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Use of feed additives to reduce enteric methane emissions in dairy cattle: meta-analysis of data retrieved through a systematic review

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Introduction: We conducted a meta-analysis of quantitative data extracted from selected peer-reviewed papers describing *in vivo* studies on enteric methane emissions from dairy cattle treated with feed additives, compared with a control group. The aim was to identify feed additives that significantly reduce enteric methane emissions, expressed as g/day, g/kg dry matter intake, g/kg milk produced, and g/kg energy-corrected milk. The feed additives considered were polyunsaturated fatty acids (PUFAs), 3-nitrooxypropanol (3-NOP), essential oils (EO), and monensin. Four electronic databases (PubMed, CAB Abstracts, Web of Science, and Scopus; 2001–2024) were used to retrieve papers, following the PRISMA 2020 statement.

Methods: Effect sizes were calculated as log response ratio percentages and analyzed using three-level random-effects models. Heterogeneity, cluster-robust variance estimation, and leave-one-out diagnostics were applied. A total of 34 studies met the inclusion criteria.

Results: Of these, 19 investigated the impact of PUFAs, yielding 45 data points; seven investigated the impact of 3-NOP, yielding 23 data points; eight investigated the impact of essential oils on enteric methane emissions, yielding 12 data points; and three investigated the impact of monensin, yielding four data points. PUFAs significantly reduced enteric methane emissions across all metrics, although high heterogeneity remained ($I^2 \approx 86\%–95\%$). 3-NOP exhibited the most substantial average reductions in enteric methane emissions; however, the significance of these effects varied depending on the metric and model formulation. In addition, basal crude protein significantly influenced the effectiveness of 3-NOP. The effects of essential oils were generally non-significant and dependent on formulation. Evidence for monensin was limited and descriptive only.

Discussion: Based on the current body of evidence, PUFAs and 3-NOP represent the most reliable nutritional strategies for mitigating enteric methane emissions in dairy cows. PUFAs supplementation has been shown to reduce methane production without measurable adverse effects on milk yield or energy-

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corrected milk. Similarly, 3-NOP produced the greatest average reductions in methane emissions without impairing milk production, although its efficacy may vary depending on diet composition and may decline over time. Future research should prioritize standardized dosing, harmonized measurement methodologies, and extended trial durations that simultaneously assess efficacy, dietary covariates, persistence, productivity, and cost-effectiveness.

KEYWORDS

dairy cattle, feed additives, methane measurement, enteric methane reduction, milk production

1 Introduction

Carbon dioxide (CO_2) and methane (CH_4) are the two most significant GHGs released due to anthropogenic activities. Their atmospheric concentrations have risen by 28% and 79%, respectively, since 1950 (Baceninaite et al., 2022). It has been estimated that animal husbandry contributes 14.5% of global GHG emissions, with ruminant livestock emitting between 80 and 95 million tonnes of CH_4 globally. Of these emissions, 80.7% originate from enteric fermentation in dairy cattle, 17.4% from manure management, and 1.2% from rice cultivation. Methane has 28 times the warming potential of CO_2 and due to methane's short lifetime in the atmosphere, its reduction within the dairy industry could lead to a significant and near-immediate mitigation of warming effects (Mayerfeld et al., 2023).

On the other hand, it has been suggested that, with appropriate agricultural practices and effective farm management, ruminant husbandry can contribute to increased carbon sequestration in soil, optimized fodder consumption, and the production of high-quality food, including meat, cheese, milk, and other dairy products rich in proteins, lipids, and micronutrients (Teague et al., 2016; Broderick, 2018).

Strategies to reduce methane emissions from enteric fermentation and respiration in dairy cattle include feed management, the selection of breeding cows with lower methane emissions (Fresco et al., 2024), and vaccination against rumen methanogenic microbes (Zhang et al., 2015), although the latter remains under investigation. More innovative approaches include modulating the rumen microbiome to promote the growth of non-methanogenic microbes (Van Lingen et al., 2017; Cardinale and Kadarmideen, 2022; Yan et al., 2023).

To evaluate the effectiveness of enteric methane emission mitigation strategies for dairy cattle, it is crucial to measure these emissions with high precision. A wide range of technologies has been developed for this purpose, varying in complexity, accuracy, applicability, and cost. Each method has advantages and limitations, but none is without flaws (Baceninaite et al., 2022). Direct measurement methods include respiration chambers, where trained cows are confined in a sealed environment to measure the methane they emit (MaChado et al., 2016). Another method is the GreenFeed system, which measures enteric methane emissions when cows access a feeding station (Wu et al., 2018; Jonker et al., 2020). Sniffer analyzers are also employed to detect enteric methane emissions (Boutes et al., 2024). In addition, a bolus can be inserted into the animal's rumen to release an inert tracer gas, sulfur hexafluoride (SF6), allowing enteric methane emissions to be quantified based on the methane-to SF6 ratio, which is calculated using a known fixed release rate (Munoz et al., 2012).

As an alternative to direct methods, predictive equations can be used to estimate enteric methane emissions based on animal parameters, such as feed intake, body weight, and milk fatty acid profile. However, these equations require refinement through a factorial approach that incorporates the contributions of maintenance, production level, and milk quality to improve prediction accuracy (Massaro et al., 2024).

As most available studies on reducing enteric methane emissions focus on nutritional interventions, this study retrieved scientific papers quantifying reductions in enteric methane yield and intensity resulting from feed nutritional interventions. Quantitative data extracted from the selected papers, comparing enteric methane emissions in animals treated with specific feed additives (PUFAs, 3-NOP, EO, and monensin) with those in a control group, were analyzed through a meta-analysis. The aim was to identify feed nutritional strategies that significantly reduce enteric methane emissions in terms of yield (expressed as g/day and g/kg dry matter intake (DMI)) and intensity (g/kg milk produced and g/kg energy-corrected milk (ECM)).

2 Materials and methods

2.1 Search strategies

Four electronic databases were used to retrieve papers on the use of feed additives for dairy cattle with an impact on CH_4 emissions: PubMed, CAB Abstracts, Web of Science, and Scopus (accessed on

30th July 2024). The search string applied was as follows: (feed additives OR supplementation OR animal feed OR essential oil OR PUFAs OR polyunsaturated fatty acids OR 3-NOP OR 3-nitrooxypropanol OR monensin) AND (animal husbandry OR farm OR farming) AND (dairy cattle OR cow) AND (Methane OR emission OR GHG OR greenhouse gas OR CH4).

The bibliographic search was restricted to studies published between 2001 and 2024. Studies published before 2001 were excluded because diet composition and formulation were described using different parameters (NRC, 2001). The statements reported in the Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 were followed throughout the study (Page et al., 2021).

2.2 Eligible criteria and study selection

Articles meeting the following inclusion criteria were selected for the meta-analysis:

a. study subjects were lactating dairy cows;
b. the control group received exactly the same diet as the treatment groups, excluding the feed additive;
c. enteric methane emission outcomes were reported as g/day, g/kg dry matter intake (DMI), g/kg milk, g/kg energy-corrected milk (ECM), or at least one of these measures; and
d. methane measurements were obtained using respiration chambers, a GreenFeed unit, or sulfur hexafluoride SF.-6. The exclusion criteria were as follows:

a. studies involving dairy cattle diagnosed with a certified disease;
b. studies in which the control group received a specified pharmacological treatment or feed additive; and
c. short papers, case reports, reviews, or studies for which no English translation was available.

Although a limited number of studies have attempted to predict enteric methane emissions using *in vitro* models (Hindrichsen et al., 2004), only *in vivo* studies were included in this meta-analysis. For studies employing a Latin square or cross-over design, the total sample size for each treatment was considered in the methane emission calculations, and study duration was reported as the sum of the adaptation period and the experimental period.

2.3 Data extraction

The data extraction process addressed enteric methane emissions expressed as g/day, g/kg dry matter intake, g/kg milk produced, and g/kg energy-corrected milk in treated and control groups. Details of the data extraction and management process are summarized below.

When selected studies provided only the standard error of the mean (SEM) for enteric methane emission values, the standard deviation (SD) was calculated using the following Equation 1:

$$SD = SEM \star \sqrt[2]{n} \quad (1)$$

where n=sample size.

When a 95% confidence interval (CI) was available for an absolute effect measure (e.g., standardized mean difference, risk difference, or rate difference), or when standard error differences (SED) were provided and the number of observations in the control and treatment groups was similar, SEM was calculated using the following Equations 2, 3:

$$SEM = \frac{(upper\ \ limit - lower\ \ limit)}{3.92} \quad (2)$$

$$SEM = \frac{SED}{\sqrt{2}} \quad (3)$$

When standard error was reported on the log-transformed scale and non-log-transformed (back-transformed) means were provided, the original standard error was calculated using the following Equation 4:

$$SE\ \ original = Mean\ \ non\ \ log \star SE\ \ Log\ \ transformed \quad (4)$$

In addition, when methane emissions were reported as L/d or L/kg, data were converted using the following Equation 5:

$$CH4\ \ in\ \ g = CH4\ \ in\ \ \ L \star 0.000668 \frac{g}{cm^3} \star 1000 \quad (5)$$

where 0.000668 g/cm³ represents methane density at 20 °C.

When data were not provided in the retrieved papers or were depicted only graphically, the corresponding authors of each study were contacted by email; however, no responses were received.

2.4 Statistical analysis, correction of coefficients, and models development

Data were analyzed using the log response ratio (lnRR) as the effect size, the I^2 statistic for heterogeneity, and three-level random-effects models. The lnRR is based on the transformation of the mean difference into a percentage measure, as relative differences between control and treated groups allow comparability across studies reported in different units. This approach is robust across measurement scales (g/day, g/kg DMI, g/kg milk, and g/kg ECM) and reduces dependence on absolute methane production levels, thereby improving comparability between studies. The lnRR approach is widely recommended in ecological and nutritional meta-analyses to address bias arising from baseline differences (Laujeunesse, 2011, Laujeunesse, 2015, Livingstone et al, 2015). The lnRR calculation and its percentage transformation are reported in the following Equations 6, 7:

$$lnRR = \ln\left(\frac{average\ \ of\ \ treated\ \ group}{average\ \ of\ \ control\ \ group}\right) \quad (6)$$

$$lnRR\ \ \% = (e^{lnRR} - 1) \star 100 \quad (7)$$

In lnRR variance calculation for paired studies, the Pearson correlation coefficient (r) was set to 0.5. lnRR variance calculation for unpaired and paired studies are reported in the following Equations 8–10:

$$lnRR\ variance\ for\ unpaired\ studies = \frac{SD\ of\ treated\ group^2}{n\ of\ treated\ group} * (average\ of\ treated\ group^2) + \frac{SD\ of\ control\ group^2}{n\ of\ control\ group} * (average\ of\ control\ group^2) \quad (8)$$

where SD = standard deviation, n = sample size.

$$lnRR\ variance\ for\ paired\ studies = \frac{SD\ of\ treated\ group^2}{n\ of\ treated\ group} * (average\ of\ treated\ group^2) + \frac{SD\ of\ control\ group^2}{n\ of\ control\ group} * (average\ of\ control\ group^2) - 2\tau * SD\ of\ treated\ group * \frac{SD\ of\ control\ group}{n\ of\ treated\ group} * average\ of\ treated\ group + average\ of\ control\ group \quad (9)$$

where SD = standard deviation, n = sample size.

