



Original Research Article

Meta-analysis quantifying the potential of dietary additives and rumen modifiers for methane mitigation in ruminant production systems

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ABSTRACT

Increasingly countries are seeking to reduce emission of greenhouse gases from the agricultural industries, and livestock production in particular, as part of their climate change management. While many reviews update progress in mitigation research, a quantitative assessment of the efficacy and performance-consequences of nutritional strategies to mitigate enteric methane (CH₄) emissions from ruminants has been lacking. A meta-analysis was conducted based on 108 refereed papers from recent animal studies (2000–2020) to report effects on CH₄ production, CH₄ yield and CH₄ emission intensity from 8 dietary interventions. The interventions (oils, microalgae, nitrate, ionophores, protozoal control, phytochemicals, essential oils and 3-nitrooxypropanol). Of these, macroalgae and 3-nitrooxypropanol showed greatest efficacy in reducing CH₄ yield (g CH₄/kg of dry matter intake) at the doses trialled. The confidence intervals derived for the mitigation efficacies could be applied to estimate the potential to reduce national livestock emissions through the implementation of these dietary interventions.

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1. Introduction

Recognising the urgent need to address climate change, nations have agreed to reduce greenhouse gas (GHG) emissions, aiming for net zero emissions by the second half of this century (UNFCCC, 2015). Livestock enteric methane (CH₄) contributes 11.6% of global GHG emissions from anthropogenic activities (Ripple et al., 2014), and it is the main source of GHG in agriculture, accounting for 43% of the GHG emissions from livestock globally (Herrero et al., 2016). Enteric CH₄ emissions represent a waste of energy by the ruminant fermentation process, and efforts are being made to identify and encourage actions to reduce these emissions (Rivera-Ferre et al., 2016).

As CH₄ has a relatively brief lifetime in the atmosphere (i.e., from 8.4 to 12 years, Ehhalt et al., 2001), mitigating CH₄ may represent a timely contribution to achieving climate stabilisation targets (Reisinger et al., 2021). Mitigation efficacy of these many strategies have been reported and often combined in broad-ranging reviews (Martin et al., 2010; Cottle et al., 2011; Asizua et al., 2014; Patra, 2016; Grossi et al., 2019), the effect of feed management on animal productivity is less well assessed. Progress towards carbon neutrality for the ruminant production sector may involve nutritional strategies, as well as whole farm systematic approaches including vaccines, improving reproductive rate, stock number and productivity, pasture management and animal genetics.

The nutritional approach, more specifically rumen manipulation, encompasses a wide range of possibilities (e.g., oils, algae, nitrate, ionophores, protozoa population control, bacteriocins, phytochemicals, 3-nitrooxypropanol, acetogens, organic acids, among others). In this meta-analysis, we focus on nutritional strategies that are more likely to contribute to carbon neutrality in the near decades. Therefore, the approach of this assessment is to use CH₄ mitigation data published since 2000 to quantify the technical potential of strategies for CH₄ mitigation by ruminants (cattle and sheep), as well as quantifying the co-benefits and

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identifying barriers to implementation. The ultimate purpose of this assessment is to estimate the CH₄ mitigation potential of nutritional strategies to inform the development of effective policies to support CH₄ abatement.

2. Materials and methods

2.1. Published literature screening

A database was created with publications from 2000 to 2020, using only reports of *in vivo* trials that measured ruminant enteric CH₄ emissions. Studies included addressed the effects of a range of dietary abatement measures (oils, algae, nitrate, ionophores, protozoa population control, bacteriocins, phytochemicals, 3-nitrooxypropanol, acetogens, organic acids). Keywords used to identify papers were as follows: “ruminant”, “enteric”, “methane emission”, and one of the potential strategies. Pertinent literature cited in each considered article was also screened for inclusion. All data were from articles published in indexed journals identified through searches conducted using the Google Scholar search engine (<https://scholar.google.com>) from 18th of August 2019 to 26th of April 2020. Data were entered in an Excel spreadsheet in a systematic fashion in which each row represented a treatment and each column represented an exploratory variable (Sauvant et al., 2008). A summary of the data of all publications is presented in Table 1. Data reported in divergent units of measure were transformed to matching units. When a study did not report all needed results and it was possible to calculate from the reported data, appropriate calculations were performed from the reported data.

The investigated factors were body weight (BW; kg), dry matter (DM; kg/d) intake, liveweight gain (g/d), milk production (kg/d), diet chemical composition (crude protein, CP; neutral detergent fiber, NDF; fat; and non-fiber carbohydrate, NFC; %DM), and digestibility of nutrients (DM, CP, fat and NDF; %). Methane emissions were reported as CH₄ production (g CH₄/animal per d), CH₄ yield (MY; g CH₄/kg DMI) and CH₄ emission intensity (MI; g CH₄/kg animal product, typically milk or liveweight gain).

2.2. Inclusion criteria

For inclusion in the meta-analysis, studies were required to have MY (g/kg DMI) measured, as well as data reported on composition of diets and intake. Besides these, other variables required in the dataset were digestibility, animal performance and rumen fermentation indicators (pH, molar proportion of volatile fatty acids, and protozoa count). Overall, 108 publications met these requirements and were included in the analysis (Table 1).

2.3. Statistical analysis

The meta-analysis was performed using the MIXED procedure of SAS (version 9.4, SAS/STAT, SAS Institute Inc., Cary, NC), considering study as a random effect. Furthermore, to account for variations in precision across studies, the inverse of the squared standard error of the mean (SEM) (Wang and Bushman, 1999) of MY was used as a factor in the WEIGHT statement of the model (St-Pierre, 2001). The slopes and intercepts by study were included as random effects, specifying an unstructured variance-covariance matrix for the intercepts and slopes (St-Pierre, 2001). Differences between means were determined using the P-DIFF option of the LSMEANS statement, which is based on Fisher's F-protected least significant difference test. Significant difference was declared at $P < 0.05$.

3. Results and discussion

An appraisal of the quantitative potential enteric CH₄ abatement of each considered dietary strategy is given below and summarized in Fig. 1. Among the strategies assessed, one may note that nutritional management can alter MPR and MY by multiple means that directly target methanogens or affect methanogenesis by altering residual hydrogen availability in the rumen.

