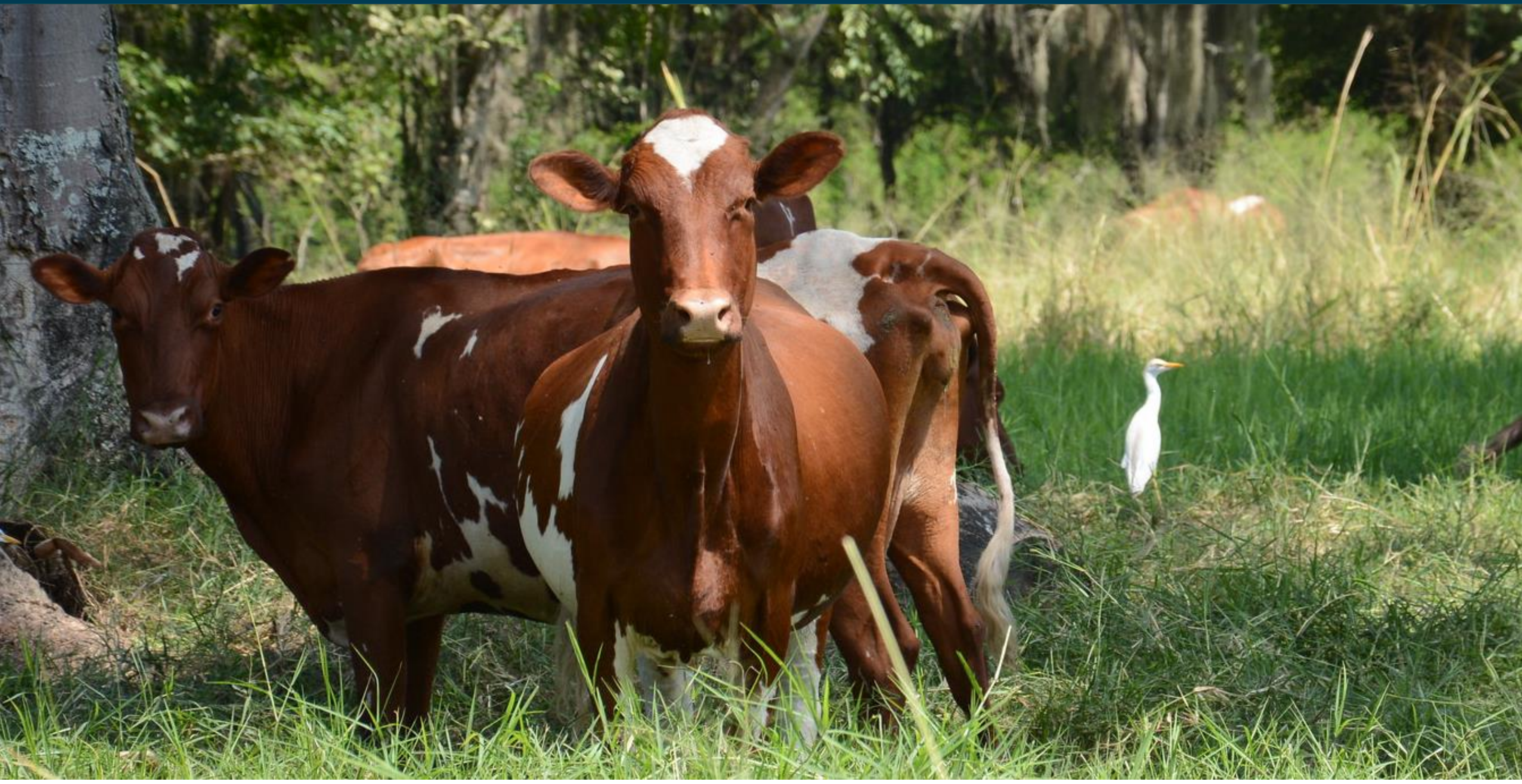

An evaluation of evidence for efficacy and applicability of methane inhibiting feed additives for livestock

November 2021

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Cover photo: LJ Urrea, CCAFS

Executive summary

The inclusion of feed additives in livestock diets or supplements is a routine global nutritional management practice. Consequently, the existing commercial feed additive marketing and delivery pathways will be able to deliver rapid market penetration of feed additives specifically developed to reduce enteric methane emissions. So, the delivery path is clear, but are the methane mitigating additives available, effective, and are there any constraints or risks associated with their use? To answer these questions an assessment of the ten leading classes of compounds being studied for methane mitigation efficacy in ruminants was made. The assessment is provided as a concise resource that can serve as an evidence base to guide investment and management decisions by all actors in the livestock additive supply chain.

Key findings of the review of these 10 additive groups include:

- Only two of the additives (3-Nitrooxypropanol) and dried Asparagopsis (red algae) have routinely delivered over 20% mitigation of enteric methane by the consuming ruminants.
- The level of confidence in efficacy is greater for 3-Nitrooxypropanol than for Asparagopsis because of a greater number of refereed publications demonstrating this efficacy.
- Dietary nitrate is the third most effective additive and can safely deliver 10% or more mitigation when consumed.
- The other classes of additive cannot be expected to deliver 10% mitigation when fed.
- There are two major constraints to any of the ten additives achieving substantial global impact on livestock emissions in the immediate future, being:
    - Insufficient evidence exists to be confident that any of these feed additives will deliver a co-benefit of increased production (growth or milk) from the animal in association with decreasing methane output.
    - Almost all studies have relied on additives mixed into a total mixed ration; that is, in a diet which provides the additive in every mouthful. There is almost no evidence of how much mitigation will be achieved if the additive is provided in a supplement that the animal may only consume once daily or once every few days, as in rangeland systems.

- To establish a business case for on-farm use of these additives, there is an absolute requirement for further research to:
    - Conduct highly replicated studies of animal production responses to inclusion of the leading additives. These need to have sufficient statistical power to detect differences of 5% or less, in keeping with the differences sporadically observed in smaller studies.
    - Quantify the mitigation achieved and optimise delivery strategies when additives are supplied in supplements provided separate from

the basal feed. This will require assessment at a range of supplementation frequencies pertinent to major feeding systems.

- Without co-benefit and pulse dose efficacy data there is no way of establishing the commercial argument for additive use or identifying the market sectors in which they may be applicable.
- The assessment identifies that the global livestock industries have a very limited suite of emerging feed additives suitable for enteric methane suppression. Therefore, it would be appropriate that basic research be expanded to extend the range of additives under development.
- A small survey of the actors in the feed additive pipeline from the manufacturers through feed millers to livestock managers, showed:
    - A poor understanding of the efficacy and co-benefits of potential additives.
    - A recognition by all livestock managers that they required more information on these additives.
    - Not one additive manufacturer identified the grazing industry as an extremely high priority market for a methane mitigating product.
    - When matched with the scientific concern about additives not being optimised for pulsed intake as supplements, the grazing livestock in the developing world may not achieve significant mitigation through feed additives in the near future.

Contents

Feedlot cattle receiving additives in total mixed rations (TMR). (Credit: Nicky Oelbrandt)

Rangeland supplement delivery of additives in northern Australia (Credit: Roger Heagarty)

Project synthesis

Project objectives

As the livestock sector seeks to achieve carbon neutrality by 2050 to meet the goals of the Paris Agreement, the research and commercial communities have accelerated efforts to identify greenhouse gas emission mitigation opportunities. With increasing attention on feed additives and the accompanying proliferation of products claiming to reduce emissions, a thorough evaluation of the evidence for feed additives intended to reduce emissions can help guide further action.

The objective of this report is to provide evidence to policy makers, industry investors and feed industry advisers about the effectiveness, applicability, and broader commercial issues regarding feed additives used for the purpose of reducing methane emissions. By scoping the full breadth from technical effectiveness to industry applicability and research needs, the report goes beyond what is normally presented in scientific reviews. We present this information as a concise resource that can serve as a basis to guide investment and management decisions by all actors in the livestock supply chain.

Key information is provided as a reference library, consisting of a four-page summary of each additive's attributes with links to the source information.

Methods and scope

This synthesis and reference library were developed assessing the 10 leading categories of methane-suppressing feed additives based on chemical grouping. Their mode of action, efficacy, and stage of development were individually summarized. The assessment excluded feed ingredients that would constitute more than 5% of the diet, such as dietary lipids. Commercial names of products were identified but the assessments are made by chemical-group, rather than by commercial product. The assessments of efficacy are principally derived from published peer-reviewed meta-analyses for most of the additives considered.

It should be noted that the report is for additives that are available and well researched at this time. There is a range of additional early-stage research products which are not yet well researched. It is also possible that new additives in some categories, that have shown poor performance to date, may be further developed (e.g., more active direct fed microbials or biochars) for higher efficacy.

To augment this evaluation, questionnaires were developed to assess interest, activity, and time to commercialization of methane mitigating additives for livestock producers, feed or supplement manufacturers and producers of feed additives.