Heterogeneity (I^2) was calculated using the following equation (Higgins et al., 2003):

$$I^2 = \frac{(Q - df)}{Q} * 100 \quad (10)$$

where Q= Cochran's Q statistic and df= degrees of freedom.

A three-level random-effects model was applied to estimate variability between studies (τ^2) and within studies (multiple effect sizes from the same study), providing more reliable heterogeneity estimates (Konstantopoulos, 2011; Van Den Noortgate et al., 2013). Standard errors and p-values for model covariates were corrected using cluster-robust variance estimation (CR2) with the Satterthwaite method to account for the small number of studies and dependent effect sizes (Tripton, 2015; Satterthwaite et al., 1946). Leave-one-out diagnostics were performed to assess whether pooled estimates were driven by individual studies (Viechtbauer and Cheaung, 2010).

When the number of independent studies for a specific feed additive was fewer than five (e.g., monensin, n = 4), multilevel models were considered statistically unreliable (Tripton, 2015; Hedges et al., 2010). In these cases, only descriptive forest plots of lnRR (%) were reported to illustrate the direction and magnitude of effects. Additionally, when covariate-adjusted model p-values were not statistically significant, forest plots of lnRR (%) with and without covariate correction were reported for descriptive purposes.

Covariates included in the model formulation to explain heterogeneity were study duration (days), methane measurement technique (SF_6, respiration chambers, or GreenFeed), experimental design (randomized or cross-over), crude protein (CP), and neutral detergent fiber (NDF) content of the basal diet. Although dosage is recognized as a key factor in feed additive efficacy, it was not included as a covariate because it was reported using heterogeneous units across studies (e.g., mg/kg DMI, g/day per cow, percentage of diet), precluding standardization (Beauchemin et al., 2020).

Statistical significance was set at $p< 0.05$. All statistical analyses were performed using R 4.5.0 (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/ accessed on September 17th, 2025) with the packages "dplyr", "readr", "writexl", "metafor", "readxl", "ggplot2", "stringr", "cowplot", "tibble", "clubSandwich", and "purr".

3 Results

3.1 Search results

A total of 4,828 studies were identified in the primary search across the four selected databases: 871 from PubMed, 122 from CAB Abstracts, 1,179 from Web of Science, and 2,654 from Scopus. Duplicates, reviews, and meta-analyses were removed using Zotero, resulting in 3,416 studies for title screening. Of these, 868 articles were selected for abstract screening, and 796 were excluded, leaving 72 articles to be assessed for eligibility. Based on the inclusion and exclusion criteria, 38 additional papers were excluded for the following reasons: 13 contained a control diet with feed additives or did not report the composition of the control diet; five used supplements not addressed in this meta-analysis; three did not report a method for measuring methane; and 17 did not meet other inclusion criteria. Ultimately, 34 studies met the inclusion criteria, from which 84 methane measurements were extracted: 45 associated with PUFAs supplementation, 23 with 3-NOP, 12 with essential oils (EO), and four with monensin. The PRISMA flow chart detailing the search and selection process is presented in Figure 1, based on Page et al. (2021).

3.1.1 Characteristic of the studies

The main characteristics of the studies included in this meta-analysis are provided in the Supplementary Material (Supplementary Table S1), available in the Zenodo repository (DOI: 10.5281/zenodo.17512816). Notably, 11 studies reported all enteric methane emission measurements (i.e., g/day, g/kg DMI, g/kg milk, and g/kg ECM), while 22 studies reported at least three different measurements to assess the impact of feed additives on CH4 emissions.

In total, 84 studies provided data measured in g/day (45 for PUFAs, 23 for 3-NOP, 12 for EO, and four for monensin); 68 studies reported data in g/kg DMI (37 for PUFAs, 17 for 3-NOP, 12 for EO, and two for monensin); 38 studies presented data in g/kg milk (30 for PUFAs, four for 3-NOP, four for EO, and none for monensin); and 45 studies provided data in g/kg ECM (26 for PUFAs, 14 for 3-NOP, five for EO, and none for monensin). These distributions highlight the overall statistical power of this meta-analysis, while also indicating uneven data availability across outcome metrics.

3.1.2 Risk of bias assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias (RoB) tool was used to assess potential bias in the studies included in this meta-analysis (Hoojimans et al., 2014). As described by Hooijmans et al., this tool is based on the Cochrane Collaboration RoB tool and has been adapted to identify biases that are particularly relevant in animal

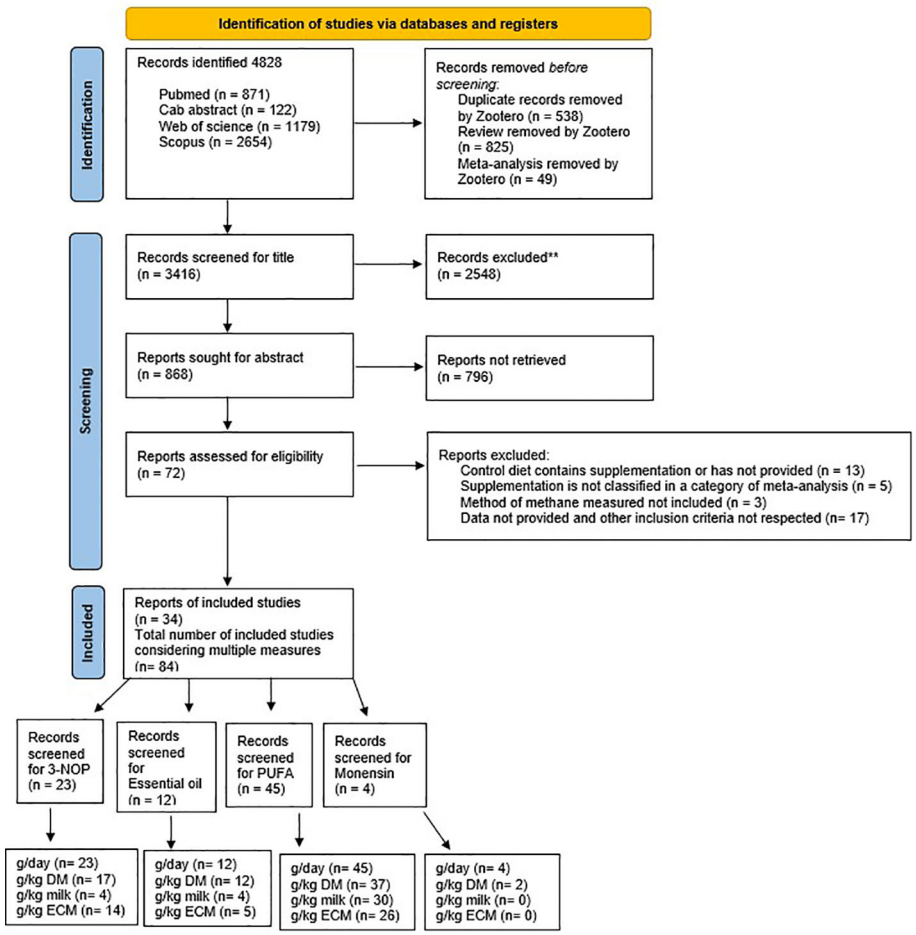

FIGURE 1
PRISMA flowchart of the study selection process.

studies. The SYRCLE RoB tool comprises 10 domains grouped into six types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. The SYRCLE assessment of the included studies is reported in Table 1. Notably, it is recommended not to assign an overall summary score to individual studies when using the SYRCLE RoB tool. Furthermore, Hooijmans et al. recommend adapting the assessment domains to the specific requirements of each review. For example, in studies employing Latin square or cross-over designs, it may not be possible to evaluate certain domains, and these items are often classified as unclear.

3.2 Effects of PUFAs on enteric methane emissions

PUFAs supplementation consistently reduced enteric methane emissions across all four metrics evaluated in this meta-analysis: absolute emissions (g/day), emissions normalized to dry matter intake (g/kg DMI), emissions per unit of milk yield (g/kg milk), and emissions per unit of energy-corrected milk (g/kg ECM). Uncorrected random-effects meta-analytic estimates based on lnRR revealed statistically significant reductions for each metric (g/day: overall REML = -18.46, 95% CI: -23.30 to -13.62; g/kg DMI: overall REML = -10.77, 95% CI: -15.37 to -6.18; g/kg milk: overall REML = -18.57, 95% CI: -24.95 to -12.19; g/kg ECM: overall REML = -12.58, 95% CI: -17.95 to -7.21), as illustrated in Figures 2-5. These results confirm the methane-mitigating potential of PUFAs in dairy cattle, with effect magnitudes varying by metric but remaining consistently negative and statistically significant.

High heterogeneity characterized all datasets (I^2 ranging from 85.78% to 94.54%), indicating substantial variability in effect sizes both within and between studies. For methane emissions expressed in g/day, variance was predominantly within studies; for emissions expressed in g/kg DMI, variance was primarily between studies; and for emissions expressed in g/kg milk and g/kg ECM, variance components were comparable across within- and between-study levels. Heterogeneity tests were highly significant across all metrics (all $p < 0.0001$).

Multilevel models incorporating CR2 correction were applied to address potential sources of heterogeneity; however, no covariates exerted statistically significant effects on PUFAs effect sizes

TABLE 1 Assessment of risk of bias in the selected studies using the SYRCLE tool (green = yes; yellow = unclear; red = no).