3.1. Oils

Among the several dietary strategies specifically developed to mitigate enteric CH₄ production, oil inclusion in the diet is the one with most papers published in the last 20 years, that were included in the meta-analysis ($n = 35$; Table 1). Our analysis revealed that the MY mitigation ranges from 12% to 20% (95% CI of the mean effect size; mean reduction of 15%). For every increase of 1% of oil (10 g/kg DM) from 2.85% to 6.20% inclusion, the MY was reduced by 1.02 ± 0.113 g CH₄/kg DMI or 4.37% ($P < 0.01$; RMSE = 4.56). A previous meta-analysis (of 17 studies) examined the reduction in MY in response to oil in the diet and reported that for each 1% oil added to the diet MY was reduced by 5.6% (Beauchemin et al., 2007).

Oils (i.e., polyunsaturated fatty acids and the medium-chain saturated fatty acids) have been previously recognised to suppress CH₄ production in ruminants (Blaxter and Czerkawski, 1966). For instance, adding oil to the ruminant diet reduces H₂ producers (i.e., protozoa; Mao et al., 2010, Guyader et al., 2015), as well as methanogen populations (Mao et al., 2010), and may act as a H⁺ acceptor through fatty acid biohydrogenation, although the effect is small (Ungerfeld, 2015). Among the papers included, only 2 (Johnson et al., 2002, Silva et al., 2018) out of 35 showed that adding oil to the diet of dairy cattle, beef cattle, or sheep did not affect enteric CH₄ production.

Oil addition within the range of the studies included reduced DMI by 1.24% to 6.17% (Fig. 2A), and reduced NDF digestibility by 6.30% to 13.0% (Fig. 2B). No effect of oil on growth rate was detected using the present database, whereas oil addition decreased milk production by 1.17% to 13.7% (95% CI). The extent of the CH₄ mitigation by dietary oil may vary with basal diet. Oil can be added to a low-fibre diet without impairing fibre digestibility, but negative effects on DMI, fibre digestibility, as well as animal performance, have been reported from adding oil to ruminants fed high-fibre diets (Machmüller et al., 2001; Machmüller et al., 2003; Benchaar et al., 2015; Beauchemin and McGinn, 2006; Hollmann et al., 2012, Troy et al., 2015). Therefore, the risk of adverse effects on fibre digestibility could restrict the use of oils as a mitigation strategy for grazing livestock.

A reduction in MI (g CH₄/kg of milk or weight gain) from 14.4% to 21.5% ($P < 0.01$; Fig. 2C) was found. Supplementation with unsaturated fatty acid rich-oils may influence the biohydrogenation, yielding the production of trans-10 18:1 fatty acid, which may result in a greater concentration of anti-lipogenic conjugated linoleic acid (trans-10, cis 12) production in the mammary gland (Odongo et al., 2007), and therefore may depress milk production (Baumgard et al., 2002). Moreover, the practicality of oil supplementation in the diet in a farm setting should be evaluated considering its benefits in CH₄ mitigation, as well as effects on animal performance and cost of feeding. Oil as a mitigation strategy can readily be applied to feedlot and dairy systems. The main barriers to adoption in grazing systems relate to reduction in fibre digestibility and logistics of delivery in extensive rangelands.

Table 1
Summary of the data used in the meta-analysis of the effect of different strategies for enteric methane abatement.

Study code	Source	Animal	Strategy	Methane analysis method	n
1	Alemu et al. (2019)	Sheep	Phytochemicals, NO ₃	GreenFeed	22
2	Beauchemin et al. (2006)	Beef	Phytochemicals, oil, organic acid	Chamber	8
3	Beauchemin et al. (2007)	Beef	Oil	Chamber	4
4	Beauchemin et al. (2009)	Dairy	Oil, protozoa control	Chamber	4
5	Benchaar (2016)	Dairy	Phytochemicals, ionophores	SF ₆	8
6	Benchaar et al. (2015)	Dairy	Oil, protozoa control	Chamber	6
7	Bird et al. (2008)	Sheep	Protozoa control	Chamber	7
8	Caetano et al. (2019)	Beef	Phytochemicals	GreenFeed	10
9	Carulla et al. (2005)	Sheep	Phytochemicals, protozoa control	Chamber	6
10	Carvalho et al. (2016)	Beef	Oil	SF ₆	9
11	Chung et al. (2013)	Beef	Phytochemicals, protozoa control	Chamber	8
12	Coopridge et al. (2011)	Beef	Ionophores	Chamber	4
13	Cosgrove et al. (2008)	Sheep	Oil	SF ₆	2
14	Ding et al. (2012)	Sheep	Oil	other	3
15	Duthie et al. (2018)	Beef	NO ₃	Chamber	18
16	El-Zaiat et al. (2014)	Sheep	Phytochemicals, NO ₃	Chamber	6
17	Fiorentini et al. (2014)	Beef	Oil, protozoa control	SF ₆	9
18	Grainger et al. (2008)	Dairy	Oil	SF ₆	6
19	Grainger et al. (2008b)	Dairy	Ionophores	Chamber/SF ₆	15
20	Grainger et al. (2009)	Dairy	Phytochemicals	SF ₆	10
21	Grainger et al. (2010)	Dairy	Ionophores	Chamber/SF ₆	10/15
22	Ganja-Salcedo et al. (2019)	Beef	NO ₃	SF ₆	10
23	Guyader et al. (2015a)	Dairy	phytochemicals, NO ₃	Chamber	4
24	Guyader et al. (2015b)	Dairy	Oil, NO ₃ , protozoa control	Chamber	4
25	Guyader et al. (2016)	Dairy	Oil, NO ₃	Chamber	8
26	Haisan et al. (2014)	Dairy	3-nitrooxypropanol (3-NOP)	SF ₆	5
27	Haisan et al. (2017)	Dairy	3-NOP	SF ₆	6
28	Hegarty et al. (2008)	Sheep	Protozoa control	Chamber	6
29	Hess et al. (2016)	Sheep	Phytochemicals, protozoa control	Chamber	6
30	Hollmann et al. (2012)	Dairy	Oil	Chamber	6
31	Holtshausen et al. (2009)	Dairy	Phytochemicals, protozoa control	Chamber/SF ₆	4
32	Hosoda et al. (2005)	Dairy	Phytochemicals	Chamber	4
33	Hristov et al. (2013)	Dairy	Phytochemicals	SF ₆	8
34	Hristov et al. (2015)	Dairy	3-NOP	GreenFeed	12
35	Hulshof et al. (2012)	Beef	NO ₃	SF ₆	8
36	Hünerberg et al. (2013a)	Beef	Oil	Chamber	8
37	Hünerberg et al. (2013b)	Beef	Oil	Chamber	4
38	Johnson et al. (2002)	Dairy	Oil	SF ₆	4
39	Jordan et al. (2006a)	Beef	Oil	Chamber	10
40	Jordan et al. (2006b)	Beef	Oil	SF ₆	12
41	Jordan et al. (2007)	Beef	Oil	SF ₆	4
42	Jose Neto et al. (2019)	Beef	Oil	SF ₆	9
43	Kim et al. (2019)	Beef	3-NOP	GreenFeed	9
44	Kinley et al. (2020)	Beef	Seaweed	Chamber	5
45	Klevenhusen et al. (2011)	Sheep	Phytochemicals, protozoa control	Chamber	6
46	Lee et al. (2015)	Beef	NO ₃	Chamber	8
47	Lee et al. (2017)	Beef	NO ₃	Chamber	7
48	Lee et al. (2017)	Beef	NO ₃	Chamber	7
49	Li et al. (2012)	Sheep	NO ₃	Chamber	5
50	Li et al. (2013)	Sheep	NO ₃	Chamber	6
51	Li et al. (2018)	Sheep	Seaweed	Chamber	6
52	Liu et al. (2011)	Sheep	Phytochemicals, oil	Chamber	8
53	Lopes et al. (2016)	Dairy	3-NOP	GreenFeed	6
54	Ma et al. (2015)	Sheep	Phytochemicals	Chamber	6
55	Ma et al. (2017)	Sheep	Phytochemicals	Chamber	6
56	Machmüller et al. (2000)	Sheep	Oil, protozoa control	Chamber	3
57	Machmüller et al. (2001)	Sheep	Oil, protozoa control	Chamber	3
58	Machmüller et al. (2003)	Sheep	Oil, protozoa control	Chamber	3
59	Malik et al. (2017)	Sheep	Phytochemicals, protozoa control	SF ₆	10
60	Mao et al. (2010)	Sheep	Phytochemicals, oil	Chamber	8
61	Martin et al. (2008)	Dairy	Oil	SF ₆	8
62	Martin et al. (2016)	Dairy	Oil	SF ₆	4
63	Martinez-Fernandez et al. (2018)	Beef	3-NOP	Chamber	4
64	McGinn et al. (2004)	Beef	Oil, organic acid, ionophores	Chamber	8
65	McGinn et al. (2009)	Beef	Oil	SF ₆	30
66	Melgar et al. (2020)	Dairy	NO ₃	GreenFeed	24
67	Moate et al. (2011)	Dairy	Oil	Chamber	4
68	Moate et al. (2014)	Dairy	Phytochemicals, protozoa control	SF ₆	10
69	Mohammed et al. (2004)	Dairy	Phytochemicals, protozoa control	Chamber	4
70	Moreira et al. (2013)	Sheep	Phytochemicals	SF ₆	3
71	Mwenya et al. (2005)	Dairy	Ionophores	Other	4
72	Newbold et al. (2014)	Beef	NO ₃	Chamber	6
73	Nguyen and Hegarty (2017)	Beef	Oil, protozoa control	Chamber	6