Evaluation of climate change mitigation efficacy

A graded and color-coded estimate of efficacy in mitigation was developed, based on the percentage reduction in methane emission resulting from the average dose used in animal studies in meta-analyses and/or reviews (Figure 1). Confidence in that efficacy was ranked (1-5), based on the robustness of data, being the number of peer-reviewed animal-based publications, and the subjectively assessed level of agreement on efficacy within that data (Figure 1).

1.

CH_4 Reduction Potential	
Very High	> 25 %
High	> 15 - 25 %
Medium	> 5 - 15 %
Low	≤ 5 %

2.

The number of academic papers that report CH_4 emission measurements from animal studies.

3.

Confidence in Efficacy * Considers agreement and quality of evidence within academic literature		
5 High agreement & Robust evidence	4 High agreement & Medium evidence	3 High agreement & Limited evidence
4 Medium agreement & Robust evidence	3 Medium agreement & Medium evidence	2 Medium agreement & Limited evidence
3 Low agreement & Robust evidence	2 Low agreement & Medium evidence	1 Low agreement & Limited evidence

* Based on Intergovernmental Panel on Climate Change (IPCC) methodology

4.

Assessment considers likely technical applicability within the next five years, but does not consider regulatory approval, public acceptance nor cost, and therefore the expected adoption.

Figure 1. Key to Table 1 for estimating and describing efficacy and confidence in the estimate for each methane mitigating additive.

The key attributes of the ten additives most researched for methane mitigation are summarized in Table 1 below. In summary, among the additives examined, the evidence is most robust for the synthetic product 3-Nitrooxypropanol (3-NOP, which provided substantial efficacy in total mixed rations, with no apparent health, or product safety risk. The natural product *Asparagopsis* has shown higher efficacy but with less supporting data, and residual levels of bromine and iodine in animal production need further study. Nitrate can achieve over 20% mitigation, but risks of animal toxicity need to be managed, so Cargill recommends inclusion levels that would only provide 10% mitigation. Beyond these three additives, there are no other additives assessed for which there is robust evidence of even a 10% mitigation. Absolutely no additive exhibits robust evidence of co-benefit impacts on productivity of more than 10%.

Project Synthesis

Table 1. Summary of mitigation efficacy, applicability, co-benefits, and constraints of feed additives. Numerical and colour codes for efficacy parameters are explained in Figure 1.

Additive	Efficacy: CH_4 reduction potential [1]	Efficacy: No. of academic papers [2]	Efficacy: Confidence in efficacy [3]	Potential animal welfare risks	Potential food safety risks	Potential co-benefits	Production system applicability [4]	Development needs
3-Nitrooxyproponal	Very High	> 20	5	None known	None known	Improved feed efficiency.	TMR systems immediately. Grazing systems in future.	Validation in large-scale TMR systems required. Formulation for grazing systems.
Asparagopsis	Very High	< 10	1	Damage to rumen wall	Bromide & iodine residues in animal tissue/products	Improved feed efficiency.	TMR systems immediately. Grazing systems in future.	Validation in large-scale TMR systems required. Formulation for grazing systems.
Nitrate	High	< 20	4	Toxicity in non-adapted animals	None known	Can reduce need for urea supplementation in animal feed.	TMR systems immediately. Grazing systems in future.	Validation in large-scale TMR systems required. Formulation for grazing systems.
Essential Oils	Low	< 20	2	None known	None known	Improved milk productivity (limited evidence & indication of reduced body growth).	TMR & grazing systems (where supplements are administered)	Peer reviewed studies of mitigation potential and productivity within TMR & supplement systems.
Saponin	Low	< 15	1	None known	None known	Improved protein supply by protozoa control.	TMR & grazing systems (where forage crops containing saponin are utilized)	Further research into CH_4 reductions, productivity impacts & saponin chemistry required.
Tannins	Low	< 15	2	None known	None known	Shift from urine to faecal excretion of nitrogen reducing risk of N_2O emissions.	TMR & grazing systems (where forage crops high in tannins are utilized)	Tannins may have a stronger role in forage-based mitigation than as feed additives.
Monensin	Low	> 20	5	None known	None known	Improved weight gain. Reduced risk of bloat & acidosis.	TMR & specialized grazing systems	Few needs – already a widely used product.
Microalgae	Low	< 5	1	None known	None known	PUFA levels in meat improved. Enhanced antioxidants in food products.	TMR & grazing systems (where supplements are administered)	Microalgae supply dependent on use in renewable energy sector.
Biochar	Low	< 5	1	None known	None known	Toxins & heavy metals absorption prevention in animals. Enhanced soil quality when excreta is applied to soils.	TMR & grazing systems (where supplements are administered)	Engineering of an acidified biochar required to achieve adequate efficacy.
Bacterial Direct Fed Microbes	Low	< 15	2	None known	None known	Improved productivity (though inconsistent). Improved calf health. Reduced incidence of E.coli in manure.	TMR & grazing systems (where supplements are administered)	Development of high efficacy bacterial strains.
Fungal Direct Fed Microbes	Low	< 15	1	None known	None known	Improved productivity (+ 3% in milk observed). Improved feed efficiency.	TMR & grazing systems (where supplements are administered)	Development of high efficacy fungal strains.

Implications for feed additives' role in global climate change

With 44% of anthropogenic methane emissions arising from livestock systems[1], managing feed to minimize enteric methane emissions is an important strategy to controlling global climate change. Aside from changing the quantity of feed supplied, the nutritional value of the forage (in grazing systems) or the quality of the ration in Total Mixed Ration (TMR) feeding systems can also be changed. Additives can readily be applied in the TMRs, but feedlots contribute only 2.1% of global livestock enteric emissions (Figure 2). Almost all published research on additives has been carried out under conditions of providing the additive in a TMR ration, that is, the additive is in every mouthful the animal eats. Consequently, most of the existing research on mitigation additives is only directly applicable to the small global emission from the feedlot sector.

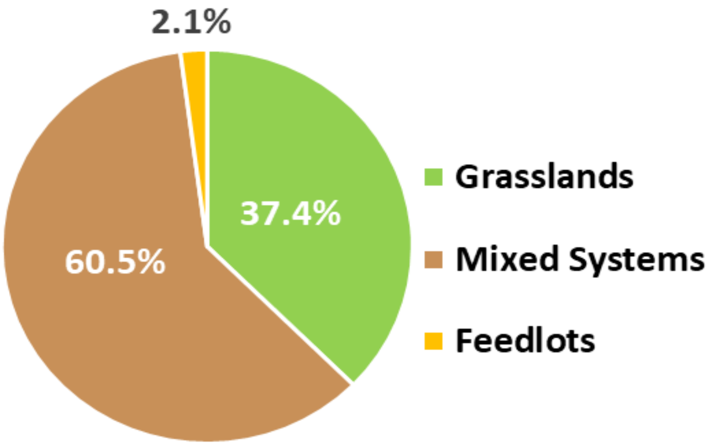


Figure 2. Proportions of enteric methane emission from grassland, mixed or feedlot animal systems; data is from 2010. FAO. 2017. Global Livestock Environmental Assessment Model (GLEAM) [online]. Rome. [Cited 18 May 2017]. www.fao.org/gleam/en/

There is scope for including additives in mixed feeding systems (60.5% of emissions) where supplements or partial mixed rations are fed. But even here there is an extreme lack of data to answer the pivotal following question. "Will the results observed from placing additives in TMR feeds be replicated when the additive is not in every mouthful, but rather is provided in a 'pulse dose', either in a supplement while grazing or in a partial ration? For example, when animals are fed at milking or in cut and carry forage systems."

In these systems the additive may (1) have to be provided at a higher inclusion rate than if mixed in the entire diet, which could affect efficacy and feed intake. Conversely, or (2) be expected to only work for a short period due to rumen dilution or destruction of the additive.

This issue of short-term efficacy is easily visualized in the time-course of emissions from dairy cattle provided 3-NOP twice daily in a pre-feed supplement (Figure 3). The difference in methane emission rate lasted for less than 6h after the additive was provided.

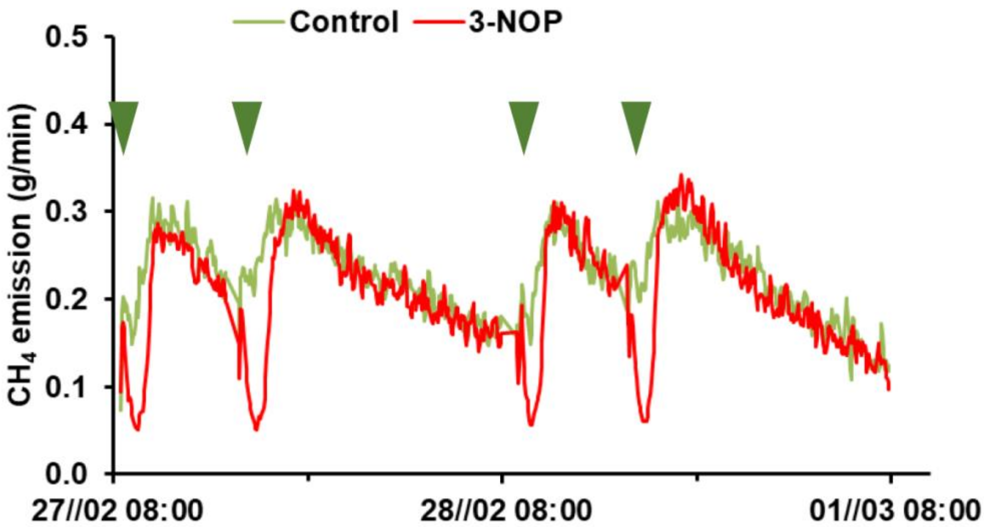


Figure 3. Moment by moment enteric CH_4 emission rates over 2 days in dairy cattle offered 3-Nitrooxypropanol (3-NOP) in supplements provided twice daily (arrows indicate supplement time). Graphs show the mitigation effect persists for less than 6 h post supplement[2].