Study ID	Reference	1. Was the allocation sequence adequately generated and applied?	2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?	3.Was the allocation to the different groups adequately concealed during?	4. Were the animals randomly housed during the experiment?	5. Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	6. Were animals selected at random for outcome assessment?	7. Was the outcome assessor blinded?	8. Were incomplete outcome data adequately addressed?	9. Are reports of the study free of selective outcome reporting?	10. Was the study apparently free of other problems that could result in high risk of bias?
1	Alvarez-Hess et al., (2019)	yes	yes	unclear	unclear	no	yes	unclear	yes	unclear	unclear
2	Bayat et al., (2015)	unclear	unclear	unclear	yes	unclear	unclear	yes	yes	yes	unclear
3	Bayat et al., (2017b)	unclear	unclear	unclear	yes	unclear	unclear	yes	yes	yes	yes
4	Bayat et al.,, 2017a	unclear	unclear	unclear	yes	unclear	unclear	yes	yes	yes	unclear
5	Bayat et al., (2022)	unclear	unclear	unclear	yes	unclear	unclear	yes	yes	yes	yes
6	Benchaar (2016)	yes	unclear	unclear	unclear	no	yes	unclear	unclear	unclear	yes
7	Benchaar (2019)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	yes
8	Benchaar and Hassanat (2024)	unclear	unclear	no	yes	unclear	unclear	yes	yes	yes	unclear
9	Benchaar et al., (2015)	unclear	unclear	unclear	yes	unclear	unclear	yes	yes	yes	unclear
10	Chung et al., (2011)	no	yes	no	unclear	yes	unclear	yes	yes	yes	yes
11	Darabighane et al., (2021)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	unclear
12	Haisan et al., (2014)	no	yes	unclear	unclear	unclear	unclear	unclear	unclear	yes	unclear
13	Hamilton et al., (2010)	unclear	yes	unclear	yes	no	unclear	no	yes	yes	yes
14	Hassanat and Benchaar (2021)	yes	yes	unclear	unclear	no	yes	unclear	yes	unclear	no
15	Hassanat and Benchaar (2021)	unclear	yes	unclear	yes	unclear	no	unclear	no	yes	no
16	Hristov et al., 2013b	unclear	yes	unclear	unclear	unclear	unclear	unclear	yes	yes	yes
17	Hristov et al., 2015)	unclear	yes	no	unclear	unclear	unclear	unclear	yes	yes	yes
18	Khurana et al., (2023)	unclear	yes	unclear	yes	unclear	unclear	unclear	yes	yes	yes
19	Kliem et al., (2019)	unclear	yes	no	unclear	unclear	unclear	unclear	yes	yes	no
20	Livingstone et al., 2015	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	unclear
21	Maigaard et al., (2024)	yes	yes	unclear	unclear	no	yes	unclear	yes	unclear	yes
22	Martin et al., (2016)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	no
23	Martin et al., (2021)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	unclear
24	Martin et al., (2008)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	unclear
25	Melgar et al., (2021)	unclear	yes	unclear	yes	no	unclear	unclear	yes	yes	unclear
26	Munoz et al., (2024)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	no
27	Munoz et al., (2021)	yes	yes	no	unclear	no	yes	unclear	unclear	unclear	no

(Continued)

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TABLE 1 Continued

Study ID	Reference	1. Was the allocation sequence adequately generated and applied?	2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?	3.Was the allocation to the different groups adequately concealed during?	4. Were the animals randomly housed during the experiment?	5. Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	6. Were animals selected at random for outcome assessment?	7. Was the outcome assessor blinded?	8. Were incomplete outcome data adequately addressed?	9. Are reports of the study free of selective outcome reporting?	10. Was the study apparently free of other problems that could result in high risk of bias?
28	Pirondini et al., (2015)	yes	yes	unclear	unclear	no	yes	unclear	unclear	unclear	no
29	Silvestre et al., (2023)	unclear	yes	unclear	unclear	unclear	unclear	unclear	yes	yes	no
30	Storlien et al., (2017)	yes	yes	unclear	unclear	no	yes	unclear	no	unclear	no
31	Tondini et al., (2024)	unclear	yes	unclear	unclear	no	yes	unclear	yes	unclear	unclear
32	Van Gastelen et al., (2020)	unclear	yes	unclear	unclear	unclear	yes	unclear	yes	unclear	no
33	Van Wesemael et al., (2020)	unclear	yes	unclear	unclear	unclear	unclear	unclear	no	yes	yes
34	Williams et al., (2020)	yes	yes	unclear	unclear	unclear	unclear	unclear	yes	yes	yes

green = yes, yellow = unclear, red = no.

(covariate tests: p = 0.3829 for g/day; p = 0.966 for g/kg DMI; p = 0.6465 for g/kg milk; p = 0.4448 for g/kg ECM). These results indicate that the study-level and dietary factors examined did not substantially explain the observed variability.

Leave-one-out (LOO) validation analyses further underscored the robustness of the models while identifying influential studies. For methane emissions expressed in g/day, studies 10, 15, and 24 most strongly influenced fit indices (AIC, BIC, and log-likelihood), whereas studies 22, 30, and 34 had negligible effects. Similar patterns were observed for other metrics: studies 10 and 24 for g/kg DMI; studies 2, 9, 14, 23, and 24 for g/kg milk; and studies 1, 3, 4, 9, 11, 14, 23, and 24 for g/kg ECM. Importantly, exclusion of these influential studies did not alter the direction or statistical significance of pooled effects, confirming the stability of the meta-analytic conclusions.

Complete model specifications, variance components, LOO diagnostics, and corrected forest plots are reported in the Supplementary Material (Supplementary Tables S2–S9; Supplementary Figures S1-S4). Overall, these results highlight PUFAs as a reliable nutritional strategy for mitigating enteric methane emissions, although the persistent heterogeneity observed across studies underscores the need for standardized reporting and longer-term trials.

3.3 Effects of 3-NOP on enteric methane emissions

3-nitrooxypropanol (3-NOP) supplementation reduced enteric methane emissions across all four outcome metrics evaluated in this meta-analysis: absolute emissions (g/day: overall REML = −22.93, 95% CI: −29.04 to −16.82), emissions normalized to dry matter intake (g/kg DMI: overall REML = −16.19, 95% CI: −23.06 to −9.31), emissions per unit of milk (g/kg milk: overall REML = −10.86, 95% CI: −23.92 to 2.19), and emissions per unit of energy-corrected milk (g/kg ECM: overall REML = −16.54, 95% CI: −22.92 to −10.16). Uncorrected random-effects models based on lnRR indicated statistically significant reductions for g/day, g/kg DMI, and g/kg ECM, whereas the reduction for g/kg milk was not statistically significant, primarily due to the limited number of available studies. After CR2 correction, confidence intervals widened and statistical significance was not consistently retained across metrics. Nevertheless, pooled lnRR estimates remained negative for all outcomes, confirming a consistent methane-mitigating effect of 3-NOP (Figures 6–9; Tables 2, 3; Supplementary Tables S10–S13).

All datasets exhibited high heterogeneity (I^2 ranging from 83.28% to 97.99%), reflecting substantial variability in methane responses within and between studies. For methane emissions expressed in g/day and g/kg DMI, variability was predominantly between studies, suggesting that differences in experimental conditions, diets, and management practices were primary drivers of effect size dispersion. For methane emissions expressed as g/kg milk and g/kg ECM, heterogeneity was more evenly distributed across within- and between-study levels, consistent with the smaller

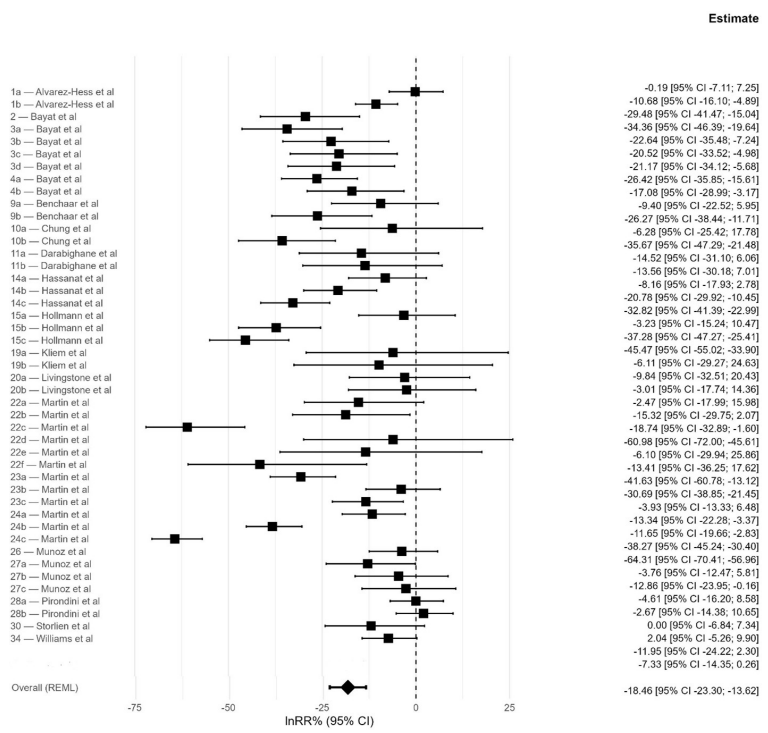

FIGURE 2
Forest plot from the meta-analysis of PUFAs use for the reduction of enteric methane emissions expressed as g/day, without application of a three-level model.

number of comparisons and the greater sensitivity of intensity-based metrics to production-related variation. Heterogeneity tests were statistically significant for g/day and g/kg DMI models (all $p < 0.0001$). Covariate tests (g/day: $p = 0.0057$; g/kg DMI: $p < 0.0001$) indicated that included moderators only partially explained the observed variability. For g/kg ECM, the heterogeneity test was not significant ($p = 0.1169$), whereas the covariate test remained significant ($p < 0.0001$), suggesting a meaningful contribution of moderators.

Multilevel models with CR2 correction identified crude protein concentration in the basal diet as the most consistent covariate associated with methane reduction magnitude for g/day, and both crude protein concentration and study design (randomized vs. non-randomized) for g/kg DMI. These findings indicate that dietary composition exerts a stronger influence on the methane-mitigating response to 3-NOP than animal-related factors or other design features. Leave-one-out analyses showed that only a limited number of studies influenced model fit indices, and their exclusion did not change the direction or magnitude of pooled effects, supporting the robustness of the findings.