(continued on next page)

Table 1 (continued)

Study code	Source	Animal	Strategy	Methane analysis method	n
74	Nolan et al. (2010)	Sheep	NO ₃	Chamber	4
75	Norris et al. (2020)	Beef	Phytochemicals, protozoa control	Chamber	8
76	Odongo et al. (2007)	Dairy	Oil	Chamber	6
77	Odongo et al. (2007b)	Dairy	Ionophores	Other	12
78	Olijhoek et al. (2016)	Dairy	NO ₃	Chamber	4
79	de Oliveira et al. (2007)	Beef	Phytochemicals	SF ₆	8
80	Patra et al. (2011)	Sheep	Phytochemicals	Chamber	4
81	Pen et al. (2007)	Sheep	Phytochemicals	Chamber	4
82	Rebello et al. (2009)	Beef	NO ₃	SF ₆	10
83	Reynolds et al. (2014)	Dairy	3-NOP	GreenFeed	6
84	Romero-Perez et al. (2014)	Beef	3-NOP	Chamber	8
85	Romero-Perez et al. (2015)	Beef	3-NOP	Chamber	8
86	Roque et al. (2019)	Dairy	Seaweed	GreenFeed	12
87	Rossi et al. (2017)	Beef	Oil	SF ₆	7
88	Santoso et al. (2004)	Sheep	Phytochemicals	Chamber	4
89	Silva et al. (2018)	Beef	Oil	SF ₆	6
90	Soltan et al. (2013)	Sheep	Phytochemicals	Chamber	6
91	Staerfl et al. (2012)	Beef	Phytochemicals, protozoa control	Chamber	6
92	Sun et al. (2017)	Beef	NO ₃	Chamber	4
93	Tiemann et al. (2008)	Sheep	Phytochemicals	Chamber	6
94	Troy et al. (2015)	Beef	Oil, NO ₃	Chamber	6
95	Van Wesemael et al. (2019)	Dairy	3-NOP	GreenFeed	10
96	van Zijderveld et al. (2010)	Sheep	NO ₃	Chamber	5
97	van Zijderveld et al. (2011a)	Dairy	Oil, Phytochemicals	Chamber	10
98	van Zijderveld et al. (2011b)	Dairy	NO ₃	Chamber	5
99	Velazco et al. (2014)	Beef	NO ₃	GreenFeed	10
100	Veneman et al. (2015)	Dairy	oil, NO ₃	Chamber	6
101	Villar et al. (2019)	Beef	oil, NO ₃	Chamber	4
102	Vyas et al. (2016)	Beef	3-NOP	Chamber	5
103	Vyas et al. (2018a)	Beef	3-NOP	Chamber	5
104	Vyas et al. (2018b)	Beef	3-NOP, ionophores	Chamber	5
105	Waghorn et al. (2008)	Dairy	Ionophores	SF ₆	16
106	Wang et al. (2009)	Sheep	Phytochemicals	Chamber	4
107	Yang et al. (2017)	Beef	Phytochemicals, protozoa control	Chamber	4
108	Zhou et al. (2011)	Sheep	Phytochemicals, protozoa control	Chamber	3

n = total number of animals; 3-NOP = 3-nitrooxypropanol.

