminant production systems.

Key words: feed additive, methane mitigation, modeling, mechanistic models, empirical models

INTRODUCTION

Mitigating methane (CH_4) emissions emerged as a crucial strategy in addressing the pressing issue of climate change. Enteric CH_4 emissions, arising mainly from ruminants, account for a substantial portion of global agricultural GHG emissions (IPCC, 2022). The urgency of mitigating CH_4 emissions is underscored by its relatively short atmospheric lifetime compared with other GHG such as carbon dioxide (CO_2) and nitrous oxide (N_2O) and its more than 80 times the warming power of CO_2 over the first 20 yr after it reaches the atmosphere (IPCC, 2022). In recent years, several CH_4 mitigation strategies have been proposed (Arndt et al., 2022; Beauchemin et al., 2022; Honan et al., 2022). Among these strategies, the manipulation of rumen fermentation through the use of antimethanogenic feed additives (**AMFA**) garnered significant attention over the past decade. These additives, often administered in small quantities, hold the potential to significantly reduce rumen methanogenesis (Honan et al., 2022). Several AMFA have been investigated for their effect on CH_4 emissions, showing considerable variation in their effectiveness (Arndt et al., 2022). Recent meta-analyses have demonstrated varied efficacy of several AMFA including 3-nitrooxypropanol (**3-NOP**; Dijkstra et al., 2018; Kebreab et al., 2023), seaweeds (Lean et al., 2021), nitrate (Feng et al., 2020), monensin (Appuhamy

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et al., 2013; Marumo et al., 2023), and blends of essential oils (Belanche et al., 2020). This variability can be attributed not only to the inherent characteristics of AMFA and application (e.g., delivery method and dose), but also to variations in ruminant production systems. These include differences in target animal type (e.g., beef vs. dairy), physiological status (e.g., growing vs. lactating), feed management system (e.g., confinement vs. grazing), and diet characteristics (e.g., forage to concentrate ratio), which vary locally and globally (Niu et al., 2018; van Lingen et al., 2019b; Belanche et al., 2023).

Given this wide diversity of production scenarios and the variation encountered in rumen fermentation conditions associated with this (Belanche et al., 2025; Hristov et al., 2025), there is a need to develop models that can help to understand and predict the effect of AMFA on enteric CH_4 emissions across various production systems and diets, reducing the reliance on costly experimental studies (Hristov et al., 2018). Specifically, models capable of predicting the variability in response to AMFA are required to support farm-level, national, regional, and global inventories accounting of GHG emissions (del Prado et al., 2025) through life cycle assessments (**LCA**) and farm GHG assessments, capturing synergies or trade-offs with additional mitigation strategies. Models may be developed directly (empirical approaches) or indirectly (evaluating mechanistic approaches) from sound measurements of factors leading to variation in the CH_4 mitigating effect (Hristov et al., 2025), which are also instrumental from a national inventory, legislative and regulatory perspective (Tricarico et al., 2025). The aim of the present study is to provide guidelines and recommendations regarding modeling approaches for quantifying the impact of AMFA on enteric CH_4 emissions of ruminants.

MODELING THE IMPACT OF AMFA AT ANIMAL LEVEL

Several modeling approaches have been applied to quantify enteric CH_4 emissions at the individual animal level. These approaches aim to assess the effects of AMFA on CH_4 production (g/d), CH_4 yield (g/kg DMI), or CH_4 intensity (g/kg product; e.g., milk or BW gain) or one or more of these factors. The distinction between metrics is critical in modeling and in determining GHG impacts, as they capture different aspects of the underlying biological processes. Methane production represents the total absolute emission from the animal, and is primarily influenced by overall DMI, dietary nutrient composition, and animal size (Appuhamy et al., 2016). Methane yield is considered a more biologically meaningful metric compared with CH_4 production as it accounts for the methanogenic potential of feed intake and digestive processes. Methane intensity, a metric that represents the net CH_4 produced per unit of productive output, allows for the assessment of ruminant production and efficiency (Eckard and Clark, 2020). In evaluating the impact of AMFA on CH_4 emissions, studies have explored all of these expressions of the outcome, to better understand the additive's value proposition (e.g., Kebreab et al., 2023). Methane mitigation approaches that solely focus on emission intensity may inadvertently increase net CH_4 emissions if ruminant production increases more than the decrease in CH_4 emissions intensity (i.e., unless total ruminant production is constrained through additional policy measures or interventions). Focusing solely on reducing CH_4 production may inadvertently decrease feed intake or feed digestion and ultimately animal productivity. Focusing solely on reducing CH_4 yield may inadvertently decrease feed digestion or lead to rumen acidosis. Thus, quantification of CH_4 mitigation requires careful consideration of metrics and its implications (for further discussion, see Arndt et al., 2022).

The general modeling approaches, including the classification of modeling types, have been defined and extensively reviewed elsewhere (France and Thornley, 1984; Dijkstra et al., 2002; Ellis et al., 2020; Figure 1). The most common approaches to modeling CH_4 emissions are empirical, involving the degree of association between diet, animal performance, environmental factors, and AMFA inclusions with CH_4 emissions. This can range from simple regressions on a single dataset to meta-analyses (St-Pierre, 2001; Sauvant et al., 2020), which combine data from multiple experimental sources. Mechanistic models, in contrast, are based on mathematical description of underlying biological pathways, such as the representation of fermentation and feedstuff degradation resulting in rumen and hindgut production of CH_4 (e.g., Mills et al., 2001; Gregorini et al., 2013). To achieve a clear description of the relevant processes involved in capturing enteric CH_4 abatement through the use of AMFA (including trade-offs and synergistic effects on animal performance as well as on other GHG sources such as excreta), mechanistic models that interpret specific biological pathways occurring in the rumen become essential. The main AMFA developed thus far act by directly inhibiting methanogenesis (e.g., 3-NOP; halogenated compounds), competing for methanogenesis substrates (e.g., nitrates), or modulating rumen fermentation to decrease H_2 production (e.g., lipids and plant secondary compounds), with varying effectiveness (Honan et al., 2022). Several AMFA have multiple modes of action to decrease CH_4 emissions. A comprehensive overview, including recommendations, is presented elsewhere (Belanche et al., 2025).

Beyond these traditional modeling approaches, the advent of the fourth agricultural revolution has ushered