Taken together, these results demonstrate that 3-NOP consistently reduces enteric methane emissions across all expression metrics. However, substantial residual heterogeneity indicates that diet- and design-related covariates only partially explain response variability. Notably, the limited data available for g/kg milk precluded multilevel modeling and LOO analyses; therefore, results for this metric are presented for descriptive purposes only.

3.4 Effects of EO on enteric methane emissions

Essential oil (EO) supplementation showed no statistically significant reduction in enteric methane emissions across all four outcome metrics evaluated in this meta-analysis: absolute emissions (g/day: overall REML = −1.58, 95% CI: −4.71 to 1.55), emissions normalized to dry matter intake (g/kg DMI: overall REML = −1.13, 95% CI: −3.70 to 1.43), emissions per unit of milk (g/kg milk: overall REML = −1.17, 95% CI: −2.96 to 0.62), and emissions per unit of energy-corrected milk (g/kg ECM: overall REML = −2.72, 95% CI: −6.79 to 1.34).

Heterogeneity varied across datasets, ranging from moderate (I^2 = 54.07%–60.13% for g/day, g/kg DMI, and g/kg ECM) to absent (I^2 = 0% for g/kg milk). For methane emissions expressed in g/day and g/kg DMI, variability was predominantly within studies, indicating that differences in experimental units or measurement protocols within individual experiments contributed more to effect size dispersion than between-study differences. For methane emissions expressed in g/kg milk (n = 4 studies) and g/kg ECM (n = 5 studies), the limited number of studies precluded reliable multilevel modeling, CR2 application, and leave-one-out (LOO) diagnostics, consistent with established guidelines for meta-analytic robustness. The g/kg milk metric exhibited complete consistency across the limited number of studies (n = 4). Heterogeneity tests were not statistically significant for g/day ($p = 0.0683$) and were significant for g/kg DMI ($p = 0.0144$).

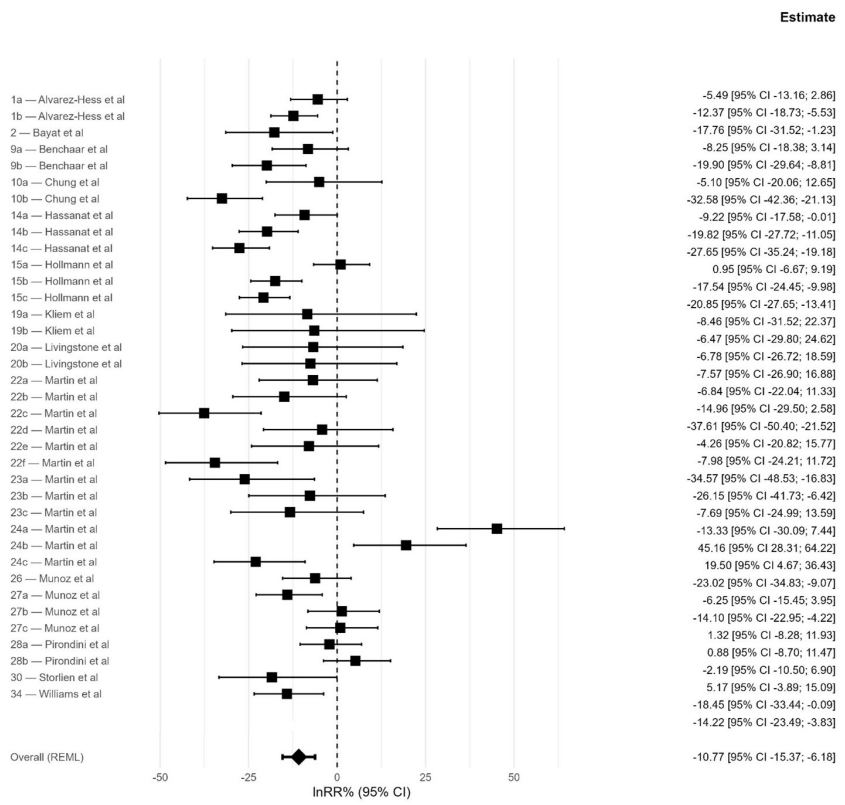

FIGURE 3
Forest plot from the meta-analysis of PUFAs use for the reduction of enteric methane emissions expressed as g/kg dry matter intake (DMI), without application of a three-level model.

Multilevel models with CR2 correction applied to the g/day and g/kg DMI datasets identified no statistically significant covariates influencing effect size (covariate tests: $p = 0.8476$ and $p = 0.7691$, respectively). Tested moderators, including dietary composition, animal characteristics, and study design, did not meaningfully account for observed variability, suggesting that unmeasured factors or inherent biological variability may contribute to the lack of EO efficacy.

LOO validation analyses for the g/day and g/kg DMI models revealed that a small number of studies exerted measurable influence on fit indices, particularly study 6 across both metrics and study 16 for g/kg DMI. Exclusion of these influential studies improved model fit but did not alter the direction, magnitude, or non-significance of pooled lnRR estimates. Other studies showed negligible impact, confirming overall model stability despite moderate within-study variability. Heterogeneity patterns remained consistent following sequential exclusions.

Taken together, these results demonstrate that EO supplementation does not yield statistically significant reductions in enteric methane emissions across any expression metric in dairy cattle. The consistent lack of effect across datasets, combined with predominantly within-study variability and the absence of influential covariates, suggests that EO has limited methane-mitigating potential under the experimental conditions represented in the literature. The small number of studies for milk-based intensity metrics (g/kg milk and g/kg ECM) further limits inferential power; therefore, results for these metrics are presented for descriptive purposes only. Complete model specifications, LOO diagnostics, and forest plots are provided in the Supplementary Material (Supplementary Figures S8–S13; Supplementary Tables S14–S17).

3.5 Effects of monensin on enteric methane emissions

Only three studies on monensin met the inclusion and exclusion criteria, with one study providing data at two time points, resulting in a total of four data points. While these outcomes are informative for descriptive purposes, they are insufficient to support reliable statistical analysis. Notably, none of the studies measured enteric methane emissions in relation to the productive performance of dairy cows. Consequently, data were available only for enteric methane emissions expressed as g/day ($n = 4$) and g/kg DMI ($n = 2$). Heterogeneity was moderate for g/day ($I^2 = 57.47\%$) but absent for g/kg DMI ($I^2 = 0\%$).

Due to the very small sample size, model formulation, CR2 correction, and LOO validation were not feasible. However, forest plots of lnRR (%) are provided in the Supplementary Material as Supplementary Figures S14, S15 for g/day (overall REML = 0.14,

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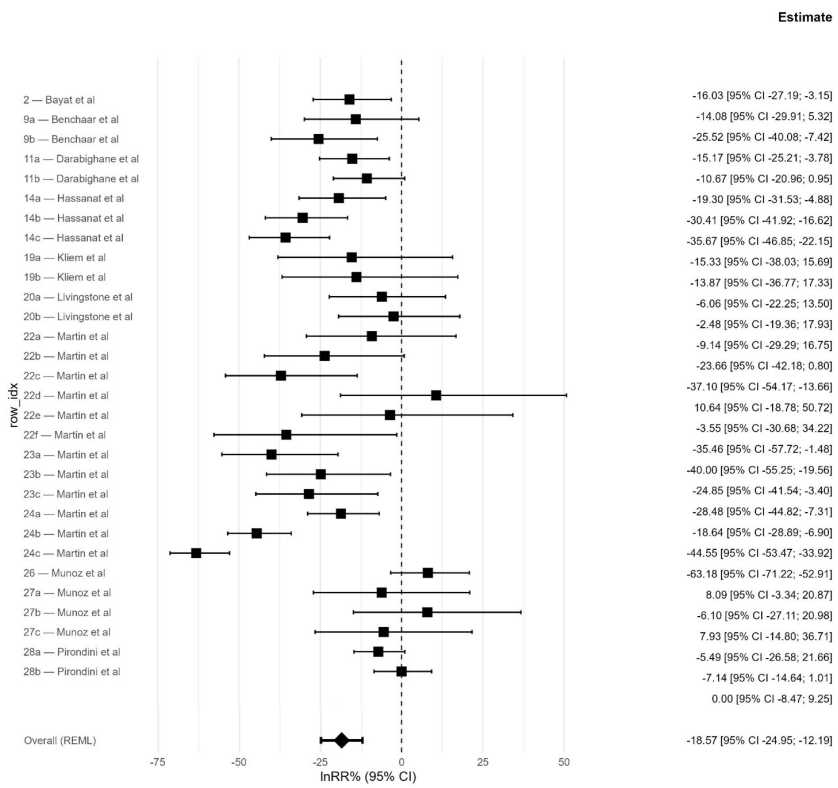

FIGURE 4
Forest plot obtained through meta-analysis of the use of PUFAs in reduction of g/kg milk methane emission with no three levels model application.

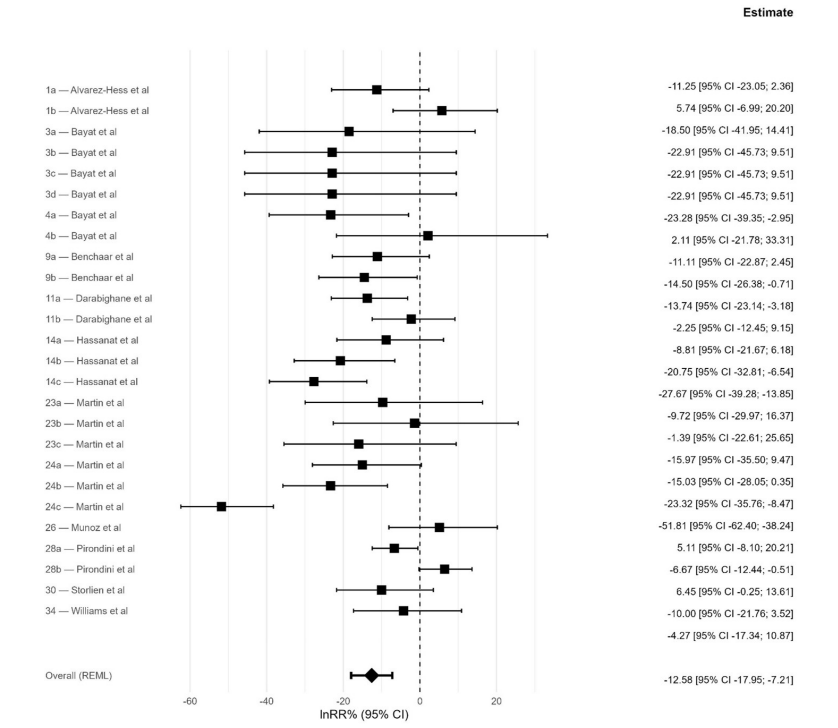

FIGURE 5
Forest plot obtained through meta-analysis of the use of PUFAs in reduction of g/kg ECM methane emission with no three levels model application.