Consequently, to achieve global enteric methane mitigation, there is an outstanding need for development of methane inhibitor delivery systems that are robust in achieving mitigation in pasture-based systems. Some efforts have already been made to prolong rumen mitigation (e.g., with 3-NOP[2] and cyclodextrin protected haloforms[3]). Preserving stability of volatile additives within the feed has also been studied[4]. However, current evidence for effective delivery of methane-suppressing feed additives is insufficient to enable their use in substantive mitigation in grazing and mixed farming sectors in any country.

There has been a strong expectation by commercial developers that the energy saved by suppressing enteric (gut) methane production would be captured in improved productivity of ruminants. As the subsequent review indicates, this does not reliably occur for most methane inhibiting additives. None of the currently available additives can consistently offer a "productivity gain" to justify feeding the additive across all ruminant production systems. Other motivations as listed below may be needed to raise use of methane suppressing additives.

- Corporate environmental reputation
- Income from carbon credits
- Government subsidies to change nutritional management
- Corporate premiums for low carbon products
- Market access for low carbon products
- Government subsidies for low carbon products
- Legislative requirements

Supplementation of grazing animals is an early step to agricultural intensification and is viewed as an industry advance in rangeland environments and the developing world. Sustainable intensification of food production is seen as the way to secure humanity's future[5], although it remains aspirational in some regions. Even assuming adequate availability and efficacy of methane-mitigating feed additives, a critical weakness remains in that almost nothing is known of the efficacy of leading products, such as 3-nitrooxypropanol and *Asparagopsis*, when consumed intermittently by free-ranging ruminants.

How prepared is the global livestock industry?

In addition to identifying the above 'gaps in the science' by review of literature, a survey-based assessment of awareness and understanding in the feed additive pipeline (additive manufacturers, feed or supplement manufacturers, and livestock managers) was undertaken. This provided a range of broad insights that are important if feed additive use is to lead to major reductions in global enteric emissions from livestock.

Regarding additive manufacturers this industry assessment found:

- Additive manufacturers are largely targeting livestock in the developed rather than developing world.
- Not one additive manufacturer identified the grazing industry as an extremely high priority market for a methane mitigating product.
- When matched with the scientific concern about additives not being optimised for pulsed intake as supplements, this suggests the developing world will not achieve significant mitigation through feed additives in the near future.

The feed and supplement manufacturing industries were found to:

- Place no or low priority on supply of methane lowering feeds currently, but they expect that priority to increase over the next 5 years.
- Be poorly informed regarding appropriate additives, with 4 or less of the 14 respondents being aware of the three additives with highest efficacy (3-NOP, Asparagopsis and nitrate).
- Have a belief that existing probiotics, essential oils and antibiotic rumen modifiers can be used for methane mitigation.

Livestock managers themselves (largely in Brazil and Indonesia, and principally cattle managers) identified that they:

- Saw greenhouse gas (GHG) reduction as a low priority but as increasing concern over the next 10 years.
- Expected methane inhibitors to deliver an increase in animal performance and feed efficiency.
- All 24 livestock managers said they would need additional information to support decisions on feed additive use for methane, with the majority anticipating seeking that information from current feed/additive suppliers.

Consequently, the industry assessment (completed after the technical reviews of additives) extends the list of research and development required (below) if feed additives are to be used to impact global climate change substantially. The industry assessment confirms the need to provide information on methane lowering additives to feed manufacturers and livestock managers. Clear market forces to promote adoption are just as important as the discovery of safe and effective additives if methane mitigation in global ruminant livestock systems is to be achieved.

Outstanding research and development needs

- For feed additives to act as agents for global change in livestock emissions, the over-riding need is for a quantitative understanding of the time-course and extent of mitigation when additives are provided in 'pulse fed' supplements rather than in every mouthful of a TMR.
- Associated with this is the need to quantify the efficacy of methane mitigating additives in grazing and mixed feeding systems where feed intake is largely uncontrolled, and levels and variation in supplement intake between animals are largely unknown.
- A strong economic argument for additive development and use requires the co-benefits from methane mitigating additives be defined. This is particularly true for animal production co-benefits such as liveweight gain and milk production. Because co-benefits are mostly 5% or less of current production, their estimation needs highly replicated studies to confirm statistical significance and so confidence in the scale of effect. Currently there is an optimism among feed or supplement manufacturers as well as livestock producers that these additives will improve animal performance and feed use efficiency. This is not strongly supported by the research.
- There is a range of additive-specific issues (such as testing for residues in animal products or for animal welfare effects) that need to be identified and addressed.
- The low number of additives identified as providing high level mitigation (>25%) even in total mixed rations is very low (3-NOP and *Asparagopsis*) and there is little sign of novel products emerging from within the other less effective additive groups reviewed. Consequently, the investment in novel additives (and non-nutritional means) to mitigate enteric emissions must be expanded.
- Improved communication is required to overcome poor understanding among feed manufacturers and livestock managers about what methane suppressing additives exist and their efficacy in reducing enteric emissions.

Assessment of the 10 leading feed additives considered for livestock methane mitigation

There has been a recent proliferation of research, publications, scientific reviews, and meta-analyses of strategies to reduce enteric methane emissions from livestock. A search on Scopus* revealed 187 unique, relevant publications arising in 2010/2011 but 461 in 2020/2021.

In this assessment, we have primarily relied on reviews and meta-analyses. Before presenting our assessment, and with a view to providing a ready resource to expand the reader's understanding, a list of recent broad-based scientific reviews of feeds and additives suppressing ruminant methane production is provided below. A list of more detailed reviews and meta-analyses for as many individual additives as were available is then presented before providing our consolidated assessment in a tabular form for each additive.

Existing reviews on dietary inhibition of rumen methanogenesis

- 🕮 **2021: Feed additives as a strategic approach to reduce enteric methane production in cattle: Modes of action, effectiveness and safety**
  Honan M, Feng X, Tricarico JM, Kebreab E. *Animal Production Science*, 51(6), 491-514.

- 🕮 **2021: Methane Emissions from Ruminants in Australia: Mitigation Potential and Applicability of Mitigation Strategies**
  Black JL, Davison TM, Box I. *Animals*, 11(4).

- 🕮 **2021: Research progress on the application of feed additives in ruminal methane emission reduction: a review**
  Sun K, Liu H, Fan H, Liu T, Zheng C. *Zooilogical Science, 9.*

- 🕮 **2021: Recent Nutritional Advances to Mitigate Methane Emission in Cattle: A Review**
  Hadipour A, Mohit A, Darmani Kuhi H, Hashemzadeh F. *Iranian Journal of Applied Animal Science,* 11(1), 1*.*

- 🕮 **2020: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation**
  Beauchemin KA, Ungerfeld EM, Eckard RJ, Wang M. *Animal*, 48(2), 21-27.

- 🕮 **2018: Dietary manipulation: A sustainable way to mitigate methane emissions from ruminants**
  Haque MN. *Journal* of *Animal Science and Technology*, 60(1), 1-10.

Scopus search for "methane AND (mitigate OR reduc*) AND (livestock OR sheep OR cattle) AND NOT (biogas)"

📖 **2013: Special topics—Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options**
Hristov AN, Oh J, Firkins JL, Dijkstra J, Kebreab E, et al. *Journal of Animal Science,* 91(11), 5045.

Existing inhibitor-specific reviews of methane mitigating feed additives

3-Nitrooxypropanol

📖 **2020: The effects of dietary supplementation with 3-nitrooxypropanol on enteric methane emissions, rumen fermentation, and production performance in ruminants: a meta-analysis**
Kim H, Lee HG, Baek Y-C, Lee S, Seo J. *Journal of Animal Science and Technology*, 62(1), 31-42.

📖 **2018: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation**
Dijkstra J, Bannink A, France J, Kebreab E, van Gastelen S. *Journal of Dairy Science,* 101(10), 9041-9047.

📖 **2018: Use of 3-nitrooxypropanol as feed additive for mitigating enteric methane emissions from ruminants: a meta-analysis**
Jayanegara A, Sarwono KA, Kondo M, Matsui H, Ridla M, Laconi EB, Nahrowi. *Italian Journal of Animal Science*, 17(3).