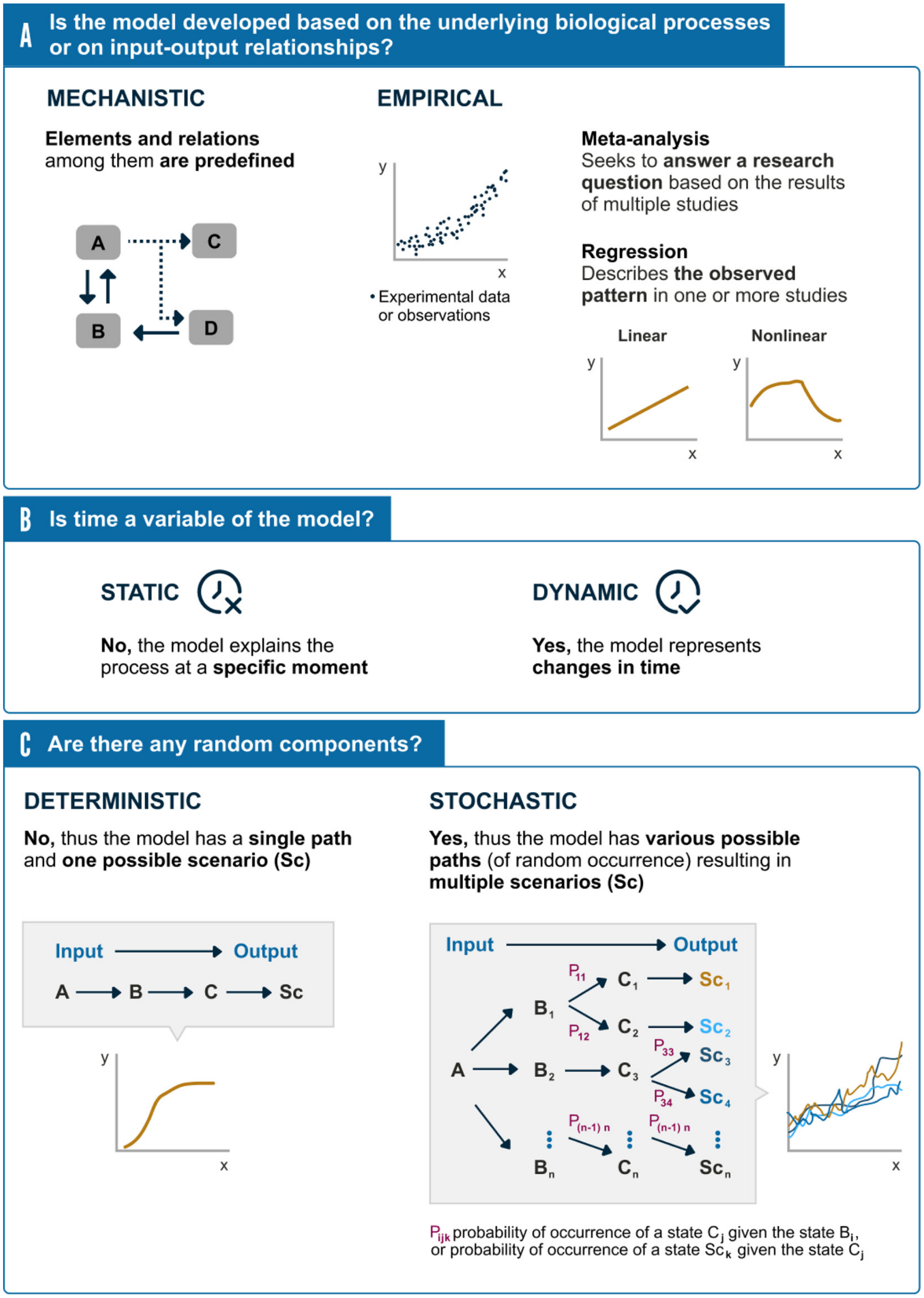

Figure 1. Illustration of types of models depending on background information used to develop them (A), the presence or absence of time as a variable (B), and the presence or absence of random parameters (C). Created by F. Garcia and Sabrina Garay; used with permission.

in advancements in big on-farm data collection, digital technology, robotics, sensors, computing, and Internet connectivity (Neethirajan and Kemp, 2021). These approaches have opened new avenues for quantifying CH_4 emissions at the animal level. Machine learning (**ML**), a branch of artificial intelligence, refers to the development and use of computer systems that are able to learn from data and adapt without following explicit instructions, by using algorithms and statistical models to analyze patterns in a training dataset and draw inferences and make predictions on new unseen data (Ellis et al., 2020; Tedeschi et al., 2021). Machine learning represents an empirical and data-driven modeling approach that is ideally suited for analyzing big data due to its ability to handle its volume, variety, velocity of data collection, and data veracity (Hackenberger, 2019). This approach is ideally suited toward prediction tasks, as opposed to documenting and understanding underlying mechanisms.

The availability and quality of data are of paramount importance in the development and implementation of reliable animal-level CH_4 emission models. Although many new ML approaches focus on predicting outcomes for individual animals, many empirical and mechanistic modeling approaches are based on literature data, which often reflect group or herd average responses to treatments. Although individual animal data may exhibit high variability, and there may be a desire to capture this variability through models developed from on-farm data, literature-derived data may provide more intentional, nonrandom variation that is valuable for group or herd level model development. Mechanistic modeling approaches may effectively quantify individual animal differences, as far as these are explainable from demonstrated underlying physiological differences. This understanding may further extend to genotypic differences if such information becomes available in the future.

Another challenge to all modeling approaches is the variation in CH_4 measurement methodologies between individual studies, which can include the use of respiration chambers (van Gastelen et al., 2015), GreenFeed breath-analyzer stations (C-Lock Inc., Rapid City, SD), nondispersive Fourier-transformed infrared (**FTIR**) breath analyzers ("sniffers") installed in milking parlors or feed bins (Lassen et al., 2012), sulfur hexafluoride tracer gas technique (Johnson et al., 1994), ventilated hoods (respiration boxes; Castelan Ortega et al., 2020), portable accumulation chambers (Goopy et al., 2011), face masks (Silveira et al., 2019), and laser detectors (Chagunda et al., 2013). These methodological differences (discussed extensively including recommendations by Hristov et al., 2025) can contribute to measurement differences and discrepancies (Hammond et al., 2016; Zhao et al., 2020) and may lead to relationships or prediction models specific to certain measurement techniques (Hristov et al., 2018).

Most studies in the field of AMFA and enteric CH_4 emissions focus on ruminants in confinement-type feeding systems compared with grazing systems (Arndt et al., 2022). Consequently, there is an imbalance with fewer grazing animals represented in the literature compared with confined animals (Vargas et al., 2022) and lack of information on enteric CH_4 emissions and AMFA abatement for ruminants in grazing systems (Ungerfeld, 2022). Of particular relevance to modeling efforts are the alternative methods of delivering AMFA and their overall effectiveness in the abatement of CH_4 emissions in grazing systems compared with confinement systems (discussed further by Hristov et al., 2025).

In general, current CH_4 emissions models do not integrate microbial -omics data (metagenomics, metatranscriptomics, metaproteomics), although recently a wealth of -omics data has become available (Muñoz-Tamayo et al., 2023). Incorporating microbial data, particularly when already available, into mathematical models of rumen metabolism may have enhanced power for predicting CH_4 emissions (Zhang et al., 2023).

Empirical Modeling

Regression analysis and meta-analysis are 2 commonly employed statistical techniques, each serving distinct purposes and grounded in unique principles. Regression analysis, a well-established method, is used to model the relationships between a dependent variable and one or several independent variables. This approach allows for the assessment of the relationships and marginal effects of those independent variables. Various types of regression (e.g., linear, nonlinear) can be applied depending on the nature of the data. The standard principles of regression analysis encompass the selection of suitable data including data scrutiny for outliers, an appropriate model, addressing collinearities, verifying underlying assumptions, and evaluating the model's performance. In many cases, studies on a common research question yield diverse and occasionally contradictory results (James et al., 2013). Therefore, meta-analysis is a quantitative strategy employed to aggregate results from different studies, providing a comprehensive overview of a research topic and yielding more robust conclusions compared with individual study results. The key principles of meta-analysis include study selection, calculation of effect sizes, weighting of studies based on factors such as sample size and quality, combining effect sizes to produce a summary statistic, assessment of heterogeneity among study results, and examining the potential impact of publication bias (St-Pierre, 2001; Madden and Paul, 2011). Essentially, the meta-analysis approach constructs a regression model that synthesizes findings from multiple studies, while considering random effects and heterogeneity among those studies.