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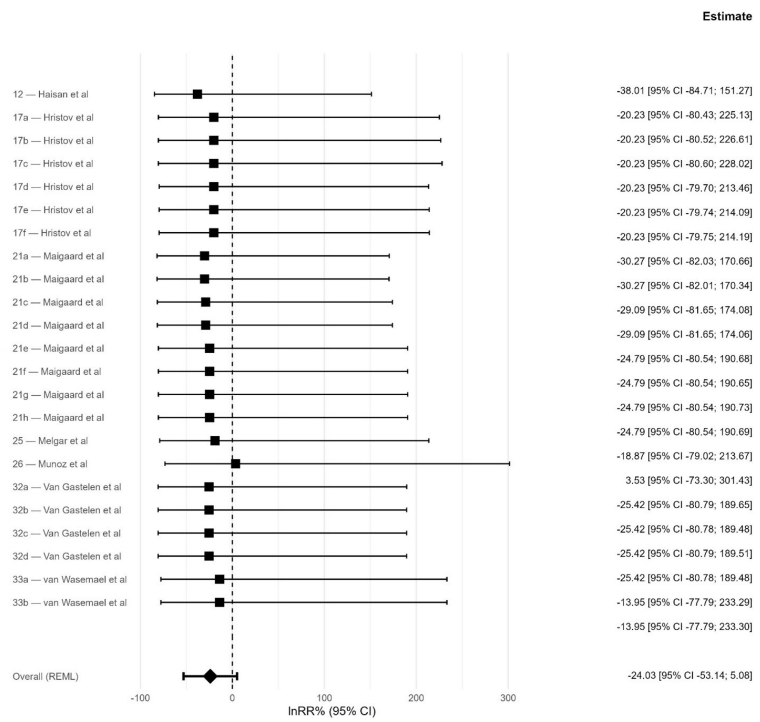

FIGURE 6
. Forest plot obtained through meta-analysis of the use of 3-NOP in reduction of g/day methane emission with three level model correction.

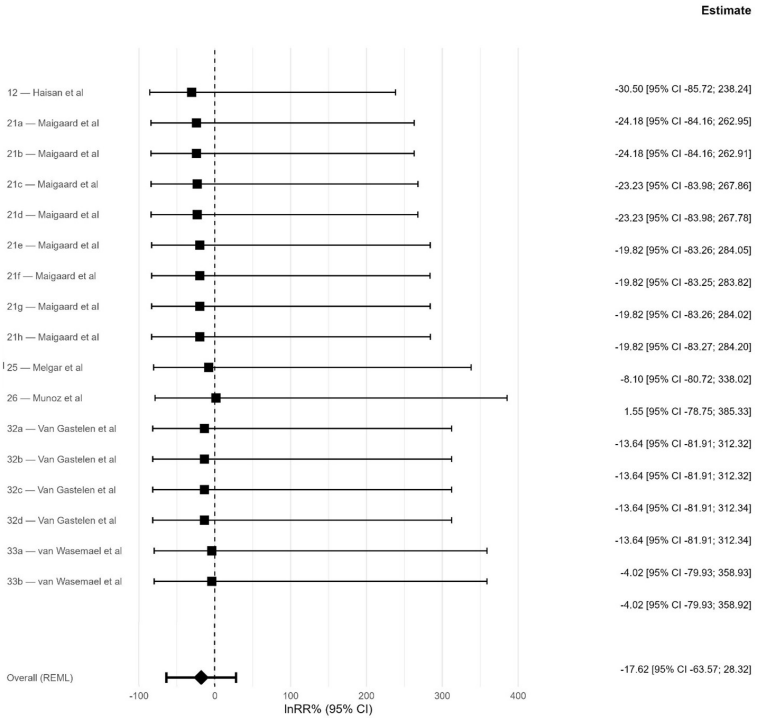

FIGURE 7
Forest plot obtained through meta-analysis of the use of 3-NOP in reduction of g/kg of DMI methane emission with three level model correction.

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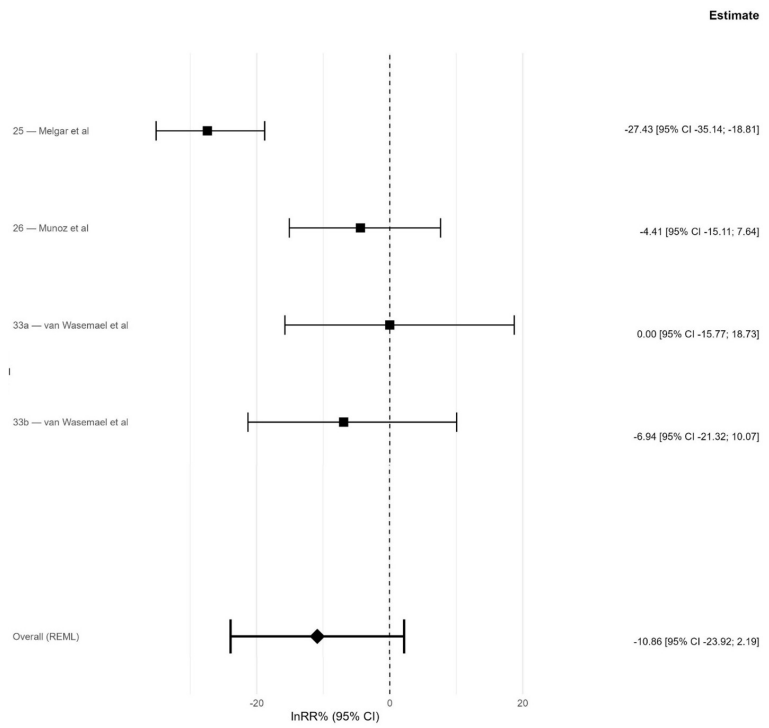

FIGURE 8
Forest plot obtained through meta-analysis of the use of 3-NOP in reduction of g/kg of milk methane emission with no three levels model application due to the low numbers of study available.

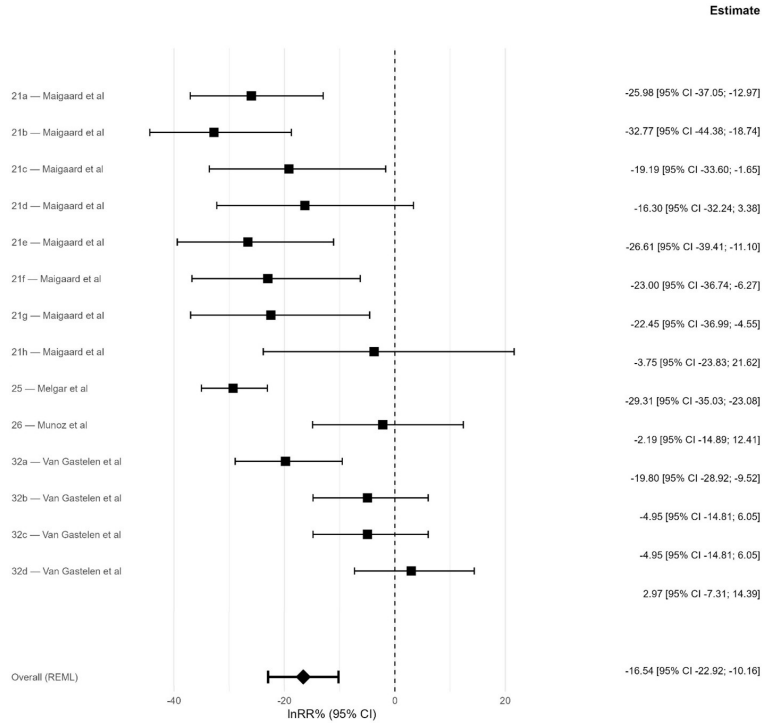

FIGURE 9
Forest plot obtained through meta-analysis of the use of 3-NOP in reduction of g/kg of ECM methane emission with no three levels model application due to a not presence of statistically significant covariates for the correction of effect sizes.

TABLE 2 Features of the three levels model applied for 3-NOP g/day enteric methane emission (I^2 = 97.99%; AIC = -19.73114; BIC = -15.55305; Log likehood = 13.86557).

Covariate	β coefficient	ES	p-value	CI low level	CI high level	ES corrected	p-value corrected
Intercept	1.379	0.4863	0.0046*	0.426	2.3321	0.355	0.058
Equipment (Respiration chambers)	0.2334	0.1145	0.0415*	0.0089	0.4579	0.0675	0.0685
Equipment (SF-6)	0.0405	0.1077	0.7067	-0.1706	0.2516	0.0491	0.550
Study type (randomized design)	-0.0942	0.1054	0.3715	-0.3007	0.1124	0.106	0.480
Time days	0.0018	0.0012	0.142	-0.0006	0.0042	0.00111	0.338
Crude protein	-0.0157	0.0033	<0.0001*	-0.0222	0.0092	0.00276	0.011*
NDF	0.0028	0.0013	0.0342*	0.0002	0.0054	0.000785	0.0984

* means p-value under 0.05 and statistically significant value.

95% CI: −4.71 to 5.00) and g/kg DMI (overall REML = −4.21, 95% CI: −8.55 to 0.14), respectively. These results should be interpreted with caution, as they are primarily descriptive in nature.

4 Discussion

4.1 Effects of feed additives on enteric methane emissions

This meta-analysis reveals distinct patterns in the methane-mitigating efficacy of nutritional additives in dairy cattle. PUFAs and 3-NOP consistently reduced methane emissions across all emission metrics, whereas essential oils (EO) and monensin exhibited limited and inconsistent effects. PUFAs and 3-NOP operate through different mechanisms. PUFAs primarily modify rumen microbial ecology and fermentation pathways due to their toxicity toward cellulolytic bacteria responsible for acetic acid production. Acetic acid is, in turn, one of the primary substrates utilized by methanogens as a hydrogen donor. In contrast, 3-NOP directly inhibits methyl coenzyme M reductase, a key enzyme in the methanogenesis pathway. Both additives produced biologically meaningful reductions in methane emissions, whether expressed as absolute output or relative to production performance parameters. In contrast, the effects of EO and monensin varied widely. For EO, this variability is likely attributable to differences in EO blend composition, as several oils are known to possess antibacterial activity. For monensin, variability likely reflects the influence of rumen microbial populations and dietary substrates. These findings highlight the importance of additive-specific characteristics and contextual factors in determining methane mitigation potential.