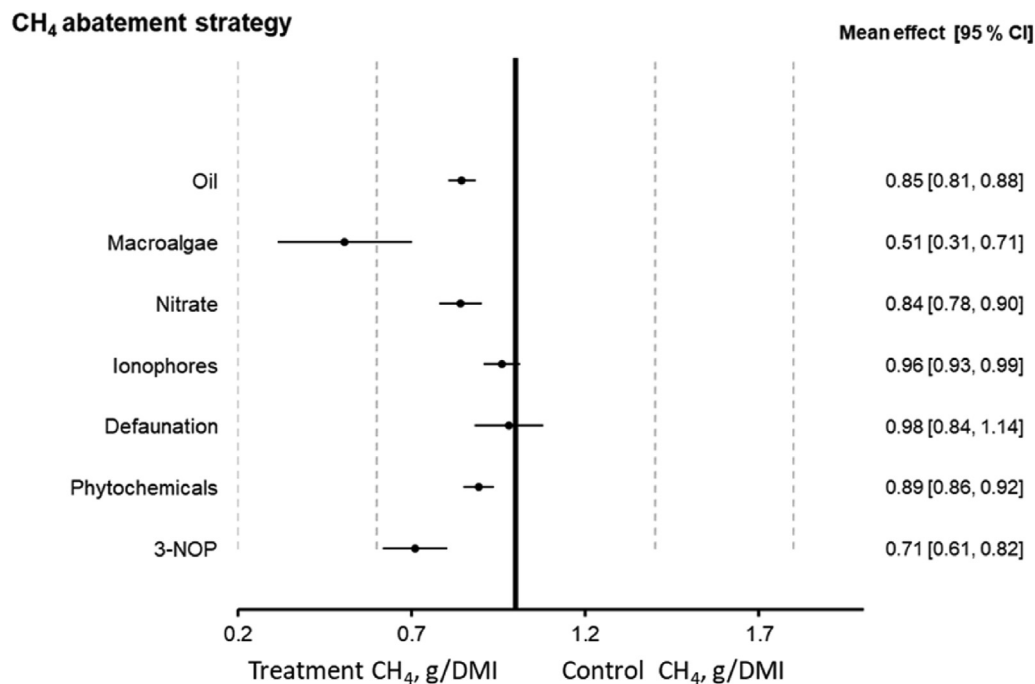


Fig. 1. Forest plot depicting the standardized mean effect of the estimated ratio of methane (CH₄) yields for mitigation strategy vs. control emissions (mean CH₄ emission in treatment with mitigation strategy divided by mean CH₄ emission in control) and the 95% confidence interval (95% CI). Values below 1.0 (vertical line) indicate that the mitigation strategy yields a reduction in CH₄ emissions.

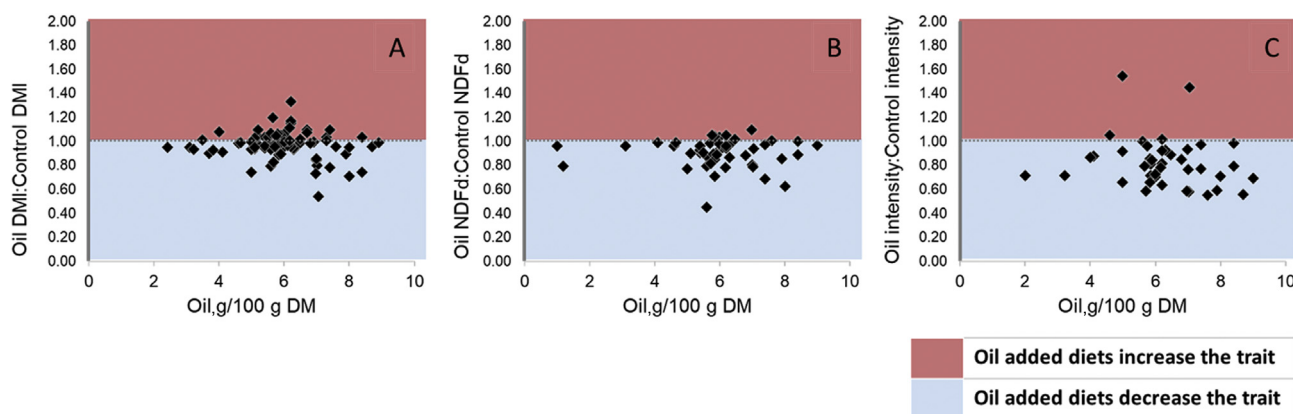


Fig. 2. Mean effect of diets containing oil on dry matter intake (DMI; A), neutral detergent fibre digestibility (NDFd; B) and methane (CH_4) intensity ($\text{g CH}_4/\text{kg animal product}$; C), expressed relative to control diets.

3.2. Seaweeds

In vivo animal trials testing seaweeds as a mitigation option have only recently been published. One study in sheep (Li et al., 2018), one in dairy cattle (Roque et al., 2019) and one in feedlot cattle (Kinley et al., 2020) are available, showing a dose dependent MY reduction from 30.0% up to 69.0% (95% CI; $P < 0.01$; $n = 3$; mean reduction of 49.0%), with *Asparagopsis* inclusion from 0.5% to 3.0%. Li et al. (2018) revealed reduction of 3.50 $\text{g CH}_4/\text{kg DMI}$ (i.e., or 23.3% CH_4 mitigation) for every gram of *Asparagopsis taxiformis* intake, with no effect on DMI or blood chemistry and pathology, but with some effect on rumen fermentation (Li et al., 2018), in sheep. Roque et al., 2019 reported 38% DMI reduction and reduced milk production when *Asparagopsis* was fed to dairy cows at 1% of DM.

Seaweeds are macroalgae, complex and diverse multicellular organisms that can grow in both marine and fresh water environments (van der Spiegel et al., 2013). The term “seaweed” has no taxonomic importance but is commonly used to refer to the marine algae (Makkar et al., 2016). Based on the pigment involved in their photosynthetic process, seaweeds can be categorised as red algae (*Rhodophyceae*), brown algae (*Phaeophyceae*), and green algae (*Chlorophyceae*) (Chapman and Chapman, 1980). There are more than 13,000 species of macroalgae (Huisman et al., 1998) and several species of macroalgae have been proposed as a novel ingredient in ruminant diets (van der Spiegel et al., 2013; Halmemies-Beauchet-Filleau et al., 2018). Seaweeds vary in chemical composition (Machado et al., 2014; Makkar et al., 2016), digestibility (i.e., 15% to 94% as reviewed by Makkar et al., 2016) and show a wide diversity and concentration of secondary metabolites (Carroll et al., 2019), including those by which CH_4 mitigation is achieved (Dubois et al., 2013; Machado et al., 2014; Kinley and Fredeen, 2015).