Micro and macroalgae

📖 **2020: Management of enteric methanogenesis in ruminants by algal-derived feed additives**
McCauley JI, Labeeuw L, Jaramillo-Madrid AC, Nguyen LN, Nghiem LD, Chaves AV, Ralph PJ. *Current Pollution Reports*, 6, 188-205.

📖 **2020: Seaweed and seaweed bioactives for mitigation of enteric methane: Challenges and opportunities**
Abbott DW, Aasen IM, Beauchemin KA, Grondahl F, et al. *Animals*, 10(12).

Nitrate

📖 **2020: Antimethanogenic effects of nitrate supplementation in cattle: A meta-analysis**
Feng XY, Dijkstra J, Bannink A, van Gastelen S, France J, Kebreab E. *Journal of Dairy Science*, 103(12), 11375-11385.

📖 **2014: A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance**
Lee C, Beauchemin KA. *Canadian Journal of Animal Science*, 94(4).

Essential oils

- 🕮 **2020: A meta-analysis describing the effects of the essential oils blend Agolin Ruminant on performance, rumen fermentation and methane emissions in dairy cows**
  Belanche A, Newbold C, Morgavi D, Bach A, Zweifel B, Yáñez-Ruiz D. *Animals*, 10(4).
- 🕮 **2014: Essential oils and opportunities to mitigate enteric methane emissions from ruminants**
  Benchaar C, Greathead H. *Animal Feed Science and Technology,* 166, 338-355.

Antibiotic rumen modifiers

- 🕮 **2013: Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis**
  Appuhamy JADRN, Strathe AB, Jayasundara S, Wagner-Riddle C, Dijkstra J, France J, Kebreab E. *Journal of Dairy Science,* 96(8), 5161-5173.

Saponins and Tannins

- 🕮 **2021: The effects of dietary saponins on ruminal methane production and fermentation parameters in sheep: a meta-analysis**
  Darabighane B, Mahdavi A, Aghjehgheshlagh FM, Navidshad B, Yousefi MH, Lee MRF. *Iranian Journal of Applied Animal Science,* 11(1), 15-21.
- 🕮 **2021: Effects of Dietary Tannins' Supplementation on Growth Performance, Rumen Fermentation, and Enteric Methane Emissions in Beef Cattle: A Meta-Analysis**
  Orzuna-Orzuna J, Dorantes-Iturbide G, Lara-Bueno A, Mendoza-Martínez G, Miranda-Romero L, Hernández-García P. *Sustainability*, 13(13).
- 🕮 **2021: Effects of saponin on enteric methane emission and nutrient digestibility of ruminants: An in vivo meta-analysis**
  Ridla M, Laconi EB, Jayanegara A. In IOP Conference Series: *Earth and Environmental Science*, 788.
- 🕮 **2012: Methane mitigation from ruminants using tannins and saponins**
  Goel G, Makkar HP. *Tropical animal health and production*, 44(4), 729-739.

Biochar

- 🕮 **2019: The use of biochar in animal feeding**
  Schmidt H-P, Hagemann N, Draper K, Kammann C. *Peer*, 7.

Direct fed microbials

- 🕮 **2019: Environmental efficiency of Saccharomyces cerevisiae on methane production in dairy and beef cattle via a meta-analysis**
  Darabighane B, Salem AZM, Mirzaei Aghjehgheshlagh F, Mahdavi A, Zarei A, et al. *Environmental Science and Pollution Research*, 26, 3651–3658

Broad-based assessment of methane mitigating feed additives for ruminant use

The following pages comprise consolidated assessments of a broad range of commercially important attributes, as per the Table below, for additives being considered for livestock methane mitigation. All references cited are provided in a combined bibliography at the rear of this report and can be immediately reached by the "See the reference list" hyperlink for each additive.

Evidence of efficacy	Magnitude of mitigation defined in meta-analyses and scientific reviews. Only relies on animal (in-vivo) studies. Meta-analyses of laboratory studies (in-vitro) may be noted but were not used in efficacy assessment.
Mode of action	What is the biochemical or microbiological basis of action?
Dose	Recommended inclusion rate of additive in feed.
Manufacturer	Current or emerging manufacturers of the additive.
Availability	Is the product available locally, globally, or restricted?
Impacts on animal production	How does the additive affect growth, milk production and feed-use efficiency or nitrogen use efficiency where known?
Applicability	What livestock sectors is this additive suitable for?
Expected market trajectory	Based on its efficacy, market availability, applicability and co-benefits or constraints.
Expected cost	US$ /kg where known.
Constraints to use	Availability, registration requirements or prohibitions, stability, and suitability of additive for delivery.
Residue in animal product	Evidence of additive derived materials in blood, milk, or meat.
Impacts on manure	Nitrogen distribution effects (manure v urine).
Potential regional distribution	Is distribution limited by feeding system, by site of production, or by regional regulations?
Life-cycle Assessment (LCA)	LCA analysis on the carbon cost of the additive itself and/or the effect on the carbon cost of livestock enterprises when using this additive.
Actions needed to accelerate the roll-out	Research, marketing, and development tasks required to advance this additive to commercial delivery.
Assessment Summary	Provides a concise summary of key factors affecting this additive's potential to contribute to GHG mitigation from agriculture.

3-Nitrooxypropanol (3-NOP)

See the reference list

Evidence of efficacy	Refereed literature shows a consistent efficacy of 3-NOP in reducing daily methane production (g/d) and methane yield (g CH_4/kg dry matter (DM)) and there is a strong positive linear association between 3-NOP dose and level of mitigation achieved[8], with CH_4/kg DMI declining linearly at 0.41 per 1 mg 3-NOP/kg feed[9]. The average dose in the literature of 123 mg 3-NOP/kg DMI was shown to cause 23% (in beef) and 39% (in dairy) reduction in emissions[10]. At lower doses persistence over several months declined[11,12] but not at expected commercial dosages. Efficacy does show some diet dependency, being greatest in low forage diets[8]. Meta-analyses of 3-NOP efficacy have been undertaken by Dijkstra et al.[10], Jayanegara et al.[13], Kim et al.[9] and de Almeida et al.[14]. De Almeida et al.[14] reported a mean 29% reduction in methane yield associated with 3-NOP inclusion.
Mode of action	In the normal final reaction of Archaeal methane production, the methyl-coenzyme M reductase (MCR) enzyme docks with methyl-coenzyme M and methane is produced. 3-NOP has a similar chemical structure to methyl-coenzyme M so it can take methyl-coenzyme M's place and bind to MCR, although no methane is produced[15]. Rather the 3-NOP oxidises the Nickel in MCR to make it no longer able to bind to methyl-coenzyme M. This oxidation by 3-NOP produces nitrate and nitrite, and ultimately 1,3-propanediol as 3-NOP is degraded in the rumen[15].
Dose	40-340 mg 3-NOP/kg DM has been used in research, but practical doses of approximately 100 ppm are expected, offering mitigation without feed intake inhibition.
Manufacturer	DSM (Koninklijke DSM N.V. or Royal DSM). The use of feeding nitro-oxy organic compounds to reduce ruminant methane emissions and or improve ruminant performance is patented (WO2012084629A1).
Availability	The commercial name of 3-NOP in the EU is Bovaer®. Bovaer is still in research trials to support global product registrations but has been used in large scale commercial trials in beef cattle[16]. It has just received a positive opinion from the European Food Safety Association (EFSA) for Bovaer® in the European Union (22/11/2021) as a step-in clearance to enable ratification for use by member countries in the EU and is awaiting registration through the FDA for North America. Many of the Latin American countries can be expected to also accept registration based on EFSA approval, with recent approval in Brazil and Chile.

	Australia, North America and New Zealand lack a registration pathway for additives with environmental claims, but the positive EFSA opinion may facilitate registration in these countries also.
Impacts on animal production	Overall, there is minimal effect on production, but due to a slight reduction in feed intake by 3-NOP fed animals, there may be an improvement in feed efficiency. Meta-analyses of 3-NOP actions reported the following overall effects from the literature: Diet Digestibility: 3-NOP supplementation did not alter fibre digestibility[14]. Feed Intake: 3-NOP supplementation reduced DMI up to 4.5 % overall[14] ($P = 0.02$) but showed no effect in many studies and was not significant in Jayanegara's meta-analysis in 2017[13]. Kim et al.[9] reported a (near significant) decline in DMI with 3-NOP inclusion in beef but no effect in dairy cattle. Live weight gain: Significant data on grain-based diets showed no advantage to live weight gain from 3-NOP inclusion[14] and there may be reductions in growth at high levels of 3-NOP inclusion[8]. Milk production: Melgar et al.[17] found no effect on milk production in high yielding Holsteins but an increase in milk fat with 3-NOP. Haisan et al.[18,19] also found no change in milk yield with 3-NOP.
Applicability	As a synthetic compound being introduced into the human food chain, registration of 3-NOP is likely to be required wherever it is used. Initial registration applications are likely to be sought for the intensively fed industries, primarily feedlot and dairy. It has recently (09/09/2021) received full market registration approvals for Brazil and Chile.The positive opinion of Bovaer® from EFSA will facilitate Bovaer's delivery into Europe. Feedlot: 3-NOP is highly suited to feedlot application as an exact control of 3-NOP/kg DMI is possible due to regulation of DM offered. Cut and carry: Inclusion of 3-NOP in concentrate or premix for ruminants fed cut pasture is highly possible as has been done in research trials[2]. Dairy: In farms where cattle are housed for much or all of the year, 3-NOP can be added directly to the ration, supplement, or premix to regulate daily 3-NOP intake. Grazing: While 3-NOP could conceivably be delivered in licks, blocks and concentrate supplements, this extremely large sector is likely to be the last for commercial development due to the need: to develop suitable delivery products; address any needs for stability and slow release in products that may demand a considerable shelf life before use; and accommodate diet effects on efficacy[10].