Within the literature, numerous papers address empirical modeling of enteric CH_4 emissions. One of the first studies reporting a regression analysis of enteric CH_4 emission was Kriss (1930), and this has been followed by a large number of subsequent studies (recently, e.g., van Lingen et al., 2019b in beef cattle; Belanche et al., 2023 in sheep). The proliferation of experimental studies on GHG emissions, driven partly by the development of different measurement techniques around the world (Hammond et al., 2016), led to the creation of different databases containing original GHG emission data such as the Emission Factor Database from IPCC (https://www.ipcc-nggip.iges.or.jp/EFDB/main.php), FAO databases (https://www.fao.org/faostat/en/#data/GT), and more recently, data specifically related to enteric CH_4 emissions (de Ondarza et al., 2023). This surge in data availability spurred the development of meta-analysis studies focusing on GHG mitigating strategies (Arndt et al., 2022), with some focusing specifically on AMFA (e.g., Feng et al., 2020; Lean et al., 2021; Table 1). Whenever feasible, these studies consider the response to AMFA while taking into account dosage levels of the additives. Furthermore, they explore other explanatory factors and consider their interaction with the mitigating effects of AMFA, including factors such as basal diet composition and animal species. The effectiveness of CH_4 mitigation strategies may significantly differ across ruminant types, although such differences in efficacy between dairy cattle, beef cattle, and small ruminants are generally less pronounced (or absent) with AMFA than with general dietary strategies (van Gastelen et al., 2019). There is a considerable variation in CH_4 production both across and within ruminant species (e.g., higher CH_4 emissions during early versus late-lactation dairy goats; Fernández et al., 2021). Consequently, expressing the impact of AMFA on a relative basis (e.g., reduction in CH_4 emission relative to the control) may be preferable to using absolute measures (e.g., decrease in g of CH_4/d). Expressing impact on a relative basis requires standardizing the AMFA dose among various studies, usually done by expressing AMFA as the amount of additive per unit feed. To explore variation in relative response at similar standardized AMFA doses, heterogeneity can be examined by including variables that potentially explain variation in relative response (e.g., categorical variables such as beef cattle and dairy cattle may be included in the model to reduce heterogeneity). A major issue in these empirical modeling studies is the general lack of consideration of the method and frequency with which the AMFA is delivered (e.g., top-dressed; mixed in TMR; infrequent supply via concentrate dispensers), which is of particular importance with ruminants on pasture (Arndt et al., 2022).

Two recent meta-analyses on the antimethanogenic effects of 3-NOP (beef and dairy cattle, Dijkstra et al., 2018; dairy cattle, Kebreab et al., 2023; Table 1) showed that CH_4 emission reduction is dependent on the dose of 3-NOP inclusion. For every 1 mg/kg DM increase of 3-NOP from its mean dietary inclusion, there was a linear decrease in CH_4 production, yield, and intensity. Moreover, these studies showed negative and positive interactions between the potential to decrease CH_4 emissions and dietary factors. Specifically, for dairy cows (Kebreab et al., 2023) the decrease in CH_4 production, yield, and intensity due to supplementation of 3-NOP was partially impaired by the presence of greater levels of dietary crude fat (not for CH_4 intensity) and fiber but was increased by the presence of greater levels of dietary starch (CH_4 yield only; Tables 1 and 2). Furthermore, the type of cattle played a role in the response to 3-NOP, with 3-NOP supplementation being more effective with dairy cows compared with beef cattle (Dijkstra et al., 2018). This was presumably explained by the generally higher feed intake level of dairy cattle compared with beef cattle, leading to higher concentrations of fermentation products (e.g., VFA; hydrogen) in the rumen. NADH oxidation and type of VFA formed appear to be controlled by hydrogen partial pressure, and the modified VFA profile subsequently affects sinks of ruminal hydrogen (van Lingen et al., 2016). This, in turn, results in relatively lower concentrations of methyl-coenzyme M and elevated inhibitory potential of 3-NOP at greater feed intake levels.

In a meta-analysis using data from beef and dairy cattle, Feng et al. (2020) demonstrated that supplementing nitrate decreased CH_4 production and yield in a dose dependent and linear manner. Specifically, for every 1 g/kg DM increase in nitrate supplementation from its mean dietary inclusion, there was a consistent further reduction in CH_4 production, which was modified by level of feed intake (CH_4 production only; Tables 1 and 2). Moreover, the relative nitrate decreasing effect on CH_4 production and yield was greater with dairy cows than with beef steers, likely because in some studies with beef steers, slow-release nitrate was used and this was presumed to have a lower CH_4 mitigating efficacy than nonprotected nitrate (Feng et al., 2020).

Lean et al. (2021) studied the effect of seaweed supplementation on CH_4 yield in both dairy and beef cattle. The study indicated that supplementation of red seaweed *Asparagopsis taxiformis* (3 experiments with 7 comparisons to control) and brown seaweed *Ascophyllum nodosum* (1 experiment with 1 comparison to control) decreased CH_4 yield by 5.3 (±3.5) g/kg DMI (Table 1). The large variability is related to the use of various seaweed species and the low number of experiments included in this analysis. However, the dose-effect relationship was not explored, leaving room for further investigation in this area.

Appuhamy et al. (2013) conducted meta-analyses to assess the effect of monensin on CH_4 production and on CH_4 yield as a fraction of gross energy (**GE**; CH_4 %GE) in dairy cows and beef steers. When adjusted for effect of dietary fiber content (beef steers) or for dietary fat

Table 1. Effects of several feed additives in meta-analyses on methane (CH_4) production (g/d), yield (g/kg DMI) and intensity (g/kg milk) and CH_4 emission factor related to gross energy (GE; CH_4 in %GE), as well as on DMI, ADG, feed-to-gain ratio, and milk production

						Changes in CH_4 relative to control				Other effect on Y =			
Reference	Calc[1]	Feed additive[2]	Dose[3]	Animal type	Mean CH_4 production[4] (g/d)	CH_4 production (g/d)	CH_4 yield (g/kg DMI)	CH_4 intensity (g/kg ECM or FPCM[5])	CH_4 emission factor (% of GE)	DMI (kg/d)	ADG (g/d)	Feed-to-gain ratio (kg/kg)	Milk yield (kg/d)
Appuhamy et al. (2013)	MD	Monensin	21	Dairy cattle	338	−7 ± 5	—	—	−0.08 ± 0.11	−0.48 ± 0.09	—	—	0.17 ± 0.22
		Monensin	32	Beef cattle	131	−19 ± 4	—	—	−0.54 ± 0.14	−0.41[6] ± 0.13	—	—	—
Belanche et al. (2020)	RR	Blend of essential oils	1	Dairy cattle	321[7]	0.954 (0.921–0.987; 95% CI)	0.982 (0.918–1.050; 95% CI)	0.925 (0.864–0.989; 95% CI)	—	1.003 (0.985 to 1.020; 95% CI)			1.020 (1.011 to 1.028; 95% CI)
Feng et al. (2020)	RMD	Nitrate	16.6	Dairy cattle	339	−16.9 ± 1.0	−15.5 ± 1.2	—	—	—	—	—	—
			16.8	Beef cattle	155	−12.2 ± 1.3	−9.0 ± 1.8	—	—	—	—	—	—
Lean et al. (2021)	WMD	Seaweed	—[8]	Dairy and beef cattle	—	—	−5.3 ± 3.5	—	—	0.28 (−0.63 to 0.07; 95% CI)	−0.01 (−0.05 to 0.03; 95% CI)	−0.38 (−0.58 to −0.18; 95% CI)	1.35 (0.91 to 1.78; 95% CI)
Kebreab et al. (2023)	RMD	3-NOP	70.5	Dairy cattle	361[7]	−32.7 ± 1.5	−30.9 ± 1.5	−32.6 ± 1.3	—	—	—	—	—

[1]Calc = calculation; MD = mean differences were calculated as treatment mean minus control treatment mean; RMD = relative mean differences were calculated as treatment mean minus control treatment mean and then expressed as a fraction (in %) of the control mean; RR = response ratio, calculated as treatment mean relative to control treatment mean; WMD = weighted mean difference between treatment and reference groups means.