The high heterogeneity observed across analyses underscores the influence of unmeasured experimental variables. Diet composition emerged as a key moderator, particularly crude protein concentration in relation to 3-NOP efficacy. This aligns with established principles of rumen biochemistry. Indeed, higher protein levels may increase hydrogen availability for alternative metabolic pathways (e.g., the propionate pathway), thereby enhancing the inhibitory effect of 3-NOP on methanogenesis.

PUFAs responses similarly reflect interactions driven by lipid profiles. The findings of this meta-analysis align with previous meta-analyses and experimental studies reporting 10%–25% reductions in enteric methane emissions, depending on PUFAs

TABLE 3 Features of the three levels model applied for 3-NOP g/kg of DMI enteric methane emission (I^2 = 93.95%; AIC = -10.01299; BIC = -6.817708; Log likehood = 10.0065.

Covariate	β coefficient	ES	p-value	CI low level	CI high level	ES corrected	p-value corrected
Intercept	2.1544	0.5518	<0.0001*	1.0729	3.326	0.362	0.0991
Equipment (Respiration chambers)	0.2323	0.0807	0.004*	0.0074	0.3905	0.0395	0.0815
Equipment (SF-6)	-0.2228	0.2235	0.3189	-0.6609	0.2135	0.153	0.352
Study type (randomized design)	-0.5092	0.1678	0.0024*	-0.8381	-0.1803	0.0327	0.0395*
Time days	-0.0002	0.0013	0.8904	-0.0027	0.0023	0.00131	0.915
Crude protein	-0.0203	0.0039	<0.0001*	-0.0280	0.0126	0.000985	0.00272*
NDF	0.0044	0.0022	0.0478*	0.000	0.0087	0.000588	0.0785

* means p-value under 0.05 and statistically significant value.

dose and type (Shingfield et al., 2013; Hristov et al., 2013a; Martin et al., 2010). In contrast, EO and monensin lacked significant covariates, reflecting their broader mechanistic variability. In particular, EO bioactive compounds (e.g., cinnamaldehyde and eugenol) exhibit compound-specific potency against methanogens, while monensin primarily shifts propionate production in high-forage diets.

These findings carry important practical implications for dairy management and emission reduction policies. PUFAs supplementation can be flexibly integrated into existing rations and offers established production benefits. Similarly, 3-NOP provides targeted, dose-dependent methane reduction compatible with precision-feeding strategies. However, the temporal decline in 3-NOP efficacy observed in longer-duration trials—likely due to microbial adaptation—highlights the need for strategies such as intermittent dosing or combined mitigation approaches.

EO and monensin, despite lower overall reliability, may serve niche applications when specific formulations align with particular dietary conditions. The variability in EO effects can be attributed to distinct combinations of bioactive compounds present in EO formulations, which differ in their mechanisms of action on rumen methanogenesis, microbial composition, and fermentation pathways (Patra and Yu, 2012; Benchaar and Hassanat, 2024). Certain EO compounds, such as garlic derivatives and cinnamaldehyde, can inhibit methanogenic archaea or modulate volatile fatty acid production, but their efficacy is highly context-dependent (Patra and Yu, 2012). The two studies showing statistically significant effects of EO on enteric methane emission reduction were Khurana et al. (2023); study ID: 18), which evaluated an EO mixture containing garlic–citrus extract, and Tondini et al. (2024); study ID: 31), which used a mixture of capsicum oleoresin, eugenol, and cinnamaldehyde. Monensin can reduce enteric methane through its ionophore activity, which alters rumen fermentation and inhibits gram-positive bacteria, thereby increasing propionate production at the expense of acetate and hydrogen availability and reducing methane formation. However, the magnitude of monensin's effect depends strongly on diet composition, animal type, and rumen microbial adaptation (Beauchemin et al., 2008; Min et al., 2022).

Several key knowledge gaps remain, including the lack of standardized additive dosing protocols, harmonized methane measurement methodologies, and evaluations of long-term treatment effects (>90 days) that account for rumen microbiome and physiological adaptation dynamics. Trial duration is particularly critical, as short-term studies may overestimate sustained mitigation potential. Diet formulation—particularly protein-to-neutral detergent fiber (NDF) ratios—should inform additive selection, as both starch and NDF contents have been identified as influential factors (Kebreab et al., 2023). In the present study, NDF was included in the model formulation, whereas starch was excluded due to the limited number of studies reporting starch values and its multicollinearity with other dietary covariates. The systematic organization of experimental trials addressing these identified gaps would improve the refinement and implementation of feed additive strategies, thereby supporting verifiable methane emission reductions in dairy cattle and enhancing the sustainability of the dairy food system.

4.2 Limitations and strengths of the study

Meta-analyses are subject to several limitations, ranging from the literature search process to the conclusions drawn from study outcomes (Walker et al., 2008). These limitations include potential gaps in paper retrieval, the selection of inclusion and exclusion criteria, and issues related to the quality and heterogeneity of included studies (Allen, 2020). This meta-analysis exhibited a high degree of heterogeneity, making model development essential to explore and account for this feature. Heterogeneity was addressed by applying a three-level model that accounted for multiple sources of variability influencing study outcomes. The models identified a significant effect only for 3-NOP, which appeared to be driven primarily by basal diet composition. Previous research has indicated that forage-to-concentrate ratio and study duration can also modulate the effectiveness of 3-NOP (Shilde et al., 2021). Another important consideration concerns study design. Direct comparisons between cross-over and randomized trials are challenging due to the lack of a clearly defined and consistent control group; however, no statistically significant effect of study type was observed in this analysis. Study duration also warrants careful evaluation. Most trials were relatively short, lasting at most 1 month (nine studies for PUFAs, three for 3-NOP, six for EO, and three for monensin, including follow-ups). Only seven studies lasted between 1 and 2 months (four for PUFAs, two for 3-NOP, and one for monensin, including follow-ups), and just eight studies extended beyond 2 months (one for PUFAs, five for 3-NOP, and two for EO, including follow-ups). These limitations were considered in the model formulation. Regarding study duration, the literature clearly highlights that potential long-term side effects of additive supplementation on animal welfare, as well as the economic costs associated with feed additive production and use, are often insufficiently addressed and typically fall outside the scope of studies included in this meta-analysis.

While meta-analyses aim to provide evidence in areas where the literature presents conflicting results, they cannot compensate for a lack of research in a specific field. Consequently, findings should be interpreted cautiously and within the appropriate context. Regular updates and periodic revisions of meta-analyses are essential to incorporate newly published research and maintain relevance (Walker et al., 2008). This is particularly relevant for the use of feed additives to improve sustainability and reduce methane emissions in dairy cattle.

Despite these limitations, meta-analysis plays a valuable role in guiding future research and improving experimental design. One of the primary challenges in traditional reviews is the difficulty of simultaneously evaluating results from multiple studies and drawing conclusions beneficial to the field. Meta-analysis addresses this challenge by complementing qualitative reviews with quantitative data analysis, achieving increased statistical power through aggregation of sample sizes across studies. To the

best of the authors' knowledge, the present work extends previous research by incorporating a large number of *in vivo* studies and providing an updated assessment of feed additive efficacy in reducing methane emissions from dairy cattle. In addition, this meta-analysis incorporated an in-depth investigation of heterogeneity through model formulation to identify factors significantly influencing effect size and study outcomes. As a result, meta-analysis can reveal modest yet meaningful effects that might otherwise remain undetected, offering insights critical for the design and interpretation of future research trials. In conclusion, meta-analysis applies advanced statistical techniques to synthesize findings and enhance the quality of information derived from multiple bibliographic sources and is therefore regarded as the gold standard in evidence-based scientific research (Seidler et al., 2020).

4.3 Conclusion

The results of this study showed that PUFAs supplementation significantly reduces methane emissions across all expression metrics (i.e., g/day, g/kg DMI, g/kg milk, and g/kg ECM) without adversely affecting animal productive performance. Regarding 3-NOP, this additive generally achieved the largest average reduction in methane emissions, although the statistical significance of the effect varied by metric. Crude protein content of the diet emerged as a significant covariate influencing 3-NOP efficacy. Additionally, some evidence suggests a potential attenuation of its mitigating effect over time, potentially due to rumen microbiome adaptation.

Essential oils (EO) produced inconsistent, formulation-specific results and were not statistically significant across methane measurement metrics, whereas evidence for the efficacy of monensin was too scarce to support reliable inference.

Despite these findings, further research employing more detailed and robust study designs is required to fully assess the long-term efficacy of feed additives in mitigating enteric methane emissions from dairy cattle. There is a clear need to establish globally accepted methods for measuring methane emissions, including comparative evaluations of the three approaches considered in this meta-analysis, as well as emerging methods such as sniffer-based technologies. In addition, studies investigating feed additives in animal nutrition should, where feasible, extend beyond a few weeks or months. Finally, dosage reporting should be standardized to facilitate identification of thresholds that optimize methane mitigation without compromising production performance. Addressing these issues would enable more robust conclusions regarding the efficacy of feed additives in reducing methane emissions in dairy cattle and support meaningful comparisons among different mitigation strategies.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Dataset available by Editor and reviewers request. Requests to access these datasets should be directed to Thomas Dalmonte, thomas.dalmonte2@unibo.it.