Expected market trajectory	As a patented product, the marketing of 3-NOP will be controlled by the manufacturer but the probable pattern for release seems to be in intensive feeding environments for milking cows and feedlot beef, and then grazing ruminants once a delivery pathway in supplements is developed.
Expected cost	While no costing data has been released by the manufacturer, DSM already supply other pure additives as well as commercial premixes globally, so have solid basis for pricing.
Constraints to use	The initial constraint is that 3-NOP is only registered for use in Brazil and Chile and therefore not commercially releasable for use elsewhere. Label development will require the manufacturer to state a target dose or dose range with directions on administration. The lack of research in grazing livestock and in sheep will further delay use from these emission sources. There is a small amount of evidence for residual mitigating impact of short term 3-NOP administration to young stock on long-term methane emissions[20], which may create a new option for supplementation.
Residue in animal product	3-NOP is metabolized rapidly, and does not accumulate in the mammalian bloodstream[9]. Moreover, 3-NOP and its metabolites were not found to have mutagenic or genotoxic potential[8]. In compliance with regulatory requirements, the manufacturer has a dossier on residues and health impacts of 3-NOP.
Impacts on manure	Reynolds et al.[21] found while high levels of 3-NOP could increase faecal DM and N output, this did not occur at more practical levels (500 vs 2500 mg 3-NOP/d). Manure from 3-NOP fed cattle did not produce less methane in a biogas digester than that of control cattle, though initial production may be delayed[22]. Owens et al.[23] concluded "composted and stockpiled 3-NOP manure can be used as a nutrient source for forage crops without requiring changes to current manure management because it has minimal influence on soil health." In summary there appears to be no adverse attributes affecting manure disposal or use as a result of dietary 3-NOP inclusion.
Potential regional distribution	3-NOP can be a globally available product, subject to registrations and future development into a product deliverable to grazing livestock. While research has primarily addressed efficacy in cattle, it may be expected to perform in a similar manner in small ruminants[24].
Life-cycle Assessment (LCA)	Feng and Kebreab[25] state "the carbon footprint of emissions associated with 3-NOP production…to be 52 kg CO_2e/kg 3-NOP produced (DSM Nutritional Products)". They modelled enterprise emissions and reported 3-NOP use across the whole dairy herd in California could reduce the GHG footprint by 13.7%.

	Alavarez-Hess et al.[26] conducted a partial life cycle assessment of 3-NOP use, including emissions associated with on-farm activities and emissions associated with production and transport of major production inputs. They studied two Australian dairy farms, one Australian beef farm, one Canadian dairy farm and one Canadian beef farm. The reductions in emissions from feeding 86 mg/kg DMI on dairy farms in Australia and Canada over 120 d were 6% and 15% respectively, and 12% and 19% for a 300 d lactation. On Australian and Canadian beef farms, the reduction in emissions from 200 mg 3-NOP/kg DMI in the diet of growing stock were 9% and 6% respectively, while considered over the whole herd mitigation was greater at 23% and 15%.
Actions needed to accelerate technology roll-out	Bovaer® is progressing through registration in Europe and the USA. In Australia and New Zealand its registration will require formation of a new class in the registration process for feed additives of environmental impact. DSM is managing the marketing, manufacture, and release of this patented product. Because productivity gains have typically been small, it may require large scale trials to confirm and quantify any productivity effects. Confirmation of increased productivity would support a business case for use that was not dependent upon the availability of a carbon market. For global impact, there is also needed to evaluate the efficacy of 3-NOP in supplements suitable for grazing livestock, rather than just in total mixed rations. This may well need product modification to enable sustained rumen efficacy, an understanding of levels and variation in supplement consumption, and research on the frequency of supplementation to achieve target herd mitigation. Current evidence suggests that CH_4 emissions return to pre-treatment levels within a few days after the administration of 3-NOP ceases.
Assessment Summary	3-NOP is a commercially patented, globally applicable synthetic methane-suppressing feed additive used at very low inclusion rates (60-200 mg/kg diet DM). It has a high and consistent mitigation efficacy across fully-fed sheep, dairy, and beef cattle although little data on delivery to, or efficacy in, grazing ruminants is available. Life cycle assessment analysis (LCA) of the product manufacture and of its system impact on whole farm emissions shows the embedded manufacturing emissions and associated on-farm system emission changes with 3-NOP are minor compared to its enteric mitigation impacts. Residues in animal products do not occur. Published meta-analyses identify there is **high agreement** and **robust evidence** of very high (>25%) efficacy in reducing enteric methane production. There is medium agreement and limited evidence that 3-NOP increases animal growth or milk production of supplemented cattle. 3-NOP is undergoing registration in Europe (Bovaer®) and North America. Registration laws may require registration by a name other than Bovaer® in some countries.

Freeze-dried *Asparagopsis* meal

See the reference list

Evidence of efficacy	The efficacy of freeze-dried *Asparagopsis* meal has been shown in the laboratory [27,28,217,219] and in sheep[30], beef cattle[31,40], and dairy cattle[32,41]. Efficacy of methane reductions has varied across these studies, due to different rations and variable concentrations of bromoform in the *Asparagopsis* used. A meta-analysis of effects of feeding predominantly *Asparagopsis* algae to cattle found a reduction in methane yield of 37% or 5.3gCH_4/kg DMI[33] (8 comparisons, 4 publications). There is an indication that efficacy is affected by dietary fibre with greater efficacy on high concentrate diets than on high forage diets[31]. The only study to test the efficacy of *Asparagopsis* fed to sheep[30], offered loosely with their total diet at inclusion in diet formulation and seaweed quality in all the aforementioned studies resulting in a large variation in levels of 1.0%, to 5.7% on a DM basis, achieved 15% through to 81% mitigation for these inclusion levels, respectively. A test of the efficacy of *Asparagopsis* in feedlot steers found up to a 98% reduction in methane[40] using a 0.38% DM inclusion rate, coupled with a 42% improvement in average daily weight gain. Similar trends were found by Roque et al (2021)[31] where beef cattle were supplemented with between 0.45% DM and 0.91% DM *Asparagopsis*, and methane was reduced by up to 80% and feed-to-gain efficiencies were increased by 14%. This study also demonstrated the efficacy of *Asparagopsis* for high-, mid- and low-forage diets, as well as persistent methane reductions for the 21-week period. Two studies have been conducted in dairy cows and are the most variable in terms of methane reductions. Roque et al (2019)[32] found as much as a 43% methane reduction when 1.84% *Asparagopsis* DM was included in the diet. Stefenoni et al (2021)[41] reported methane reductions as high as 80% using 0.5% DM. There is a large variation antimethanogenic response. A recent meta-analysis showed a mean methane reduction of 49% inclusive of variable feeding rates across the *Asparagopsis* studies[14]. However, this analysis did not include the two most recent studies to be published, both with methane reductions up to 80%[31,41].
Mode of action	The bioactive array of algal chemicals is complex[35] and many compounds have potential methane-suppressing effects. Some, but not all, seaweeds have the capability of synthesizing and encapsulating halogenated methane analogues (such as bromoform) within specialized gland cells[219]. In *Asparagopsis,* bromoform has