[2]3-NOP = 3-nitrooxypropanol.

[3]Mean doses evaluated, expressed in mg/kg DM (3-NOP), g/d (essential oils blend), or g/kg DM (nitrate).

[4]Mean production (control diet without feed additives).

[5]FPCM = fat- and protein-corrected milk.

[6]No separate data for beef steers only; value for dairy cows and beef steers combined.

[7]No data for CH_4 production of control treatment only; mean value is for control and treatment combined.

[8]Several different expressions and units of dose of seaweed among data sources; no data available for average CH_4 production.

Table 2. Estimate of overall effect size and explanatory variables of different antimethanogenic feed additives from models for relative mean difference (RMD) in methane (CH_4) production (g/d), yield (g/kg of DMI) and intensity (g/kg ECM)

Reference	Feed additive[1]	Dose[2]	Animal type	Relative mean difference[3] (%)		
				CH_4 production (g/d)	CH_4 yield (g/kg DMI)	CH_4 intensity (g/ kg ECM)
Kebreab et al. (2023)	3-NOP	70.5	Dairy cattle	−32.4 − 0.282 × (3-NOP − 70.5) + 0.915 × (NDF − 32.9) + 3.080 × (CFAT − 4.2)	−30.8 − 0.226 × (3-NOP − 70.5) + 0.906 × (NDF − 32.9) + 3.871 × (CFAT − 4.2) − 0.337 × (starch − 21.1)	−33.0 − 0.275 × (3-NOP − 70.5) + 0.723 × (NDF − 32.9)
Feng et al. (2020)	Nitrate	16.6	Dairy cattle	−20.4 ± 1.89 − 0.911 ± 0.141 × (Nitrate − 16.7) + 0.691 ± 0.294 × (DMI − 11.1)	−15.5 ± 1.15 − 0.73 ± 0.20 × (Nitrate − 16.7)	—
		16.8	Beef cattle	−10.1 ± 1.52 − 0.911 ± 0.141 × (Nitrate − 16.7) + 0.691 ± 0.294 × (DMI − 11.1)	−9.0 ± 1.76 − 0.73 ± 0.20 × (Nitrate − 16.7)	—

[1]3-NOP = 3-nitrooxypropanol (mg/kg DM).
[2]Mean dose evaluated, expressed in mg/kg DM (3-NOP) or g/kg DM (nitrate).
[3]Relative mean differences were calculated as treatment mean minus control treatment mean and then expressed as a fraction (in %) of the control mean. Explanatory variables are centered on their means. CFAT = crude fat; NDF and starch in % of DM; DMI and ECM in kg/d.

content and DMI (dairy cows), monensin decreased CH_4 emissions by 19 ± 4 g/d in beef steers (at 32 mg/kg DMI) and by 6 ± 3 g/d in dairy cows (at 21 mg/kg DMI). When corrected for DMI, monensin decreased CH_4 %GE by 0.33 ± 0.16 percentage units for beef steers and 0.23 ± 0.14 percentage units for dairy cows (Tables 1 and 3). In the combined beef steers and dairy cattle dataset, monensin dose was identified as a significant factor influencing CH_4 production, contributing to differences observed between beef and dairy cattle, particularly because higher amounts of monensin were included in the diet of beef cattle as opposed to dairy cattle.

Lastly, in a meta-analysis, Belanche et al. (2020) concluded that supplementing a blend of essential oils (Agolin Ruminant) reduced dairy cattle CH_4 production (−8.8%), yield (−12.9%), and intensity (−9.9%) in longer-term studies (>4 wk of treatment), whereas shorter-term studies had minor and inconsistent effects. It is essential to note that most data used in this meta-analysis were from unpublished studies or in vitro studies, hampering a comprehensive interpretation. Three subsequent longer-term studies assessing the same essential oils blend did not confirm the results of Belanche et al. (2020). In an 8-wk experiment with dairy cattle, supplementing these essential oils did not affect CH_4 production and CH_4 yield (Carrazco et al., 2020). It only affected CH_4 intensity when expressed per unit milk based on afternoon milking, but not when expressed per unit milk based on morning and afternoon milking. In a 13-wk experiment with dairy cattle, CH_4 production and yield, but not CH_4 intensity, decreased upon supplementation with the same essential oils blend (Bach et al., 2023). Upon feeding the essential oils blend for 10 wk to dairy cattle, CH_4 production, yield, and intensity were not affected (Silvestre et al., 2023). Importantly, in contrast with the meta-analysis of Belanche et al. (2020) where longer-term studies (>4 wk) had a greater CH_4 mitigating effect than shorter-term studies, none of these 3 subsequent peer-reviewed studies found any indication of a difference in impact of essential oil supplementation on CH_4 emission with week after first dosing. The discrepancies between results of this meta-analysis and the 3 longer-term experiments remain to be explained but emphasize the crucial role of carefully selecting data in performing meta-analyses.

Mechanistic Modeling

Mechanistic models of enteric fermentation describe the process of microbial degradation of feed and microbial biomass synthesis in the rumen, and sometimes in the large intestine of ruminants. They quantify methanogenesis by representing microbial activity and tracking the amount and profile of fermentation end products, including CH_4 and (often) hydrogen production (Tedeschi

Table 3. Estimates of overall effect size and explanatory variables of monensin from models for mean difference (MD) in methane (CH_4) production (g/d) and CH_4 emission factor related to gross energy (GE; CH_4 in % GE)

Reference	Feed additive	Dose[1]	Animal type	Prediction[2]: CH_4 production (g/d)	Prediction[2]: CH_4 emission factor (% GE)
Appuhamy et al. (2013)	Monensin	21	Dairy cattle	$-6 \pm 3 + 1.4 \pm 0.6 \times (DMI - 18.6) - 4.3 \pm 1.5 \times (EE - 38)$	$-0.23 \pm 0.14 + 0.03 \pm 0.02 \times (DMI - 12.9)$
		32	Beef cattle	$-19 \pm 4 - 0.05 \pm 0.03 \times (NDF - 379)$	$-0.33 \pm 0.16 + 0.03 \pm 0.02 \times (DMI - 12.9)$

[1]Mean dose evaluated, expressed in mg/kg DM.

[2]Explanatory variables are centered on their means. EE = Ether extract. NDF and EE in g/kg DM; DMI in kg/d.

et al., 2022). Involvement of several subsequent calculation steps (e.g., substrate degradation and subsequent microbial growth, substrate passage) does not inherently classify a model as mechanistic. What distinguishes mechanistic models from empirical or ML models is the ability of the former to describe the underlying processes responsible for the microbial activity outcomes (Dijkstra et al., 2002). In employing a mechanistic approach, one aims to capture the mode of action of AMFA and account for the variation in its efficacy within the model. This is in contrast to empirical approaches, where efficacy is solely derived from observational data at the animal and diet levels, relying on explanatory variables such as feed intake, diet type, and diet chemical composition. The difference between these modeling approaches does not hinge on the type of model inputs required but rather on the fundamental distinction in how the latter elucidates the mechanistic basis of the system (Bannink et al., 2016). Similar differences apply when comparing mechanistic models with ML models. However, modeling approaches may complement each other. It is recommended to explore synergy between different modeling methodologies to advance both our predictive capabilities of CH_4 production in ruminants and our understanding of the fermentation system (e.g., Ellis et al., 2020).