Previous studies have identified that the red macroalgae *A. taxiformis* has a high efficacy in CH_4 abatement in vitro (Kinley and Fredeen, 2015; Machado et al., 2016; Machado et al., 2018) and in vivo (Li et al., 2018) due to its high content of bromoform, a halogenated CH_4 analogue (Lanigan, 1972). Halogenated CH_4 analogues inhibit enzymatic activity of the methyltransferase enzyme by reacting with the reduced vitamin B_{12} cofactor required in one of the final steps of CH_4 formation, decreasing the cobamide-dependent pathway (Wood et al., 1968).

A previous study reported moderate potential of seaweed to be market-ready as a ruminant feed within 2 to 3 years (Halmemies-Beauchet-Filleau et al., 2018) and preparations are well underway for marketing of a commercial product in Australia. The

biochemical profile varies between species of seaweeds (Machado et al., 2014; Carroll et al., 2019), and it seems that *Asparagopsis* is the most effective macroalgae for CH_4 mitigation. However, the effect of feeding macroalgae to ruminants on diet digestibility, animal performance and health, together with CH_4 abatement are yet to be extensively addressed using in vivo trials. Recently, studies reported residue in the milk (increased iodine and bromide concentrations) as a result of feeding *Asparagopsis* to dairy cattle (Stefenoni et al., 2021), thus, establishing the recommended concentration of *Asparagopsis* in the diet is necessary to enable its safe use as a feed additive.

Moreover, the mitigation effect of seaweed appears to vary among its species in vitro, and is influenced by basal diet (Machado et al., 2014, 2016; Maia et al., 2016), thus future in vivo studies should focus on the influence of basal diet on the seaweed mitigation effect. The animal performance response in a pasture-based setting is yet to be defined, as well as delivery method. Additionally, lifecycle assessment will be important, to quantify the net climate change effects of this strategy, including the seaweed production process. Among caveats to tackle before the adoption of seaweed as a mitigation strategy are the concerns that high harvesting rates in the wild may disrupt the equilibrium of coastal ecosystems (Makkar et al., 2016), and that cultivation of seaweeds may release bromoform, an ozone-depleting substance (Carpenter and Liss, 2000; Quack and Wallace, 2003).

3.3. Nitrate

Our meta-analysis revealed that NO_3^- supplementation decreased MY by 15.7% on average, compared with a control diet ($16.1 \pm 0.855 \text{ g}$ vs. $19.1 \pm 0.853 \text{ g CH}_4/\text{kg DMI}$; $P < 0.01$; $n = 25$) in ruminants. Dietary NO_3^- inclusion from 17.2 to 22.1 g/kg DM (95% CI) led to MY reduction from 10.0% to 22.1% (95% CI; $P < 0.05$; $n = 25$). Unlike oils, NO_3^- supplementation does not impair DMI ($P = 0.86$; $n = 25$; Fig. 3A) or fibre digestibility (i.e., NDF digestibility; $P = 0.86$; $n = 12$; Fig. 3B), which is a beneficial outcome for grazing systems. Moreover, dietary NO_3^- supplies non-protein nitrogen to the rumen biota, reducing the need for other dietary non-protein nitrogen sources (Hulshof et al., 2012; Li et al., 2012; Villar et al., 2020). The overall reduction in MI ($\text{g CH}_4/\text{kg}$ of milk or weight gain) from NO_3^- inclusion ranged from 10.7% to 18.7% ($P < 0.01$; $n = 11$, Fig. 3C).

The mechanism by which NO_3^- may lower ruminal CH_4 production is through competition with methanogenesis for reducing equivalents. Because NO_3^- has a higher affinity for H_2 than does CO_2 in the rumen (Jones, 1972; Latham et al., 2016), CH_4 production is

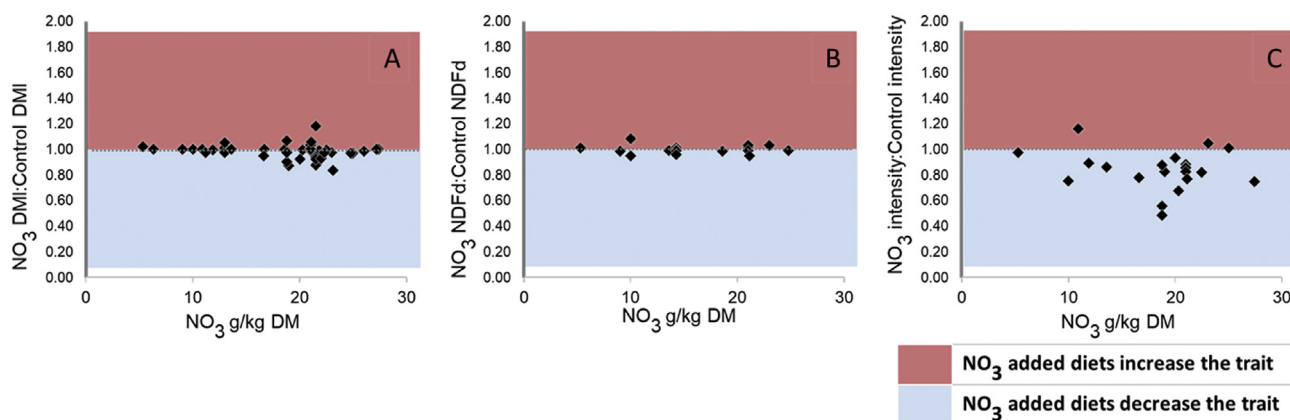


Fig. 3. Mean effect of the estimated ratio of diets containing nitrate (NO_3) and control diets in dry matter intake (DMI; A), neutral detergent fibre digestibility (NDFd; B) and methane (CH_4) intensity (g/kg animal product; C).

reduced by feeding NO_3 to ruminants. In the rumen, NO_3 is initially reduced to NO_2 (nitrite) and then to NH_3 , decreasing the availability of H_2 for methanogens (Lewis, 1951; Nolan et al., 2016).