Author contributions

TD: Visualization, Software, Formal analysis, Writing – original draft, Data curation, Methodology, Conceptualization, Investigation. NB: Data curation, Software, Methodology, Investigation, Writing – original draft. SG: Writing – original draft, Investigation, Visualization. AP: Visualization, Conceptualization, Writing – original draft. VI: Conceptualization, Investigation, Visualization, Writing – original draft. YV: Visualization, Writing – original draft, Conceptualization. GG: Visualization, Investigation, Writing – original draft. IA: Writing – original draft, Investigation, Visualization. AF: Investigation, Conceptualization, Writing – original draft, Methodology, Supervision. AS: Writing – original draft, Conceptualization, Visualization. FA: Writing – original draft, Conceptualization, Validation. AD: Validation, Resources, Visualization, Conceptualization, Supervision, Writing – review & editing, Writing – original draft.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1754069/full#supplementary-material

References

Allen, M. (2020). Understanding the practice, application, and limitations of meta-analysis. *Am. Behav. Sci.* 64, 74–96. doi: 10.1177/0002764219859619

Alvarez-Hess, P. S., Williams, S. R. O., Jacobs, J. L., Hannah, M. C., Beauchemin, K. A., Eckard, R. J., et al. (2019). Effect of dietary fat supplementation on methane emissions from dairy cows fed wheat or corn. *J. Dairy Sci.* 102, 2714–2723. doi: 10.3168/jds.2018-14721

Baceninaite, D., Dzermeikaité, K., and Antanaitis, R. (2022). Global warming and dairy cattle: how to control and reduce methane emissions. *Animals.* 12, 2687. doi: 10.3390/ani12192687

Bayat, A. R., Kairenius, P., Stefanski, T., Leskinen, H., Comtet-Marre, S., Forano, E., et al. (2015). Effect of camelina oil or live yeast (Saccharomyces cerevisiae) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. *J. Dairy Sci.* 98, 3166–3181. doi: 10.3168/jds.2014-7976

Bayat, A. R., Tapio, I., Vikki, J., Shingfield, K. J., and Leskinen, H. (2017a). Plant oil supplements reduce methane emissions and improve milk fatty acid composition in dairy cows fed grass silage-based diets without affecting milk yield. *J. Dairy Sci.* 101, 1136–1151. doi: 10.3168/jds.2017-13545

Bayat, A. R., Ventto, L., Kairenius, P., Stefanski, T., Leskinen, H., Tapio, I., et al. (2017b). Dietary forage to concentrate ratio and sunflower oil supplement alter rumen fermentation, ruminal methane emissions, and nutrient utilization in lactating cows. *Transl. Anim. Sci.* 1, 277–286. doi: 10.2527/tas2017.0032

Bayat, A. R., Vikki, J., Razzaghi, A., Leskinen, H., Kettunen, H., Khurana, R., et al. (2022). Evaluating the effects of high-oil rapeseed cake or natural additives on methane emissions and performance of dairy cows. *J. Dairy Sci.* 105, 2. doi: 10.3168/jds.2021-20537

Beauchemin, K. A., Kreuzer, M., O'Mara, F., and McAllister, T. A. (2008). Nutritional management for enteric methane abatement: a review. *Aust. J. Exp. Agric.* 48, 21–27. doi: 10.1071/EA07199

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., and Wang, M. (2020). Review: fifty years of research on rumen methanogenesis-leessons learned and future challenges for mitigation. *Animal* 14, 2–16. doi: 10.1017/S1751731119003100

Benchaar, C. (2016). Diet supplementation with cinnamon oil, cinnamaldehyde, or Monensin does not reduce enteric methane production of dairy cows. *Animal.* 10, 418–425. doi: 10.1017/S175173111500230X

Benchaar, C. (2019). Feedign oregano oil and its main component carvacrol does not affect ruminal fermentation, nutrient utilization, methane emissions, milk production, or milk fatty acid composition of dairy cows. *J. Dairy Sci.* 103, 1516–1527. doi: 10.3168/jds.2019-17230

Benchaar, C., and Hassanat, F. (2024). Diet supplementation with a mixture of essential oils: effects on enteric methane emissions, apparent total-tract nutrient digestibility, nitrogen utilization, and lactational performance. *J. Dairy Sci.* 108, 3560–3572. doi: 10.3168/jds.2024-25447

Benchaar, C., Hassanat, F., Martineau, R., and Gervais, R. (2015). Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. *J. Dairy Sci.* 98, 79993–78008. doi: 10.3168/jds.2015-9398

Boutes, A., Barbier, E., and Routier, M. (2024). The use of sniffer analyzer to measure enteric methane emissions under commercial conditions in dairy cow. *J. dairy Vet. Sci.* 17, 2. doi: 10.19080/JDVS.2024.17.555960

Broderick, G. A. (2018). Review: optimizing ruminant conversion of feed protein to human food protein. *Animal.* 12, 1722–1734. doi: 10.1017/S1751731117002592

Cardinale, S., and Kadarmideen, H. N. (2022). Host genome-metagenome analyses using combinatorial network methods reveal key metagenomic and host genetic features for methane emission and feed efficiency in cattle. *Front. Genet.* 13. doi: 10.3389/fgene.2022.795717

Chung, Y. H., He, M. L., McGinn, S. M., McAllister, T. A., and Beauchemin, K. A. (2011). Linseed suppresses enteric methane emissions from cattle fed barley silage, but not from those fed grass hay. *Anim. Feed Sci. Technol.* 166, 321–329. doi: 10.1016/j.anifeedsci.2011.04.022

Darabighane, B., Tapio, I., Ventto, L., Kairenius, P., Stefanski, T., Leskinen, H., et al. (2021). Effects of starch level and a mixture of sunflower and fish oils on nutrient intake and digestibility, rumen fermentation, and ruminal methane emissions in dairy cows. *Animals.* 11, 1310. doi: 10.3390/ani11051310

Fresco, S., Boichard, D., Fritz, S., and Martin, P. (2024). Genetic parameters for methane production, intensity, and yield predicted from milk mid-infrared spectra throughout lactation in Holstein dairy cows. *J. Dairy Sci.* 107, 11311–11323. doi: 10.3168/jds.2024-25231

Haisan, J., Sun, Y., Guan, L. L., Beauchemin, K. A., Iwaasa, A., Duval, S., et al. (2014). The effects of feeding 3-nitrooxyproanol on methane emissions and productivity of Holstein cows in mid lactation. *J. Dairy Sci.* 97, 3110–3119. doi: 10.3168/jds.2013-7834

Hamilton, S. W., DePeters, E. J., McGarvey, J. A., Lathrop, J., and Mitloenher, F. M. (2010). Greenhouse gas, animal performance, and bacterial population structure responses to dietary Monensin fed to dairy cows. *J. Environ. Qual.* 39, 106–114. doi: 10.2134/jeq2009.0035

Hassanat, F., and Benchaar, C. (2021). Corn silage-based diet supplemented with increasing amounts of linseed oil: effects on methane production, rumen fermentation, nutrient digestibility, nitrogen utilization, and milk production of dairy cows. *J. Dairy Sci.* 104, 5375–5390. doi: 10.3168/jds.2020-18853

Hedges, I. V., Tripton, E., and Johnson, M. C. (2010). Robust variance estimation in meta-regression with dependent effect size estimates. *Res. Synth. Methods* 1, 39–65. doi: 10.1002/jrsm.5

Higgins, J. P. T., Thompson, S. G., Deeks, J. J., and Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ.* 327, 557. doi: 10.1136/bmj.327.7414.557

Hindrichsen, I. K., Wettstein, H. R., Machmueller, A., Soliva, C. R., Knudsen, K. E. B., Madsen, J., et al. (2004). Effects of feed carbohydrates with contrasting properties on rumen fermentation and methane release *in vitro*. Can. *Vet. J.* 84, 265–276. doi: 10.4141/A03-095

Hoojimans, C. R., Rovers, M. M., De Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., and Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* 14, 43. doi: 10.1186/1471-2288-14-43

Hristov, A. N., Lee, C., Cassidy, T., Heyler, K., Tekippe, J. A., Varga, G. A., et al. (2013a). Effect of Origanum vulgare L. leaves on rumen fermentation, production, and milk fatty acid composition in lactating dairy cows. *J. Dairy Sci.* 96, 1189–1202. doi: 10.3168/jds.2012-5975

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kenbreab, E., Waghorn, G., et al. (2013b). Mitigation of methane and nitrous oxide emissions from animal operations: a review of enteric methane mitigation options. *J. Anim. Sci.* 91, 5045–5069. doi: 10.2527/jas.2013-6583

Hristov, A. N., Oh, J., Giallongo, F., Frederick, T. W., Harper, M., Weeks, H. L., et al. (2015). An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. *Proc. Natl. Acad. U.S.A. 25* 112, 10663–10668. doi: 10.1073/pnas.1504124112

Jonker, A., Antwi, C., Gunter, S. A., Hristov, A., Martin, C., Minnée, E. M. K., et al. (2020). "The 'GreenFeed' automated methane measurement system to determine enteric methane emissions from ruminants," in *Chapter 3, pages 11–14 in Guidelines for estimating methane emissions from individual ruminants using: GreenFeed, 'sniffers', hand-held laser detector and porta ble accumulation chambers* (Ministry for Primary Industries, Wellington, New Zealand). Available online at: https://www.mpi.govt.nz/resources-and-forms/publications/ (Accessed July 30, 2024).

Kebreab, E., Bannink, A., Pressman, E. M., Walker, N., Karagiannins, A., Van Gastelen, S., et al. (2023). A meta-analysis of effects of 3-nitrooxypropanol on methane

producton, yield, and intensity in dairy cattle. *J. Dairy Sci.* 10, 927–936. doi: 10.3168/jds.2022-22211

Khurana, R., Brand, T., Tapio, I., and Bayat, A. R. (2023). Effect of a garlic and citrus extract supplement on performance, rumen fermentation, methane production, and rumen microbiome of dairy cows. *J. Dairy Sci.* 106, 4608–4621. doi: 10.3168/jds.2022-22838

Kliem, K. E., Humphries, D. J., Kirton, P., Givens, D. I., and Reynolds, C. K. (2019). Differential effects of oilseed supplements on methane production and milk fatty acid concentrations in dairy cows. *Animal.* 13, 309–317. doi: 10.1017/S1751731118001398

Konstantpoulos, S. (2011). Fixed effects and variance components estimation in three-level meta-analysis. *Res. Synth. Methods* 2, 61–76. doi: 10.1002/jrsm.35

Laujeunesse, M. J. (2011). On the meta-analysis of response ratios for studies with correlated and multi-group designs. *Ecology.* 92, 2049–2055. doi: 10.1890/11-0423.1

Laujeunesse, M. J. (2015). Bias and correction for the log response ratio in ecological meta-analysis. *Ecology.* 96, 2056–2063. doi: 10.1890/14-2402.1

Livingstone, K. M., Humphries, D. J., Kirton, P., Kliem, K. E., Givens, D. I., and Reynolds, C. K. (2015). Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows. *J. Dairy Sci.* 98, 4000–4011. doi: 10.3168/jds.2014-8987