Published mechanistic models of the rumen fermentation process have been reviewed by Tedeschi et al. (2014). Although Tedeschi et al. (2014) identified the difference between 2 main types of modeling (i.e., adopting a static or a dynamic approach), the implications of this choice were less thoroughly discussed. Static approaches lack a representation of how model elements change over time, although they may still adopt rate parameters. These approaches typically focus on representing processes basis on daily information (i.e., assuming a daily steady-state). Consequently, they do not require time as a model variable and solutions can usually be obtained algebraically. Dynamic models include time as a variable to account for changes in model elements over time, and usually, if enzymatically driven or other nonlinear relationships are involved, these models require numerical integration to solve. A distinctive feature of dynamic mechanistic models is that the changes in model elements over time are concentration-dependent (i.e., dependent on the rumen concentration of substrate, microbial mass, and potentially AMFA). The (diurnal) dynamics of these concentrations determine model outcomes. This is a major difference from static models, which may prove to be important when aiming to describe the impact of diurnal variation on rumen H_2 dynamics and methanogenesis, as well as alternative routes of H_2 utilization. It is also crucial for modeling the impacts of diurnal variation in substrate availability on microbial growth efficiency, the type of

VFA produced and associated H_2 delivery to methanogens, rumen acidity, and rumen fluid volume.

Examples of mechanistic models of enteric CH_4 production have been reviewed recently by Tedeschi et al. (2022). Although the rumen fermentation models of Baldwin et al. (1987) and Dijkstra et al. (1992), including subsequent model versions derived from these (Tedeschi et al., 2014), adopted a truly dynamic approach, they do not incorporate the prediction of AMFA efficacy, except for including the effect of monensin on rumen fermentation and CH_4 production (Ellis et al., 2012, 2014). There are 2 recent examples of dynamic models with the specific purpose of predicting efficacy of AMFA but with a less detailed representation of rumen fermentative processes. A model including the effects of 3-NOP and nitrate was developed by van Lingen et al. (2021) for in vivo conditions, and a model including the effect of bromoform-containing *A. taxiformis* was based on in vitro data by Muñoz-Tamayo et al. (2021). These models have not yet been applied in inventory or assessment studies (del Prado et al., 2025).

Selection of Empirical and Mechanistic Models

It is not recommended to search for a one-size-fits-all, universally superior modeling approach for estimating the efficacy of AMFA. Both empirical methods (as demonstrated by Niu et al., 2018) and mechanistic approaches, whether static or dynamic (as illustrated by Kass et al., 2022), can be employed to predict enteric CH_4 emissions. The choice of approach should depend on the particular functionality or modeling objective of interest, and recommendations can be made regarding which approach aligns best with those objectives and what aspects should be represented in the model. An overview of various mechanistic model functionalities and modeling aims that are relevant to the prediction of AMFA efficacy, as well as their implications for the processes and model elements that should be incorporated, is given in Table 4. The level of detail crucial for representing rumen functionality, the desired degree of aggregation (rather than modeling the entire rumen), and the potential advantages of a mechanistic or dynamic approach over an empirical or static approach, are all influenced by the specific objectives. In cases where efficacy is known to be dependent on rumen kinetics and on diurnal variations in effective concentration of AMFA, a dynamic mechanistic modeling approach may be preferable to capture these complexities. However, such mechanistic models need to be evaluated against independent observational data before use. A model containing the required features but predicting with much greater error than a simpler model, should not be used in practical applications. Attempting to address such intricacies of kinetics and diurnal

Table 4. Mechanistic modeling functionalities relevant to the prediction of enteric methane emissions and the mitigating effect of antimethanogenic feed additives

Factor/functionality	Model variables/elements	Process to be represented[1]
Influencing factor		
Feed intake	Substrate availability, microbial growth and CH_4 production	Microbial mass, or static
Dietary characteristics	Substrate-specific microbial growth and CH_4 production	Substrate-specific microbial mass, or static
Feed ingredient characteristics	Intrinsic substrate degradation characteristics per ingredient	Substrate-specific microbial mass, or static
Rumen fermentation conditions	Rumen characteristics (volume, outflow, absorption, acidity)	Fluid volume, rumen acid mass, rumen buffering
Animal aspects	Feed intake behavior, rumen characteristics	Should be aligned to the processes mentioned for the other influencing factors
Functionality		
OM digestion/substrate fermentation	Microbial activity, microbial population size	Microbial mass, or static
Methanogenesis	Methanogen activity and related substrate requirements	Methanogen mass, or static
Rumen fermentation patterns	Microbial activity and its thermodynamic control	NAD/NADH ratio, hydrogen mass, or static
Hydrogen dynamics	Diurnal pattern feed intake, fermentation, methanogen activity	Acid production, methanogen mass, hydrogen mass
Additive efficacy	Mode of action, effective concentration, methanogen activity	Additive mass, methanogen mass, additive "binding affinity or activity"
Microbial adaptation	Change in microbial/methanogen activity in time	Microbial activity, additive "binding affinity or activity"
Hindgut fermentation	Elements and variables should be according to rumen principles	The process represented should be according to rumen principles

[1]Static means that no representation is adopted of the rumen mass of a source or processing entity to describe the process. For example, microbial growth rate described as a fraction of rate of feed intake or rate of substrate degradation, or CH_4 production rate described as CH_4 yield from fermented substrate, are both to be considered static (instead of dynamic) representations of these particular (sub)processes. Static models typically do not include time as a variable.

Table 5. Aspects that require additional documentation and for which justification is needed on the assumptions made when opting for an empirical instead of a mechanistic approach to estimate efficacy with the various modes of action of antimethanogenic feed additives

Mode of action	Aspects for which quantification is required and should be documented and justified
Single mode of action	
Hydrogen sinks	Sink of hydrogen being single mode of action
	Dynamics of rumen clearance as function of allocation pattern
	Dependency on level of feed intake
	Variation in dietary inclusion and intake/dosing
	Side-effects on microbial activity
Methanogens inhibitors	Inhibition of methanogens being single mode of action
	Diurnal variation in allocation with feed
	Dependency on level of feed intake
	Dependency on diet type and hydrogen dynamics (rumen fermentation conditions)
	Variation in dietary inclusion and intake/dosing
	Side-effects on overall microbial activity
Multiple modes of action	
Broad antimicrobial activity or altering the rumen environment	Change in rumen fermentation pattern (and fermentation-inert high-energy source) being single mode of action
	Dependency on diet type (rumen fermentation conditions)
	Dependency on level of feed intake
	Persistency of efficacy/expected adaptation rumen microbiota
	Variation in dietary inclusion and intake/dosing
	Side-effects on microbial activity

variations through an empirical approach would be challenging due to the lack of comprehensive observational databases. Nevertheless, the latter approach may still be preferred for practical reasons and better alignment with inventory and accounting methodology (del Prado et al., 2025). Yet, simplicity alone should not be a justification for choosing an approach; rather this decision should be accompanied by clear documentation explaining why, to what extent, and under what conditions the empirical approach is appropriate. Various aspects need to be handled in such documentation. Table 5 lists some important aspects for each mode of action of AMFA (Belanche et al., 2025). Some aspects are relevant for all modes of action, while others are specific to a particular one.