Since 2015, the Australian Emissions Reduction Fund has included a method through which carbon credits can be generated for feeding of NO_3 to grazing ruminants (DoE, 2015). The major limitation of feeding NO_3 to ruminants is the possibility of accumulation and absorption of intermediates of NO_3 reduction (i.e., NO_2 into the bloodstream). Besides being a precursor to carcinogenic compounds, NO_2 can impair the capacity of blood to transport oxygen to an animal's tissues due to methaemoglobinaemia (Lewis, 1951; Sindelar and Milkowski, 2012; Bedale et al., 2016). In most studies used in the present analysis, blood methaemoglobin concentrations in nitrate-supplemented animals were higher than non-supplemented ones (Velazco et al., 2014; Guyader et al., 2016; Rebelo et al., 2019). None of the included studies that measured blood methaemoglobin levels observed clinical symptoms of methaemoglobinaemia, i.e., cyanosis and hypoxia that may arise at methaemoglobin >20% of total haemoglobin (Mensinga et al., 2003).

In this regard, management and nutritional strategies to reduce the risk of NO_2 poisoning may be used, including adapting animals to NO_3 , i.e., microbial acclimation (Lee and Beauchemin, 2014; Nolan et al., 2016), slowing the rate of NO_3 reduction reaction in the rumen (e.g., by encapsulating in lipid; de Raphelis-Soissan et al., 2016), as well as combining different mitigation strategies (e.g., NO_3 + oil; Nolan et al., 2016; Lee et al., 2017; Villar et al., 2019), so less NO_3 is required in the diet. Evaluating the combined effect of using nitrate and an oil source for CH_4 mitigation, the change in MY varied from 38.6% MY reduction up to 3.5% MY increase; (95% CI; $P < 0.05$; $n = 4$). Although these results were based on only four papers that evaluated the interaction of oil and NO_3 , it seems that the combination of NO_3 supplementation with oil would reduce the likelihood of nitrate poisoning.

3.4. Ionophores

The present study revealed that including an ionophore (monensin was used in the majority of studies) in cattle diets reduced MY by only 4% (95% CI from 0.5% to 7.4%; $P = 0.05$; $n = 10$; Fig. 1). Moreover, the use of ionophores as a mitigation strategy is limited to the period prior to microbiome adaptation. The current study showed only a modest MI reduction (g/ CH_4 per kg liveweight gain: 95% CI from 0% to 14.7%; $P = 0.04$; $n = 5$). Ionophores affect ammonia production, changing the fermentation dynamics towards improving energetics and N use in the rumen, as well as

controlling acidosis. Ionophores are used mainly in feedlots and dairy herds (i.e., cattle fed high grain diets), and CH_4 mitigation appears to be only a small co-benefit of the use of ionophores.

Ionophores are compounds of diverse chemical structures that are able to anchor to the lipid bilayer of cell membranes of organisms and translocate protons (H^+) and metal ions through the membrane as futile ion fluxes leading to eventual death of the microbial cell (i.e., gram + bacteria and protozoa) (Russell and Strobel, 1989; Chow et al., 1994). Typically, this shifts the microbial population toward gram-negative bacteria that are less sensitive to ionophores, at the expense of H^+ -, ammonia-, and lactate-producing organisms, resulting in higher propionate production, less CH_4 , greater protein availability and higher ruminal pH (Russell and Houlihan, 2003).

Ionophores have been commonly used as a performance enhancer in ruminants, for 4 decades. Several ionophores are registered and approved for use as feed additives (e.g., monensin, lasalocid, narasin, laidlomycin), but this varies between countries. Monensin, the most widely used ionophore in ruminant nutrition, is produced by *Streptomyces* spp. Over time, rumen microbes adapt, reducing the ionophore response, including the CH_4 mitigation (Callaway et al., 2003). Rotating ionophores and antibiotics (daily, weekly or biweekly) may improve the longevity of the effect of ionophores on feed efficiency (Guan et al., 2006; Crossland et al., 2017). As ruminal bacteria become resistant to ionophores, one may argue that ionophore resistance poses a public health threat, as genes linked to ionophore resistance in ruminal bacteria have not yet been identified.

3.5. Protozoa population control

The meta-analysis found that when a protozoa population-controlling additive was used, the protozoa population reduced by 23% (95% CI = 12% to 35%; $P < 0.01$; $n = 22$) but rumen MY diminished by only 2% on average (95% CI = -0.16% to 14.0%; $P = 0.03$; $n = 22$; Fig. 1), noting wide variation inherent to diet. The reduction in MY may be due to a reduced methanogen population, an altered pattern of volatile fatty acid production and hydrogen availability; and greater dry matter digestion in the rumen. Our results did not show reduction in DM digestibility ($P = 0.91$) or NDF digestibility ($P = 0.87$). The decline in methanogenesis associated with removal of protozoa is greatest on high concentrate diets and this is in keeping with protozoa being relatively more important sources of hydrogen on starchy diets, as many starch-fermenting bacteria do not produce H_2 .

Some methanogens in the rumen exist as endo- and ecto-symbionts with ciliate protozoa (Finlay et al., 1994; Tokura et al., 1997) and such symbionts may account for up to 37% of the rumen methanogens (Finlay et al., 1994). Although protozoa are a significant proportion of the biomass in the rumen ecosystem, they are not essential (Newbold et al., 2015). On the contrary, some co-benefits of rumen defaunation (removing protozoa) have been reported, such as increases in growth rate and live weight gain of ruminants (Eugène et al., 2004; Newbold et al., 2015) especially when the feed is deficient in protein relative to energy content. In addition, rumen protozoa are significant H₂ producers, due to their preferred production of acetate and butyrate rather than propionate.

In brief, one may use physical and chemical techniques to achieve defaunation of the rumen: the most commonly used techniques are the isolation of animals from their mothers at birth, and the use of surfactants and other chemicals (e.g., sodium lauryl sulfate, alkanes, synperonic NP9, calcium peroxide, copper sulfate), as well as emptying and washing the rumen (Hegarty et al., 2008; Newbold et al., 2015). Moreover, some feed additives used to mitigate CH₄ or as efficiency enhancers may control the protozoan population including ionophores, oil, and NO₃ supplementation. It is noteworthy that none of the available techniques is considered practical and/or efficient for commercial application to date.

3.6. Phytochemicals

For the purpose of the present study, a wide array of heterogeneous plant secondary compounds with antimethanogenic properties were grouped as phytochemicals (Patra and Saxena, 2010) including tannin-rich feeds, essential oils, and saponins but excluding macroalgal bromoform.