	been shown to be the principal compound suppressing methane emissions[29]. Bromoform ($CHBr_3$) and other halogenated methane analogues such as bromochloromethane (BCM) work to inhibit methanogenesis by binding to and sequestering the prosthetic group required by methyl co-enzyme M reductase (MCR), which is ultimately responsible for the last step in methanogenesis[37, 221,222,223].
Inclusion Rate	It has been hypothesized that *Asparagopsis* inclusion rates should not exceed 1% DM due to reductions in dry matter intake at higher inclusion levels, with many studies using 0.2–0.5% *Asparagopsis* DM in diet DM. Additionally, inclusion levels ranging from 0.20–0.91% DM may also contribute to increased animal productivity concomitant with methane inhibition[31,40]. An appropriate inclusion level will need to be based on diet formulation and consistency of concentration of bromoform within the *Asparagopsis* material.
Manufacturer	*Asparagopsis* is sourced from both wild harvest and from commercial farms with an increasing number of *Asparagopsis* producers developing in both the northern (e.g., Greener Grazing, Blue Ocean Barns, Symbrosia, Volta Greentech) and southern hemisphere (e.g., Sea Forest, CH_4 Global, Seascape Restorations, The Aquaculture Group). However, the majority of growers are currently (October 2021) in the development phase and are building up stocks for commercial sale. The use of *Asparagopsis* for methane mitigation and productivity gains in ruminants is protected by patents controlled exclusively by FutureFeed (https://www.future-feed.com/) and commercial suppliers must be licensed by FutureFeed. Specifically, there are patents around a 'Method for reducing total gas production and/or methane production in a ruminant animal' (Ref: WO2015109362; TW8808; PCT/AU2015/000030) and for 'Growth performance improvements in pasture and feedlot systems' (Ref: WO2018018062; TW9069; PCT/AU2016/050689). Further details of patent coverage are available from https://www.future-feed.com/our-patents.
Availability	It is expected that commercial availability of *Asparagopsis* will begin in early to mid 2022.
Impacts on animal production	In a meta-analysis of cattle studies, Lean et al.[33] found no effect of (predominantly) *Asparagopsis* algae on growth rate, nor any effects on DMI. Across these cattle studies, *Asparagopsis* improved feed conversion efficiency by 7%. However, severe feed refusals at approximately 6% inclusion in DM[30] and a reduction in feed intake at 1.84% vs 0.92% in diet DM[32] have been observed, and a reduction in intake at 0.5% vs 0.25% in DM[41], suggests inclusion levels should be restricted to <1% in diet DM. Lactating dairy cows appear to be the most sensitive to *Asparagopsis* inclusion in the diet, with DMI reductions of 38% at a 1.84% in DM inclusion rate and a reduced milk production as a direct consequence[32]. When incorporated in a TMR, *Asparagopsis* inclusion should be kept under 1% DM to avoid decreases in intake of the seaweed resulting

	from reduced diet DMI (R. Kinley pers. comm.), but reduced intake has on occasion been linked to an increase in feed-to-gain conversions in growing beef steers[31]. At inclusion levels below 0.5% animal performance response has been variable, with Kinley et al.[40] finding 0.18 and 0.37% inclusion in DM resulted in a 53% and 42% increase in daily weight gain respectively. In contrast, Li et al.[30] found no effect of *Asparagopsis* inclusion on live weight change of 2-year-old sheep. Daily milk production[41] was not reduced by *Asparagopsis* at 0.25% inclusion in DM[41] but was reduced by 0.5% inclusion, and was reduced at 1.84% *Asparagopsis* in DM in another study[31].
Applicability	Preparation and storage conditions are important to minimize bromoform volatilization[39]. Freeze dried product is currently the gold standard, although other forms of processing, including an *Asparagopsis* oil suspension, are being investigated[4]. There are co-benefits from *Asparagopsis* farming because seaweed production can be improved by utilizing waste nutrients (P, N, CO_2) from other forms of aquaculture (finfish, shellfish, prawns), if co-located. *Asparagopsis* farming can also enhance regional economies by using local labour, and *Asparagopsis* is seen as a multifunction feed ingredient in a circular economy. However, potential impacts of harvest on biodiversity and ecosystem health need to be considered in natural aquatic systems.
Expected market trajectory	There are multiple *Asparagopsis* producers advancing in capability and scale in Australia, New Zealand, North America, and Europe, and it is expected that the algae farming industry in SE Asia will be engaged to produce *Asparagopsis* and expand the capability for large scale global supply. Industrial production facilities in temperate latitudes are also under development.
Expected cost	The market will determine pricing. One Oceania supplier has been quoted at US$26/kg (AU$35) although price is expected to drop rapidly from the current high when product supply increases.
Constraints to use	Bromoform can be produced in drinking water naturally or as a result of chlorination after desalination and is considered a probable human carcinogen, with low levels of inhalation and oral ingestion thought to occur from drinking water and pools (refer to: https://www.epa.gov/sites/default/files/2016-09/documents/bromoform.pdf). Bromoform is recognized as an animal carcinogen (ACGIH: A3) and has been associated with renal and liver toxicity[42]. However, Kinley et al.[40] found no bromoform residue in meat or kidneys of cattle fed *Asparagopsis*. There is currently (October 2021) no literature on the metabolic fate of bromoform in the ruminant digestive system. Reductive dehalogenation, the breakdown of halogenated

	compounds such as bromoform, has been shown in certain types of bacteria such as *Dehalobacter* and *Desulfovibrio* species[224]. Bromoform released into the atmosphere from any source can contribute to ozone depletion[43,225]. Due to the need to preserve *Asparagopsis* bromoform concentrations in the feed for enteric methane reductions, loss from livestock feeds will always be minimized. While excess iodine intake from marine algae is possible, iodine level in algae can be reduced by processing[44] and by managed cultivation.
Residue in animal product	No bromoform residues were found in samples of kidney, liver, faeces, fat, muscle tissue, or milk, taken from sheep[30] and beef cattle[31,40] which had been offered *Asparagopsis* at the low effective levels. Low levels of bromoform have been found in milk (even from cows not consuming *Asparagopsis*), although no significant increases in milk bromoform concentrations have been found in cows fed *Asparagopsis* [32,41]. In a study with lactating dairy cows the level of safety of inclusion of *Asparagopsis* in cattle feed was tested under extreme conditions[45]. Trace levels of bromoform were found in a milk sample from a cow normally consuming up to 18 kg/d DMI which was restricted to only 1.2 kg DMI and provided with an extreme concentration of *Asparagopsis* on the day prior to morning milk collection. The milk bromoform content was less than half the USEPA drinking water standard for bromoform[45]. Iodine residues have not been tested widely and the information is very limited. Roque et al. (2021)[31] tested beef strip loins for iodine residues and found 0.00015 mg/g of iodine in steers fed 0.5% OM *Asparagopsis* for 147 days. Total *Asparagopsis* iodine fed per day was approximately 199.1 mg/day, which indicates a very low iodine transfer rate between *Asparagopsis* and meat for human consumption. Stefenoni et al. (2021)[41] reported milk iodine concentrations of 0.00297 mg/g but did not report levels of iodine in *Asparagopsis* fed or the transfer rate between that and milk.
Impacts on manure	Marine and freshwater macroalgae have long been used as soil conditioners, both in raw[46] and biochar[47] form. Furthermore, liquified seaweed or its extracts have been widely used as bioactives for soil/plant health[48]. Being of marine origin, *Asparagopsis* is rich in bromine and iodine. No bromoform residue has been found in manure samples, although impacts on compost and/or anaerobic digestors warrant investigation. Muizelaar et al (2021)[45] found low levels of bromoform in the urine of dairy cows near the USEPA standard for drinking water for up to the first 10 days of receiving high levels of dietary *Asparagopsis*, although in subsequent urine testing bromoform could not be detected. As livestock have no way of storing bromine or iodine derivatives,

	these must ultimately be excreted but there is no indication that this has adverse effects on soil. Other algal bioactives from unprocessed or pyrolyzed seaweeds potentially are beneficial to soil health.
Potential regional distribution	There are tropical (*A. taxiformis*) and temperate (*A. armata*) *Asparagopsis* species being farmed. These can be grown in diverse marine habitats as well as cultured in artificial growth environments including land-based systems, capitalizing on the different growth stages of the life cycle. Key market regions identified by FutureFeed are Australia, NZ, EU, USA and Canada.
Life-cycle Assessment (LCA)	Many LCAs have been conducted for growing algae for biofuel production[49] but these growth systems are quite different to those which will be used for *Asparagopsis*, for which no LCA is currently available. At the time of this publication, there are three independent articles under review.
Actions needed to accelerate technology roll-out	Currently (2021) most *Asparagopsis* grown is being used as seed material to scale up production. FutureFeed expects supply for early adopters in beef feedlots in 2022, and in dairies in 2022/23. Commercial expansion will require: • Clarification of any biosafety issues (bromine, iodine levels in animal products) • Shelf-stability of product and data and guidelines on inclusion rates for all industries • delivery mechanisms for grazing industries • Description of mitigation when provided as infrequent pulse in supplement instead of a total mixed ration • Clarification of Asparagopsis percentage in DM verses bromoform intake as the basis for describing inclusion level • Appropriate jurisdictional regulatory approval for use as a feed ingredient in each country.
Assessment Summary	There is growing capacity to farm *Asparagopsis* in natural marine locations or tanks. The feeding of *Asparagopsis* to ruminants for methane mitigating purposes is patented. *Asparagopsis* has at least one natural methane inhibiting compound (bromoform). Pure bromoform can have undesirable impacts on the animal as well as the atmospheric ozone layer. Whether bromoform levels in livestock *Asparagopsis* supplements are of a safety or environmental concern and the potential for residual bromine and iodine in food products are under investigation. While there is a need or more animal studies, *Asparagopsis* is highly effective in reducing methane emission and there is **high agreement** and **modest evidence** (<10 publications) that low levels of inclusion of <0.5% *Asparagopsis* in a TMR feed will achieve very high mitigation (>25%) of rumen methane output. There is also limited evidence and low agreement that *Asparagopsis* increases growth rate in ruminants, but it does show a consistent improvement in feed efficiency.