Empirical (including ML approaches) and mechanistic models both hold significant value. Meta-analyses are useful for identifying general patterns and leveraging farm data, which can then be explored in greater detail using a mechanistic approach. Conversely, empirical models may benefit from mechanistic modeling in identifying limitations or omissions, and how they could be improved. Simplification of mechanistic models is possible by leveraging insights from nonmechanistic approaches, to create lookup tables or relatively simple correction methods for AMFA efficacy. For example, Bannink et al. (2020) used a dynamic mechanistic model (i.e., Tier 3 method in Dutch GHG Inventory; Bannink et al., 2018) to determine CH_4 emissions factors for individual feedstuffs in dairy cattle diets. This model was adapted to account for the proportion of corn silage in roughage and for level of feed intake, which have been incorporated into practical farm assessment and C footprint tools. Similarly, mechanistic models such as those developed by van Lingen et al. (2021) and Muñoz-Tamayo et al. (2021) may be used to simplify the representation of AMFA, but examples are lacking in literature. Key measurements are needed for the further development of models predicting AMFA efficacy (Hristov et al., 2025). In addition to diet type and AMFA dosage, these measurements should at least include a representative estimate of daily rates of rumen substrate fermentation and VFA formation, the types of VFA formed, and rumen H_2 concentration.

Benefits of synergy between ML and mechanistic modeling approaches have been described as well (Ellis et al., 2020). Permutation importance (Altmann et al., 2010) is a ML strategy to examine how important any given feature (driving variable) is for prediction of a specified outcome by the ML model. Such an examination of ML model behavior may lend insight into features of importance in a dataset that might not initially be intuitive. As an example from another field, permutation importance conducted by You et al. (2022) demonstrated the importance of regional outdoor temperature in prediction of pellet quality within a commercial feed mill. Such information may lead to further research on the effect of air quality being pulled into the mill to cool pellets being manufactured. Likewise, such examinations of ML model behavior could assist interpreting nutritional or AMFA response data variation in the future, fueling ideas for improvement in both empirical and mechanistic models. Zhang et al. (2023) recently used a combination of ML and statistical modeling approaches to facilitate the selection of genera of rumen bacteria for inclusion in a modeling approach to improve CH_4 prediction accuracy for ruminants, without overfitting, providing valuable insights for future research, mitigation strategies and other types of modeling.

The relevance of a mechanistic modeling approach depends on (1) data availability to allow detailed quantitative representation of aspects of rumen fermentation conditions and microbial activity, (2) the need to explain specificity of AMFA efficacy for certain farm types, animals, or feed management, and (3) the methodological preferences or requirements for national GHG inventories or farm assessment tools. A mechanistic approach is recommended when the goal is to relate AMFA efficacy to fundamental elements of the rumen fermentative system that influence variability in efficacy. These core rumen elements may be represented in a static manner (i.e., irrespective of the state of the other rumen elements). Examples include microbial biomass synthesis calculated as a fixed fraction of fermented substrate, or fixed amounts of VFA produced from fermented substrate. Alternatively, they can be represented dynamically, which makes them a function of the state of other rumen elements. Examples include rumen microbial mass and substrate mass influencing both substrate degradation rates and hydrogen and CH_4 productions, and the production of rumen hydrogen influencing methanogenesis while also being affected by VFA production, as governed by the principles of thermodynamic control of microbial metabolism regulated by rumen hydrogen (van Lingen et al., 2019a). Details of the underlying causes of variation in efficacy of AMFA and the associated modeling aims are to be formulated carefully, as this will guide the choice of modeling approach.

ML Modeling

Machine learning modeling in dairy farm data has expanded over the past 2 decades, with Shine and Murphy (2022) identifying its applications in various domains such as feeding, healthcare, animal behavior, milking, and resource management. In this mapping study, the largest number of studies addressed issues related to the physiology and health of dairy cows (32% of 129 publications). Nearly half of these studies (48%) analyzed and included features derived from on-farms sensors. Although ML is a newer method in the context of animal production and its use is still emerging for evaluating AMFA, there have been instances where ML has been deployed to predict CH_4 emissions using "big data" on farms. For example, Shadpour et al. (2022) predicted CH_4 production based on milk yield, fat yield, protein yield, and milk mid-infrared (**MIR**) spectroscopy data via an artificial neural network using 181 weekly average CH_4 records from 158 Canadian dairy cows and 217 records from 44 Danish dairy cows. Observed data represented CH_4 estimates from the GreenFeed system and sniffer methods, respectively. However, when the algorithm was independently evaluated on data from 20 Canadian dairy cows, the results indicated a low correlation (r = 0.229) and high root mean square error (154 g/d), indicating the need for more or diverse data to enhance predictions. McParland et al. (2024) attempted a similar analysis including milk MIR as a driving variable as well from 384 lactations of 277 dairy cows. They concluded that including milk yield and DIM in the neural network prediction model resulted in superior predictions relative to just MIR spectroscopy data alone.

Other studies have approached CH_4 emission predictions by correlating video and image data of cows' intake times, estimated using artificial intelligence techniques such as computer vision, with observed CH_4 emissions. For example, Ramirez-Agudelo et al. (2022) developed prediction models using video and image data, and then evaluated their models against measures from respiration chambers. In a different approach, Wallace et al. (2019) and Zhang et al. (2023) used ML to link the rumen microbiome structure with host genetics and phenotype (including CH_4) and were able to predict CH_4 emissions from the core microbiome. As mentioned above, there may be a useful emerging synergy between this sort of analysis and more traditional empirical and mechanistic approaches.

Despite the relatively scarce attention given to the influence of AMFA on CH_4 emissions within ML research, the potential applications are substantial. For example, ML techniques such as random forest or causal forest analyses (Knaus et al., 2021) could be instrumental in identifying variations in response to AMFA, aiding in the understanding of these responses even before experimental or mechanistic understanding. With the advancement of on-farm sensors and big data coupled with ML analysis, there is also a promising future for more accurate on-farm evaluation of AMFA strategies and quantification of their actual impact on animals, extending beyond controlled experimental trials on which other modeling approaches are usually based.

Recommendations

- Large variation in CH_4 production exists between and within ruminant species. In situations of such large variation, the effect size of AMFA in meta-analyses can be expressed and analyzed in a relative manner. This requires the AMFA dose to be expressed in a standardized way and should include an evaluation (i.e., heterogeneity analysis) of the impact of between- or within-ruminant species variation on the variation in relative response.
- Alongside the dosage level, the AMFA delivery method and frequency should be included in the quantitative evaluation of the CH_4 mitigating impact of these additives.

- Quality of experimental data is central in empirical modeling. In classic regression analyses, as well as in meta-analytical approaches, to evaluate the impact of AMFA on CH_4 emission, it is recommended to use data from easily available, preferably peer-reviewed sources. Data from unpublished sources, in particular where essential details are lacking (e.g., experimental design, CH_4 measurement technique, length of experiment, and so on) should not be used.
- It is recommended to develop and employ mechanistic models of the impact of AMFA on CH_4 emission when aiming to relate its efficacy with fundamental elements of the rumen fermentative system. This approach can enhance the understanding of their mode of action of AMFA and, consequently, provide insight into variations in efficacy.
- The selected model type and modeling approach must be clearly described and justified, encompassing the objectives, boundaries, and limitations of the relevant model.
- The use of ML modeling is still emerging for evaluating AMFA. It is recommended to thoroughly explore the opportunities presented by ML, given the emergence of "big data" on farms. After development of ML models, particular attention should be given to evaluation of these ML models on independent data.
- Different modeling approaches possess distinct advantages and disadvantages. It is recommended to investigate the complementarity of diverse modeling methodologies to enhance our understanding of the fermentation system as well as to improve the predictive capabilities of AMFA impacts on CH_4 emission in ruminants.