Our meta-analysis indicated no effect of phytochemical inclusion on DMI of dairy, beef cattle, and sheep (mean effect of 1.00 ± 0.00386; 95% CI 0.992 to 1.01; P = 0.81; n = 33; Fig. 4). Among studies included in the analysis, 24% trialed saponins, 50% used tannins and 21% fed essential oils, while 5% of these studies examined other phytochemicals (e.g., flavonoids). The estimated mean reduction in MY through phytochemical supplementation was 10% compared with the control diet (16.7 ± 1.11 g vs. 18.6 ± 1.12

CH₄/kg DMI; P < 0.01; n = 33), with tannins and saponins having the greatest effect (Fig. 4). The observed mean reduction in MY due to phytochemical supplementation ranged from 8% to 14% (Fig.1; 95% CI; P < 0.01; n = 33). Phytochemical inclusion in the diet of ruminants affected fibre digestibility (mean reduction in NDF digestibility of 4.69%; 95% CI 0.86 – 8.56; P = 0.02; n = 21), without affecting total tract DM digestibility (mean effect 95% CI 0.97 – 1.01; P = 0.97; n = 21). Overall, phytochemical supplementation tended to reduce CH₄ intensity in ruminant animals (mean effect 0.922 ± 0.0351; 95% CI 0.83 to 1.00; P = 0.08; n = 21).

When using phytochemicals as feed additives, one should pay close attention to the dose and purity, as they may possess anti-nutritional characteristics at higher concentrations. The aim is to find the equilibrium between the beneficial CH₄ abatement and optimum nutrient utilisation. This balance is particularly complex to attain, as the composition and quantity of phytochemicals varies widely within natural sources (e.g., legumes), even when fed as extracts. More than 200,000 plant secondary compounds have been identified (Hartmann, 2007), and some have antimethanogenic properties.

Saponins are high molecular-weight glycosides that occur in a variety of plants, with triterpene saponins (i.e., saccharide chain units linked to a triterpene) more abundant in nature than steroidal saponins (Hostettmann and Marston, 2005). The CH₄-suppressing traits of saponin-rich plants are principally related to the inhibition of rumen ciliate protozoa, which may enhance efficiency of synthesis of microbial protein (Patra and Saxena, 2009). Similar beneficial effects on N and energy ruminal metabolism have been observed when feeding tannins to ruminants (Norris et al., 2020). Tannins are high molecular weight polyphenolic compounds soluble in water and have capacity to interact with proteins (and carbohydrates) due to the presence of a large number of phenolic hydroxyl groups forming complexes (Patra and Saxena, 2010). They exist as hydrolysable tannins (HT) and condensed tannins (CT); both have antimethanogenic effects, however CT are more commonly used as a feed additive because HT represent a high risk of toxicity to the animal (Field and Lettinga, 1987; McSweeney et al., 2001).

Tannin-rich plants include legumes that may be used to improve pasture productivity as well as nitrogen level in the diet. The

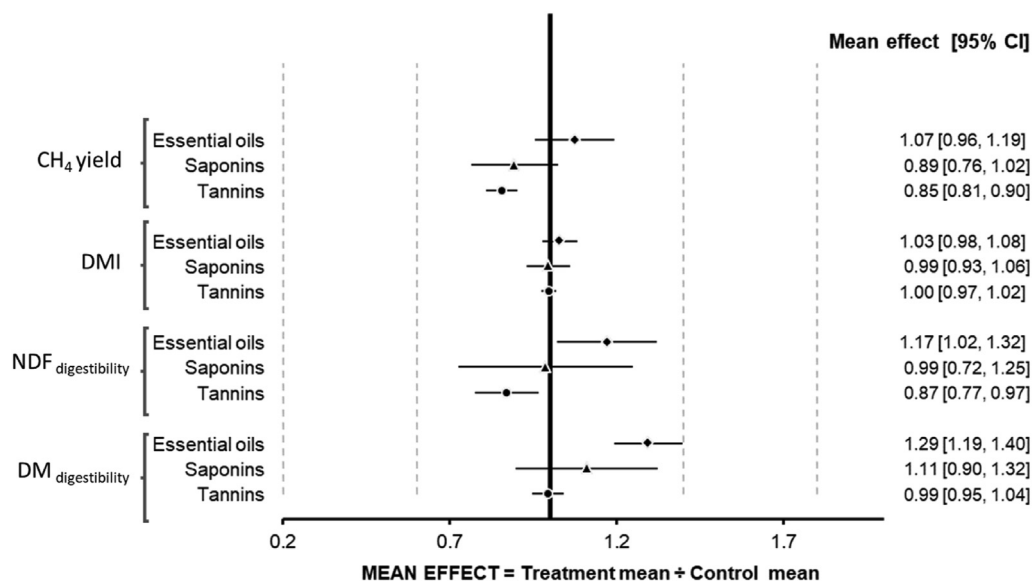


Fig. 4. Mean effect of the estimated ratio of diets essential oils (diamond), saponins (triangle), and tannins (circle) and control diets in methane (CH₄) yield, dry matter intake (DMI), NDF (neutral detergent fibre) digestibility and DM (dry matter) digestibility.

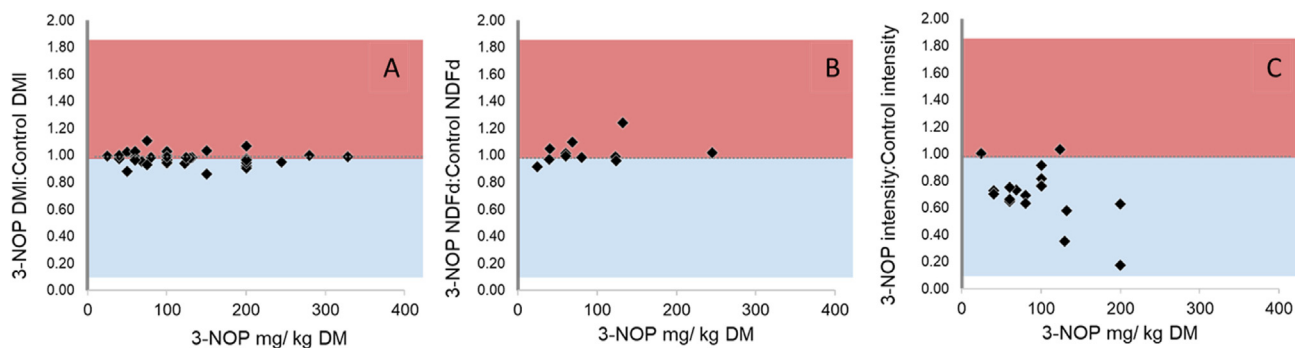


Fig. 5. Mean effect of the estimated ratio of diets containing 3-nitrooxypropanol (3-NOP) and control diets in dry matter intake (DMI; A), neutral detergent fibre digestibility (NDFd; B) and methane (CH_4) intensity (C).

tropical legumes *Desmanthus* and *Leucaena leucocephala* have significant antimethanogenic properties, and are considered a promising approach for mitigation of enteric CH_4 in beef production in the northern Australian rangelands (Suybeng et al., 2019; Tomkins et al., 2019; Vandermeulen et al., 2018). *Leucaena* is not currently recommended in southern Australia due to its propensity to become a weed.