Nitrate

Nitrates (NO_3) are highly soluble salts and are readily reduced in the anaerobic rumen. Their reduction to ammonia uses hydrogen that would otherwise be used in methane production, so they competitively reduce methane emissions. They also provide a source of nitrogen for microbial protein synthesis in the rumen. However, there is some dose-dependent risk of toxicity to the animal from accumulation of the chemical intermediate nitrite (NO_2).

See the reference list

Evidence of efficacy	Based on stoichiometric relationships, 1% NO_3 in the diet DM should reduce methane production for grazing animals by 12.5% (from 20.7 to 18.1 g CH_4/kg DMI) assuming the methane yield for grazing cattle[50]. This percentage would be higher in feedlot diets where methane yield is already low. In a meta-analysis of cattle data, Feng et al.[51] reported an overall mitigation of 11.4% per 1% nitrate mitigation or 13.2% if slow-release nitrate studies were excluded. Where slow-release nitrates have been fed (to protect from nitrite toxicity), the appearance of NO_3 in faeces suggests the nitrates were not adequately released in the rumen[52,53]. Simulation of on-farm usage in three enterprises[26] showed less than a 5% methane mitigation in 2 of 3 enterprises.
Mode of action	Microbial reduction of nitrates (NO_3^-) through to ammonia (NH_3) provides a thermodynamically favourable electron (and hydrogen) sink in the rumen compared to reduction of CO_2 to CH_4. Consequently, nitrate can be expected to competitively reduce methane production in a predictable manner, such that for every mole (62 g) of nitrate consumed, methane production would be reduced by one mole (16 g). While this strict relationship is not always seen, typically the mitigation is >80% of that expected. It has been hypothesized that nitrate may also support methane oxidation[54], but this should not change the total impact of nitrate on CH_4 production.
Dose	Because a potentially toxic intermediate, nitrite (NO_2^-), is produced and can accumulate in the rumen and enter the blood[55], the dose is typically limited to 2% nitrate in the diet DM. Dosage can be higher if the rumen microbiota is adapted to increasing concentrations of nitrate, but to minimise risks to animal health commercially 1% inclusion is recommended for SilvAir by Cargill.

Manufacturer	Nitrates are widely produced for fertilizer use. Because of the risk of ammonium nitrate being used in explosives, its availability is often restricted and so calcium nitrate is the preferred source. There are multiple suppliers of calcium nitrate with the global fertilizer manufacturer Yara preparing a feed-grade source. Calcium nitrate for use as animal feed is trademarked by Cargill as "SilvAir".
Availability	Nitrate salts are widely available globally for agronomic use.
Impacts on animal production	In nutrient-adequate diets, there is often no performance response to nitrate in grazing[56], feedlot[57] or dairy[58] animals. Nitrate can suppress feed intake at higher inclusion levels[57,59] and can cause sorting of feed[53], so ruminants must be introduced slowly to 2% nitrate in dietary increments.
Applicability	The two most likely applications of dietary nitrate are in feedlots, where it can be mixed in a total mixed ration in either dry form or in liquid supplement to avoid toxicity, and in ruminants grazing low protein forages such as in the dry season tropics[60]. In either situation it can be used to replace urea. Achieving 2% nitrate in the total diet via supplements is difficult due to the large mass required, the risk of nitrite accumulation from rapid ingestion, as well as the finding that the desired mitigation may not be achieved[56].
Expected market trajectory	The uptake of nitrate has been minimal, but its two most likely points of uptake are in feedlots (where it can be more safely mixed and fed in rations), and in the tropics where it may provide a source of nonprotein nitrogen in supplements.
Expected cost	While calcium nitrate and urea have similar costs (US$730 vs US$606/t landed Australia) the lower N content of calcium nitrate (15.5% N v 44.6% N) means the cost/unit N is almost 2.5-fold higher for calcium nitrate as a N supplement. Since there are few reports of productivity gains from calcium nitrate over urea, the carbon credit value of the methane deferred would be the economic justification for using nitrate.
Constraints to use	The two principal constraints to nitrate feeding are the purchase cost and the risk of poisoning and death of animals from nitrite absorption. For this reason, slow-release forms have been evaluated[53,61], but as nitrates are already an expensive source of feed nitrogen relative to urea, incurring further costs to regulate rumen release is unlikely to be economically feasible.

	A further practical constraint to nitrates as feed supplement is that because the N% is low (15-23% N), providing nitrate in a loose-lick or lick block for grazing animals requires at least twice the space in a supplement mixture as the same quantity of N as urea. Given that rangeland cattle only consume 100-150 g of supplement/d it becomes very difficult to get in the desired level of N supplement as nitrate. Further, the supplement is often consumed in a short period, so the risk of nitrite toxicity is increased and there can be substantial variation in supplement intake in pastured animals. In the manufacturing sector avoidance of potentially explosive nitrates is important to minimize warehousing and processing safety requirements. The feeding of nitrates for methane mitigation is covered by global patents (e.g., WO2011010921A2 originally implemented by Cargill).
Residue in animal product	Nitrates naturally occur in feeds and are naturally produced in mammalian metabolism. A study of nitrate and nitrite disposal in sheep showed substantial accumulation in the skin[55]. No evidence of potentially toxic nitrosamines was found in tissues of nitrate-fed cattle[57].
Impacts on manure	Dietary nitrate is readily reduced in the rumen and Villar et al.[55] found that in sheep over 6 days, 14% of nitrate-N was excreted in faeces and 49% in urine, with some of the urinary nitrogen present as urea. A portion of the nitrate was also recycled back into the gut. In beef heifers, encapsulated nitrate has been shown to increase the nitrate content in urine and faeces but not total nitrogen excretion[62].
Potential regional distribution	Globally available as sodium, potassium, calcium or ammonium nitrate fertilizer. The commercial feed-grade product SilvAir is already available and in commercial demonstrations in Brazil and Europe, while incentive schemes are being implemented.
Life-cycle Assessment (LCA)	Calcium nitrate manufacture shows a carbon cost of 650 grams of CO_2/kg SilvAir using LCA methods (value from Cargill). LCA of the production and use of ammonium nitrate or calcium ammonium nitrate shows the GHG impact primarily arising from on-farm emissions but that the global warming potential for their production is approximately 3 times that of urea[63].

Actions needed to accelerate technology roll-out	Nitrate is a chemically simple, widely available synthetic nutrient that has a high, predictable and well understood mechanism for reducing methane production. It is suitable for implementation in total mixed ration feeding systems, but more studies at a commercial scale are required. It is unlikely to be applicable in extensive environments due to the difficulty in safely delivering the required quantity through supplements, without incurring extra cost through developing slow-release formulations. Cargill have negotiated two approaches to overcome economic costs, being (1) government subsidy and (2) industry subsidy for Silvair use. In the absence of productivity gains, this may also be required in other adopting countries.
Assessment Summary	Inclusion of nitrate for methane mitigation is covered by global patent protection. The calcium nitrate form is not constrained by (explosion) risks associated with ammonium nitrate and is globally available as a fertilizer. There is **high agreement** and **robust evidence** (>25 publications) of high efficacy in mitigating enteric methane, with mitigation of approximately 10% per 1% nitrate by weight in the diet, using a maximum of 2% inclusion to minimize risks of nitrite toxicity. Its safest use is in a total mixed ration as it is difficult to supply adequate nitrate through a low intake supplement. There is little reason to expect nitrate to improve animal productivity in nitrogen adequate diets, but it may provide an alternative to urea as a supplement to stimulate feed intake and performance of ruminants eating diets lacking rumen degradable nitrogen. Dietary nitrate is not associated with residue in animal products.

Antibiotic Rumen Modifiers

Antibiotic rumen modifiers include ionophores (such as monensin, lasalocid and salinomycin) as well as phosphoglycolipid antibiotics such as bambermycin and antibiotics such as virginiamycin, a streptogramin antibiotic. They do not all have the same mode of action so their effects on methane emissions are inconsistent. However, monensin is the most commonly used antibiotic rumen modifier that has been most heavily researched and therefore the comments below are largely based on monensin findings.

See the reference list

Evidence of efficacy	A recent meta-analysis of monensin use in beef and dairy found that when adjusted for DMI differences, monensin reduced daily methane production by 5% (22 studies) and Ym (% of Gross energy intake lost as CH_4) by 4%[64] (20 studies). Immediate mitigation is routinely observed upon introduction, but efficacy can diminish over time and is sometimes not found in long term studies[65].
Mode of action	There are two mechanisms by which monensin reduces methane production. 1) It reduces feed intake[64] and 2) It affects the balance of hydrogen production and use by differential effect on the microbiota, reducing the quantity of hydrogen available for methane production. It has no direct effect on the methane producing organisms[66].
Dose	Typically evaluated at 16-50 mg/kg DM
Manufacturer	Monensin is produced and marketed as a coccidiostat for poultry as well as for improving feed efficiency in ruminants outside of the European Union. Its patent, and many other rumen modifier patents, have now expired and there is a large number of generic producers world-wide supplying independently produced post-patent products.
Availability	Globally available. Original production by Elanco Animal Health but now generic products are widely distributed under multiple brands.