SYNERGIES AND TRADE-OFFS

To assess whether 2 or more AMFA will have a synergistic or antagonistic effect on reducing CH_4 emissions, researchers typically use a factorial design for treatment allocation and employ statistical tools, such as mixed-model ANOVA (Kutner et al., 2005) to examine the interactions between AMFA. If significant interactions are observed, the specific type of interaction may be visualized by interaction plots to illustrate conditional effects of one treatment given the presence of another, and quantitatively determined by comparing least squared estimates. Among studies exploring potential synergies between 2 CH_4 mitigating strategies using AMFA, most concluded there was an absence of interaction between strategies (i.e., effects were shown to be additive). In cattle, there was no interaction between fat source and nitrate on CH_4 emissions (Guyader et al., 2015; Klop et al., 2016), no interaction between fat source and 3-NOP on CH_4 emissions (Zhang et al., 2021; Kjeldsen et al., 2024; Ma et al., 2024), and no interaction between monensin and 3-NOP on CH_4 emissions (Vyas et al., 2018). In contrast, Maigaard et al. (2024) reported antagonistic effects of 3-NOP, nitrate, and fat on CH_4 emissions in dairy cattle. Although the absence of a significant interaction between AMFA on CH_4 emissions is frequently reported, observed numerical differences are often of practical interest. For example, upon dietary inclusion of 3-NOP, Kjeldsen et al. (2024) observed a decline of 21.5% and 28.0% in CH_4 yield without or with fat (cracked rapeseed) dietary inclusion, respectively, but no significant interaction between both factors. This suggests a restricted statistical power to detect significant differences.

The potential for different CH_4 mitigation strategies to influence each other's effectiveness likely depends on how their mechanisms of action interrelate (Belanche et al., 2025). However, these interactions between 2 categorical variables (factorial levels) can result as either greater than (Figure 2B) or lesser than (Figure 2C) the sum of the individual effects when combined—this is referred to as ordinal interactions. In an ordinal interaction, the impact of each combined strategy exceeds that of its negative control. In contrast, disordinal interactions occur when the impact of a strategy changes direction depending on the presence of another strategy (Figure 2D). Standard statistical tests used to detect interactions do not usually predefine the expected pattern of interaction before the analysis. Ordinal interactions usually have lower statistical power than disordinal interactions given the same experimental unit (Lakens and Caldwell, 2021), indicating a larger sample size is required for the detection of ordinal interactions. Therefore, power analysis and sample size calculation for interactions, especially with the goal to explore interactive CH_4 mitigating effects, should be considered when designing an experiment (Durmic et al., 2025; Hristov et al., 2025). In this respect, a systematic framework for optimizing sample size in dairy cow methane studies, including a practical web-based tool that simplifies the process of sample size calculation, has recently been presented (Ramirez-Agudelo and Kebreab, 2024).

In addition to examining the synergies and trade-offs associated with strategies addressing enteric CH_4 emissions, similar considerations may extend to other GHG sources, such as N emissions originating from excreta, barn floors, and stored or applied slurry or manure. Values for these sources are documented in for example the DATAMAN database (Hassouna et al., 2023). Experimentally assessing these alternative sources provides specific recommendations for designing experiments focused on mitigating enteric CH_4 (see Hristov et al.,

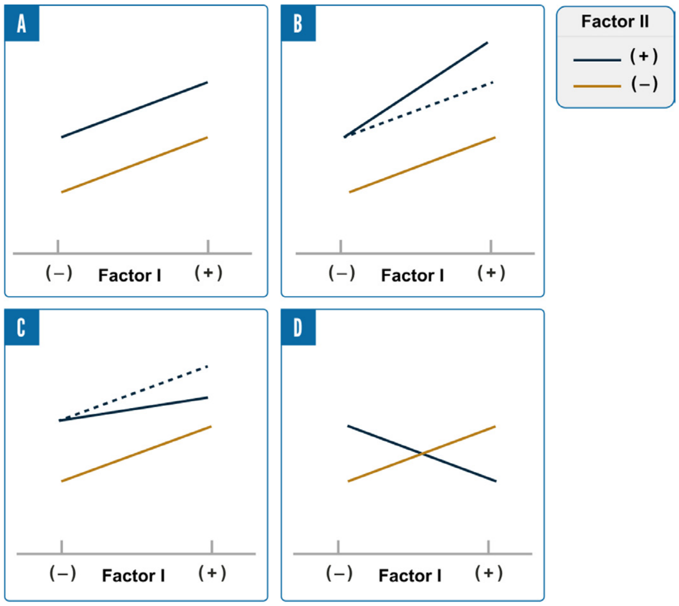

Figure 2. Illustration of various types of interactions between 2 factors, each with 2 levels. The x-axis marks 2 levels of factor I: (–) for the negative control and (+) for the treatment. Different line colors represent 2 levels of factor II: an orange solid line for the negative control (–) and a blue solid line for the treatment (+). The dashed line in scenarios (B) and (C) indicates the hypothesis of no interaction between factors I and II. Four different scenarios are depicted: (A) No interaction between factor I and factor II, where the combined effect of I (+) and II (+) is additive; (B) ordinal interaction, where the combined effect of I (+) and II (+) is greater than the addition of the 2 individual effects; (C) ordinal interaction, where the combined effect of I (+) and II (+) is less than the addition of the 2 individual effects; and (D) disordinal interaction, where the conditional effect of I (+) and II (+) switches over, and the combined effect is less than or has no effect compared with the effect of either individual factor. Created by M. Niu and Sabrina Garay; used with permission.

2025). Moreover, acknowledging the impact on these diverse GHG sources is crucial when developing farm assessment and inventory tools seeking to incorporate the mitigating effects of enteric CH_4 (see del Prado et al., 2025).

Recommendations

- Statistical models typically have low power to detect ordinal interactions, and only with a sufficiently large sample size can synergistic effects be reliably identified. Power analyses and sample size calculations should always be part of designing an experiment to study the interaction effects of AMFA, if possible, based on prediction of mitigation potential from initial in vitro tests or previous in vivo studies. Furthermore, it is recommended to employ meta-analysis methods to integrate results of individual studies, thereby improving the estimates of the effect size of additives as well as interactions on CH_4 emissions.

MODEL APPLICATION

It is important to recognize that the objectives of models described herein vary. In research, animal-level models are developed to formulate hypotheses, to understand mechanisms, and to document, account for and explore response variation. In particular, the quantitative exploration of the efficacy of AMFA can guide future scientific inquiries and enhance comprehension of the conditions under which an additive might be more or less effective at the individual animal level. For example, the meta-analysis of Dijkstra et al. (2018) indicated that the CH_4 mitigation effect of 3-NOP decreased with increasing dietary NDF content. Recognizing this potential impact of diet composition on 3-NOP efficacy, an in vivo study with dairy cattle by van Gastelen et al. (2022) investigated whether the CH_4 mitigation potential of 3-NOP is affected by the basal diet composition (starch rich vs. fiber rich), as an example of interplay between modeling and experiments, which confirmed modeling results. Models at the animal level are also constructed for direct practical application – such as calculating and predicting CH_4 emissions for LCA and inventory purposes, although the unit in which animals are expressed may differ (described extensively, including recommendations, in del Prado et al., 2025). These models may not necessarily be the same ones used for understanding mechanisms, but they certainly benefit from the latter with the method of choice or approach, accommodating trade-offs or synergies that are expected for nonenteric GHG sources. Nevertheless, at the animal level, these models serve crucial roles in decision support and opportunity analysis on-farm, particularly if they aid in response-based predictions rather than being requirement-based (Dijkstra et al., 2007). They enable the formulation of "what if" scenarios, facilitating decision making processes aimed at reducing GHG emissions and increasing animal efficiency, among other potential improvements (Jacobs et al., 2022).