Essential oils are not based on long chain fatty acids but are bio-active molecules with antimicrobial properties that can directly inhibit methanogens and hydrogen-producing microorganisms. They include garlic oil, thymol, cinnamaldehyde, peppermint, menthol and eucalyptus oils, as well as commercial blends. The type of essential oil determines the effect on CH_4 production. It is important to consider the potential anti-nutritional effect of essential oils and the adaptation of rumen microbes to essential oils, the change in flavour of animal products due to presence of residues in meat and milk, as well as acceptability by the animals, which could affect DMI (Rae, 1999; Calsamiglia et al., 2007).

Due to high variation in the concentrations and types of antimethanogenic compounds between plant species, as well as spatial and temporal inconsistency, it is not possible to provide generic recommendations about the dietary inclusion of phytochemicals for CH_4 mitigation.

3.7. 3-Nitrooxypropanol (3-NOP)

Reviewing previous studies, the in-feed doses of 3-NOP fed to ruminants ranged from 40 to 340 mg 3-NOP/kg DM (64.2 to 122 mg 3-NOP/kg DM; 95% CI) and responses are highly dose dependent. From our meta-analysis, 3-NOP supplementation decreased ruminant CH_4 emission by 23.3% compared with a control diet (15.1 ± 0.995 g vs. 19.7 ± 1.11 $\text{CH}_4/\text{kg DMI}$; $P < 0.01$; $n = 14$). The mean reduction in the MY ranged from 18 to 39% (95% CI; $P < 0.01$; $n = 14$; Fig. 1). All individual studies used in the meta-analysis noted the efficacy of 3-NOP in lowering enteric CH_4 emissions (Table 1).

Previously, CH_4 abatement achieved with dietary 3-NOP in ruminants was associated with a decrease in DMI (Romero-Perez et al., 2014; Vyas et al., 2016), and this was borne out in the current analysis where 3-NOP supplementation reduced DMI up to 4.5% ($P = 0.02$; Fig. 5A; $n = 14$). The reduction in DMI itself is not a concern if it results in the same liveweight gain by the animal, which would indicate improved feed use efficiency. In the present study, the 3-NOP supplementation did not alter fibre digestibility (i.e., NDF; $P = 0.25$; Fig. 5B; $n = 5$). The reduction in MY with 3-NOP ranged from 6.5% to 38% in dairy cattle (mean of 22.2%; $P < 0.01$; $n = 7$) and from 1.5% to 59% in beef cattle (mean of 30.0%; $P < 0.01$; $n = 7$). In contrast, previous studies suggested stronger

antimethanogenic effects of 3-NOP in dairy cattle than in beef cattle (Dijkstra et al., 2018). Additionally, 3-NOP has a greater CH_4 suppressing effect in high-forage than high-grain feedlot diets (Kim et al., 2019). One may expect a larger variation within grazing beef cattle compared to dairy production systems, because of the greater complexity involved in delivering any feed additive to cattle in a grazing situation. The overall reduction in CH_4 intensity (g CH_4/kg of milk or weight gain) from 3-NOP ranged from 13.2% to 39.9% ($P < 0.01$; Fig. 5C).

The commercially developed compound 3-NOP provides a novel and promising feed additive to mitigate CH_4 . It is a structural analogue of the nickel enzyme methyl CoM reductase produced by the methanogenic archaea, thus it inhibits the last step of CH_4 formation in the rumen (Duin et al., 2016). Previous studies have shown that 3-NOP is a potent CH_4 suppressant, effective in a wide range of diet types, exhibiting no DMI nor digestibility reduction in beef or dairy cattle (Romero-Perez et al., 2014; Haisan et al., 2017; Jayanegara et al., 2017). Research has found that 3-NOP is metabolised rapidly, and does not accumulate in the mammal's bloodstream (Thiel et al., 2019). Moreover, 3-NOP and its metabolites were not found to have mutagenic or genotoxic potential (Thiel et al., 2019b). Thus, 3-NOP does not seem to represent a food security threat or risk to animal health.

Thus, 3-NOP may offer a reliable and effective strategy for CH_4 abatement in beef, sheep and dairy cattle, yet as a relatively novel feed additive, there may be resistance to adoption. The magnitude of the mitigation differ between ruminant types. Optimal doses of 3-NOP are yet to be defined, to support registration as a permitted feed additive, enabling the use of 3-NOP in the meat, wool and dairy industries.

3.8. Other CH_4 mitigation strategies

Among the reviewed CH_4 reduction strategies, it was found that comprehensive data regarding the use of bacteriocins (for review: Garsa et al., 2019), organic acids and prebiotics (e.g., acetogens, yeasts) (Martin et al., 2010; Patra, 2016) is too sparse to support adoption in the next 10 years. Moreover, these technologies generally yield modest results in CH_4 abatement, thus they were not included in the present meta-analysis.

4. Conclusions

This meta-analysis assessed dietary strategies for CH_4 mitigation in ruminant production systems. Seaweed, 3-NOP and NO_3^- are the most effective feed additives for abatement of ruminant CH_4 emissions, and show promise as mitigation strategies available to the livestock sector within the short term.

Further investigation is required to assess combinations of different strategies for CH₄ mitigation using a systematic approach, and to devise delivery method to enable their use in grazing systems.

Author contributions

Amelia Katiane de Almeida: Formal analysis, Data Curation, Conceptualization, Interpretation of Results, Writing - Original Draft. **Roger Hegarty:** Interpretation of Results, Writing - Original Draft, Review & Editing. **Annette Cowie:** Conceptualization, Interpretation of Results, Writing - Review & Editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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