MaChado, F. S., Tomich, T. R., Ferreira, A. L., Cavalcanti, L. F. L., Campos, M. M., Paiva, C. A. V., et al. (2016). Technical note: a facility for respiration measurements in cattle. *J. Dairy Sci.* 99, 4899–4906. doi: 10.3168/jds.2015-10298

Maigaard, M., Weisbjerg, M. R., Johansen, M., Walker, N., Ohlsson, C., and Lund, P. (2024). Effects of dietary fat, nitrate, 3-nitrooxypropanol and their combinations on methane emission, feed intake, and milk production in dairy cows. *J. Dairy Sci.* 107, 220–241. doi: 10.3168/jds.2023-23420

Martin, C., Coppa, M., Fougere, H., Bougouin, A., Baumont, R., Eugene, M., et al. (2021). Diets supplemented with corn oil and wheat starch, marine algae, or hydrogenated palm oil modulate methane emissions similarly in dairy goats and cows, but not feeding behavior. *Anim. Feed Sci. Technol.* 272, 114783. doi: 10.1016/j.anifeedsci.2020.114783

Martin, C., Ferlay, A., Mosoni, O., Rochette, Y., Chilliard, Y., and Doreau, M. (2016). Increasing linseed supply in dairy cow diets based on hay or corn silage: effect on enteric methane emission, rumen microbial fermentation, and digestion. *J. Dairy Sci.* 99, 3445–3456. doi: 10.3168/jds.2015-10110

Martin, C., Morgavi, D. P., and Doreau, M. (2010). Methane mitigation in ruminants: from microbiome to the farm scale. *Animal.* 4, 351–365. doi: 10.1017/S1751731109990620

Martin, C., Rouel, J., Jouany, J. P., Doreau, M., and Chilliard, Y. (2008). Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. *J. Anim. Sci.* 86, 2642–2650. doi: 10.2527/jas.2007-0774

Massaro, S., Giannuzzi, D., Amalfitano, N., Schiavon, S., Bittante, G., and Tagliapietra, F. (2024). Review of equations to predict methane emissions in dairy cows from milk fatty acid profiles and their application to commercial dairy farms. *J. Dairy Sci.* 107, 5833–5852. doi: 10.3168/jds.2024-24814

Mayerfeld, D., Fulwider, W., and Parrish, A. (2023). *Methane emissions from livestock and climate change*. Available online at: https://cropsandsoils.extension.wisc.edu/articles/methane-emissions-from-livestock-and-climate-change/ (Accessed July 30, 2024).

Melgar, A., Lage, C. F. A., Nedelkov, K., Raisanen, S. E., Stefanoni, H., Fetter, M. E., et al. (2021). Enteric methane emission, milk production, and composition of dairy cows fed 3-nitrooxypropanol. *J. Dairy Sci.* 104, 357–366. doi: 10.3168/jds.2020-18908

Min, B. R., Lee, S., Jung, H., Miller, D. N., and Chen, R. (2022). Enteric methane emissions and animal performance in dairy and beef cattle production: strategies, opportunities, and impact of reducing emissions. *Animals. 7(12)* 8, 948. doi: 10.3390/ani12080948

Munoz, C., Munoz, I. A., Rodriguez, R., Urrutia, N. L., and Ungarfeld, E. M. (2024). Effect of combining the methanogenesis inhibitor 3-nitrooxypropanol and cottonseeds on methane emission, feed intake, and milk production of grazing dairy cows. *Animal.* 18, 101203. doi: 10.1016/j.animal.2024.101203

Munoz, C., Villalobos, R., Peralta, A. M. T., Morales, R., Urrutia, N. L., and Ungerfeld, E. M. (2021). Long-term and carryover effects of supplementation with whole oilseeds on methan emission, milk production and milk fatty acid profile of grazing dairy cows. *Animals.* 11, 2978. doi: 10.3390/ani11102978

Munoz, C., Yan, T., Wills, D. A., Murray, S., and Gordon, A. W. (2012). Comparison of the sulfur hexafluoride tracer and respiration chamber techniques for estimating. *J. Dairy Sci.* 95, 3139–3148. doi: 10.3168/jds.2011-4298

National Research Council (NRC) (2001). *Nutrient requirements of dairy cattle. 7th revised edition* (Wahington DC: National Academic Press). doi: 10.17226/9825

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., and Hoffmann, T. C. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 372, 71. doi: 10.1136/bmj.n71

Patra, A. K., and Yu, Z. (2012). Effects of essential oils on methane production and fermentation by rumen microbes. *Appl. Microbiol. Biotechnol.* 95, 339–353. doi: 10.1128/AEM.00309-12

Pirondini, M., Colomcini, S., Mele, M., Malagutti, L., Rapetti, L., Galassi, G., et al. (2015). Effect of dietary starch concentration and fish oil supplementation on milk yield and composition, diet digestibility, and methane emissions in lactating dairy cows. *J. Dairy Sci.* 98, 357–372. doi: 10.3168/jds.2014-8092

Satterthwaite, F. E. (1946). An Approximate Distribution of Estimates of Variance Components. *Biometrics Bulletin.* 2, 110–114. doi: 10.2307/3002019

Seidler, A. L., Hunter, K. E., Cheyne, S., Berlin, J. A., Ghersi, D., and Askie, L. M. (2020). Prospective meta-analyses and Cochrane.s role in embracing next-generation methodologies. *Cochrane Database Syst. Rev*. 10, 1-3. doi: 10.1002/14651858.ED000145

Shilde, M., Von Soosten, D., Huther, L., Meyer, U., Zeyner, A., and Danicke, S. (2021). Effects of 3-nitrooxypropanol and varying concentrate feed proportions in the ration on methane emission, rumen fermentation and performance of periparturient dairy cows. *Arch. Anim. Nutr.* 75, 79–104. doi: 10.1080/1745039X.2021.1877986

Shingfield, K. J., Bonnet, M., and Scollan, N. D. (2013). Recent developments in altering the fatty acid composition of ruminants-derived foods. *Animal.* 7, 132–162. doi: 10.1017/S1751731112001681

Silvestre, T., Martins, L. F., Cueva, S. F., Wasson, D. E., Stepanchenko, N., Raisanen, S. E., et al. (2023). Lactational performance, rumen fermentation, nutrient use efficiency, enteric methane emissions, and manure greenhouse gas-emitting potential in dairy cows fed a blend of essential oils. *J. Dairy Sci.* 106, 7661–7674. doi: 10.3168/jds.2022-23181

Storlien, T. M., Prestlokken, E., Beauchemin, K. A., McAllister, T. A., Iwaasa, A., and Harstrad, O. M. (2017). Supplementation with crushed rapeseed causes reduction of methane emissions from lactating dairy cows on pasture. *Anim. Prod. Sci.* 57, 81–89. doi: 10.1071/AN15287

Teague, W. R., Apfelbaum, S., Lal, R., Kreuter, U. P., Rowntree, J., Davies, C. A., et al. (2016). The role of ruminants in reducing agriculture's carbon footprint in North America. *J. Soil Water Conserv.* 71, 156–164. doi: 10.2489/jswc.71.2.156

Tondini, S. M., Bayat, A. R., Khelil-Arfa, H., Blanchard, A., and Yanez-Ruiz, D. R. (2024). Effect of a blend of cinnamaldehyde, eugenol and capsicum oleoresin on methane emission and lactation performance of Nordic Red dairy cows fed grass silage-based diets. *Livest. Sci.* 284, 105494. doi: 10.1016/j.livsci.2024.105494

Tripton, E. (2015). Small sample adjustments for robust variance estimation with meta-regression. *Psychol. Methods* 23, 425–438. doi: 10.1037/met0000011

Van Den Noortgate, W., Lopez-Lopez, J. A., Marin-Martinez, F., and Sanchez-Meca, J. (2013). Three-level meta-analysis of dependent effect sizes. *Behav. Res. Methods* 45, 576–594. doi: 10.3758/s13428-012-0261-6

Van Gastelen, S., Dijkstra, J., Binnendijk, G., Duval, S. M., Heck, J. M. L., Kindermann, M., et al. (2020). 3-nitrooxypropanol decreases methane emissions and increase hydrogen emissions of early lactation dairy cows, with associated changes in nutrient digestibility and energy metabolism. *J. Dairy Sci.* 103, 8074–8093. doi: 10.3168/jds.2019-17936

Van Lingen, H. K., Edwards, J. E., Vaidya, J. D., Van Gastelen, S., Saccenti, E., Van den Bogert, B., et al. (2017). Diurnal dynamics of gaseous and dissolved metabolites and microbiota composition in the bovine rumen. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00425

Van Wesemael, D., Vandaele, L., Ampe, B., Cattrysse, H., Duval, S., Kindermann, M., et al. (2020). Reducing enteric methane emissions from dairy cattle: two ways to supplement 3-nitrooxypropanol. *J. Dairy Sci.* 102, 1780–1787. doi: 10.3168/jds.2018-14534

Viechtbauer, W., and Cheaung, M. W. L. (2010). Outlier and influence diagnostic for meta-analyses. *Res. Synth. Methods* 1, 112–125. doi: 10.1002/jrsm.11

Walker, E., Hernandez, A. V., and Kattan, M. V. (2008). Meta-analysis: its strengths and limitations. *Cleve. Clin. J. Med.* 75, 431–439. doi: 10.3949/ccjm.75.6.431

Williams, S. R. O., Hannah, M. C., Eckard, R. J., Wales, W. J., and Moate, P. J. (2020). Supplementing the diet of dairy cows with fat or tannin reduces methane yield, and additively when fed in combination. *Animal.* 14, 464–472. doi: 10.1017/S1751731120001032

Wu, D., Zhang, J., Zhao, Y., Wang, Z., and Li, G. (2018). Measurement of methane emissions from individual dairy cows using the GreenFeed system. *J. Dairy Sci.* 101, 10479–10488.

Yan, M., Pratama, A. A., Somasundaram, S., Li, Z., Jiang, Y., Sullivan, M. B., et al. (2023). Interrogating the viral dark matter of the rumen ecosystem with a global virome database. *Nat. Commun.* 14, 5254. doi: 10.1038/s41467-023-41075-2

Zhang, L., Huang, X., Xue, B., Peng, Q., Wang, Z., Yan, T., et al. (2015). Immunization against rumen methanogenesis by vaccination with a new recombinant protein. *PloS One* 10, e0140086. doi: 10.1371/journal.pone.0140086