Decisions within farm management are often made at a broader level than the individual animal due to the various trade-offs involved at the level of feed, manure and soil, on-farm as well as off-farm. These include trade-offs among different GHG (CH_4, N_2O, CO_2) originating from enteric emissions and urinary and fecal outputs (e.g., Gregorini et al., 2016; van Lingen et al., 2018). Instead of trade-offs, synergies may occur in a limited number of situations, such as increased animal performance with enteric CH_4 mitigation (e.g., Belanche et al., 2020; van Gastelen et al., 2024), although these require confirmation and carefully obtained experimental evidence (Hristov et al., 2025). There are also trade-offs to consider between farm systems: animal, cropping, and manure management, and the financial ramifications of changing management practices. For example, Van Middelaar et al. (2014) assessed 3 feeding strategies aimed at decreasing enteric CH_4 production in dairy cattle (i.e., reducing maturity stage of grass and grass silage, supplementation with extruded linseed, and supplementation with nitrate). The evaluation considered financial income at the farm level and GHG emissions at the chain level. Although extruded linseed resulted in the most significant decrease in enteric CH_4 emissions at the animal level, the strategy of nitrate supplementation led to the greatest reduction in total GHG emissions at the chain level, whereas the approach of reducing the maturity stage of grass and grass silage yielded the highest farm income. Thus, it is imperative to integrate accurate estimation of CH_4 emission responses at the animal level, known for their considerable variability, into predictions for higher organizational levels such as the farm or region to obtain sound predictions of possible trade-offs.

The type of feed management system is particularly important when applying models to predict enteric CH_4 emissions (Congio et al., 2023). A specific case is that of pasture-based ruminant production systems. In such systems, grazed forage is the most representative source of nutrients for ruminants, with its significance ranging from complete dependency in full grazing systems to varying degrees of supplementation with concentrates or conserved roughage (Hodgson, 1990). Dry matter intake is the key variable explaining enteric CH_4 emissions in ruminants; however, it is less accurately estimated in grazing systems, often using markers, than in confinements where gravimetric methods are employed (Jonker and Waghorn, 2020). This inaccuracy in DMI measurements in pasture-based systems limits the practical application of models that include DMI at the farm level (Congio et al., 2023). To enhance the applicability of models in predicting CH_4 emissions in pasture-based systems, variables that are highly correlated with DMI, which are more readily available on farm, can be an option. Milk yield may be the only current alternative of this kind of covariate, but research using biometric images and computer vision to predict BW and BW gain, sometimes in combination with ML prediction techniques, has been advancing rapidly (Greenwood et al., 2018; Cominotte et al., 2020), and these variables could be considered as proxies in models in the near future. Another characteristic that differentiates grazing from confinement is the factors driving DMI. Although in confinement systems DMI is mostly determined by dietary nutrient composition, in grazing systems sward structure variables (e.g., sward height, herbage mass) are equally important in explaining the variability of both DMI and CH_4 emissions (da Cunha et al., 2023). In partial grazing systems, where animals are supplemented, the AMFA is often mixed into just a portion of the total daily feed (i.e., supplement), usually consumed once or twice daily, which results in a pulse dosing effect and possible low efficacy of the AMFA to mitigate CH_4 emissions. This contrasts with a more continuous feeding effect observed when the AMFA is fed in TMR. For example, this pulse-dose effect is observed when the AMFA is top-dressed on a TMR offered once daily (Romero-Perez et al., 2014). They reported that animals consumed the mix (which included 3-NOP plus carrier) within 10 min after presentation when it was top-dressed on a TMR. Although the authors observed a linear reduction of CH_4 emissions from beef cattle fed increasing levels of 3-NOP, the reduction in CH_4 yield was rather small at the 3-NOP concentration levels applied (4.4%, 9.3%, and 33.1% at 51, 161, and 345 mg 3-NOP/kg DM, respectively) compared with the meta-analysis prediction of Dijkstra et al. (2018; i.e., 7.1%, 19.7%, and 65.3%). An extreme way of pulse dosing of 3-NOP was done by Reynolds et al. (2014), where 3-NOP in rumen fistulated cattle was delivered directly in the rumen twice daily. Daily CH_4 production was reduced by 3-NOP but inhibitory effects were transitory only, and the effect on a daily basis was much lower than when predicted using the meta-analysis of Kebreab et al. (2023). This means that the delivery method used when applying AMFA on-farm is an important aspect to evaluate when applying models to predict their efficacy.

Finally, in practice, the AMFA will often be used on-farm for extended periods of time. The long-term (i.e., several months at least) impact of AMFA may differ from that in short-term (often 2–wk) experiments. This topic has received little attention in modeling efforts, likely due to a paucity of data from long-term experiments. In an evaluation of antimethanogenic effects of monensin using a meta-analysis, Appuhamy et al. (2013) did not find a significant modifying effect of the monensin treatment period length (treatment period varied between 15 and 180 d). In a meta-analysis of antimethanogenic effects of a blend of essential oils, the effects were stronger in studies with more than 4 wk of treatment, than in shorter studies (Belanche et al., 2020). Evaluating the possibility of adaptation in long-term studies is hampered by almost unavoidable variation in other factors impacting CH_4 production, including changes in diet composition, lactation stage, and so on (e.g., Van Gastelen et al., 2024). Possible adaptation to AMFA, both in terms of mode of action and evaluation in experiments, is discussed including providing recommendations in Hristov et al. (2025) and Belanche et al. (2025), respectively.

Recommendations

- Models of the impact of AMFA on CH_4 emissions vary widely. The recommended choice for practical applications in inventories and farm assessments should be based on: (1) data availability; (2) desired level of detail for predicting rumen fermentative processes; (3) the preferred methodology in inventory and farm assessment and (4) predictive ability or quality of the model.

- It is recommended to integrate accurate estimations of CH_4 emission responses at the animal level, which is known for its considerable variability, into predictions for higher organizational levels to achieve sound and robust quantification of potential trade-offs associated with AMFA use.
- The efficacy of many AMFA depends on the frequency of supply. In pasture systems (full or partial), infrequent consumption may lead to a pulse-dose effect and altered efficacy. It is recommended to assess the mode of action of the additive of interest and, in case of transient effects within a day, not to apply general models predicting the additive's impact on CH_4 emissions in pasture systems. Instead, separate models accounting for transient effects need to be developed.
- Special emphasis should be placed on quantifying the potential of adaptation (either reversing or taking longer to occur) in the antimethanogenic effects of AMFA.

CONCLUSIONS

This study provides a set of recommendations for modeling approaches aimed at quantifying the impact of AMFA on CH_4 emissions in ruminants. In summary, the quality of data is pivotal in modeling approaches, and the use of peer-reviewed sources is strongly encouraged. Special attention is recommended for assessing additive dosage, delivery method, and transient effects in quantitative evaluations. The type of model and modeling approach adopted in relation to specific objectives must be clearly defined, while further exploring the synergy of diverse modeling methodologies, including ML, to enhance our understanding and predictive capabilities regarding the impact of AMFA on CH_4 emission in ruminants. Integral quantitative assessment is recommended to evaluate the CH_4 mitigating effect of AMFA, considering synergies or trade-offs, especially in relation to other GHG sources.

NOTES

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Nonstandard abbreviations used: 3-NOP = 3-nitrooxypropanol; AMFA = antimethanogenic feed additives; Calc = calculated; CFAT = crude fat; EE = ether extract; FPCM = fat- and protein-corrected milk; FTIR = Fourier-transformed infrared; GE = gross energy; LCA = life cycle assessments; ML = machine learning; MIR = mid-infrared; MD = mean difference; RMD = relative mean difference; RR = response ratio; WMD = weighted mean difference.

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