Impacts on animal production	Feedlot cattle fed monensin-containing diets gained 1.6% faster, consumed 6.4% less feed and required 7.5% less feed/100 kg gain than cattle fed control diets[67]. In dairy cows monensin is highly effective in protecting against bloat[68]. From a meta-analysis of 71 studies, Duffield et al.[69] concluded monensin significantly decreased dry matter intake by 0.3 kg/d, increased milk yield by 0.7 kg/d and improved milk production efficiency by 2.5%. Monensin decreased milk fat percentage by 0.13% with no effect on milk fat yield. Monensin is also effective in reducing bloat in beef[68] and in dairy cattle where it has been delivered by slow release intraruminal device for cows grazing alfalfa, clover or wheat to reduce the incidence of frothy bloat.
Applicability	Monensin is manufactured and used widely (not in EU) in total mixed rations and supplements.
Expected market trajectory	Given the availability of monensin and its multiple points of manufacture it is unlikely that it’s modest impact on emissions will cause a growth in market penetration for mitigation outcomes.
Expected cost	Monensin (active ingredient) is approximately US$28/kg and sold in 10-40 g monensin/100g mix DM premixes.
Constraints to use	Monensin is moderately toxic to horses[70] and dogs so care must be taken with regard to access by non-target species. As monensin is an antibiotic, its administration through feed to cattle has been banned in the EU and could be potentially restricted in other jurisdictions in the future.
Residue in animal product	Monensin has been found in milk at levels not considered a risk to human health[71] and tissue residue levels have been specified for meat and milk[72].
Impacts on manure	Monensin is incompletely absorbed from the ruminant digestive tract and is partially metabolized and excreted[73]. Monensin that is not absorbed is excreted in its original form in faeces, where it is further degraded. Residues in lagoons and shallow ground water have been detected[74]. Its presence can also change soil and root characteristics, but this does not necessarily imply that it has an adverse impact on soil health.
Potential regional distribution	Global (out of patent) except it is prohibited for use in the European Union.

Life-cycle Assessment (LCA)	Webb et al.[75] conducted a partial LCA of cattle production for GHG emissions, fossil energy use, water use, and reactive N loss to evaluate production systems utilizing additive combinations with growth promoting technologies. • The use of ionophores reduced GHG emissions and water usage by 1%, had no effect on energy use and increased reactive N loss by 1%. • The use of ionophores in combination with anabolic implants reduced GHG emissions (8%), energy usage (5%), water usage (6%) and reactive N losses (5%). The use of ionophores in combination with implants and a beta-agonist reduced GHG emissions (6%), energy usage (3%), water usage (4%) and reactive N losses (1%).
Actions needed to accelerate technology roll-out	Monensin is widely used in feedlots and dairy cattle in jurisdictions where it is approved for use. No additional actions are required to promote monensin use in the developed world, primarily due to its large market footprint and limited impact on methane emissions, but there is potential to expand its use in the developing world.
Assessment Summary	Antimicrobial rumen modifiers are a mature market, being a strong part of diets for monogastrics (for coccidiosis control) and ruminants fed partial or total mixed rations for improved feed efficiency, rumen acidosis protection, and reduced bloat risk. All require registration and many are off patent. When monensin is used in total mixed rations and supplementary feeds, there is **robust evidence** with **high agreement** that it reduces methane yield (g/MJ of energy intake) by up to 5% from cattle over sustained feeding periods. There is also robust evidence with high agreement that monensin improves feed efficiency in both dairy and beef cattle by 4-8%.

Essential Oil Blends

Essential oils (EOs) are volatile aromatic compounds produced by plants (herbs and spices) as complex mixtures of secondary metabolites. They are not fatty acids but are hydrophobic (water repelling) compounds, usually present in mixtures with 20−60 components including alcohols, aldehydes, hydrocarbons, ketones, esters and ethers[76,77]. They are not biologically essential for animals but rather, from alchemy times, they were understood to contain the essence (essential components of flavour/aroma) of the plant from which they are extracted.

See the reference list

Evidence of efficacy	There has been intensive study of key individual EOs (e.g., cinnamaldehyde from cinnamon or allicin from garlic) as well as of diverse EO blends, so reporting efficacy of this heterogeneous group of additives is difficult. In a review, Cobellis et al.[77] concluded "the most consistent results on methane production were observed using EO from thyme, oregano, cinnamon, and garlic or their principal components (thymol, carvacrol, cinnamaldehyde, and allicin respectively)". In a meta-analysis of 21 refereed sheep publications, Torres et al.[78] found EO did not affect feed intake or reduce daily methane production.
Mode of action	There are multiple mechanisms by which EO may influence methane emissions, but these are not independent, and none are robustly evident as reducing CH_4 emissions across published literature. 1) Effects on intake. No significant effects in sheep[78], while Corbellis et al.[77] found of 18 sheep and cattle studies reporting DM intake, only two increased intake and one decreased intake. 2) Reduced protozoal population. Significant (16%) reduction in populations in sheep over 21 papers[78]. In a meta-analysis, protozoal density was reduced in 19 of 24 treatments in laboratory studies, but only in 4 of 17 animal studies[77]. 3) Reduced methanogen population in the laboratory (19 of 24 treatments) but not strongly in animal studies (2 of 17 studies)[77]. 4) Changed fermentation pattern. Increased propionate (+0.59% units) and decreased acetate (-1.0% units) molar proportions in sheep across 21 studies. But considering cross-species animal studies Corbellis et al.[77] found only 3 of 23 studies had increased propionate concentration.

Dose	Agolin: 1 g/d/cow Crina Ruminants: 150-200mg/kg bodyweight Mootral: 10-15 g/cow/d GasoLess: not specified Nutrimix: not specified Garvo: not specified
Manufacturer	There are numerous commercial EO blends available but currently two major blends are marketed specifically for methane reduction. Agolin (https://agolin.ch/products/#ruminant). In a meta-analysis of published and unpublished studies of Agolin supplementation of dairy cows, at a rate of 1 g/d, Belanche et al.[80] reported increased milk yield (3.6%) and feed efficiency (4.4%) and an 8.8% reduction in methane production, with no reduction in feed intake. As more than half of the studies were apparently on commercial farms and their data used without details being available, cautious interpretation of these results is required, as they are not confirmed by other EO literature. Mootral (https://mootral.com/) This allicin (from garlic) and citrus extract is specifically marketed for methane mitigation. It was shown to reduce methane emissions in sheep[81], with preliminary assessments in dairy[82] but it did not reduce emissions from feedlot cattle[32]. In addition, there is a suite of regionally available essential oil blends, with some listed below: Crina (https://www.dsm.com/anh/en_NA/products/solutions/crina.html) This EO blend has not been so well assessed for methane inhibition and makes no claims regarding methane but has been adopted as a monensin replacement in the EU. Gasoless (https://www.idena.fr/en/products-expertise/ruminants/) Nutrimix (https://www.idena.fr/en/products-expertise/ruminants/) Garvo (https://www.garvo.nl/contact/)
Availability	EOs are available worldwide.

Impacts on animal production	In only 2 out of 18 studies using a wide range of EO mixtures did Corbellis et al.[77] find a significant increase in feed intake with EO. Across 11 and 15 studies respectively, Torres et al.[78] found diverse mixtures of EO significantly reduced growth rate (-10 g/d) and carcass weight (560 g) in sheep. In dairy cows, Hart et al.[83] reported a significant increase in milk yield (without changing milk composition) and a 10% reduction in methane emissions with Agolin. In a meta-analysis of Agolin alone, where more than half the studies used were unpublished, Belanche et al.[80] claimed a similar reduction in methane production (8.8%) and improvement in milk yield and feed efficiency of <5%.
Applicability	EOs are transportable so they are widely available, although as indicated, the animal performance and mitigation responses are not consistent across products. Essential oils have low chemical stability and high volatility, thus there is a concern that exposure to oxygen, light or temperature may reduce their antioxidant and antimicrobial capacity. Pelleting may also adversely impact the biological activity of EO. EOs in their liquid form can be firstly mixed in with the diet concentrate or in a mineral mix and incorporated into the TMR diets of small and large ruminants (dairy and beef). Torrecilhas et al.[84] observed that EOs remained stable for up to 30 days after inclusion in the diet. Moreover, EO can be microencapsulated (in dry form) leading to an extended shelf-life and maintaining the metabolic activity for extended periods[85]. This technology permits EO to be added to mineral or protein/energy supplements that are offered to ruminants in an open environment (rain, sun, wind, etc.) without losing efficacy.
Expected market trajectory	The major market opportunity for EO came when monensin was banned in Europe and EO and probiotics entered the market as an alternative to this antibiotic, to reduce acidosis and other digestive upsets in ruminants fed high-grain diets.
Expected cost	In Brazil, EO blends are often sold as part of a mineral and vitamin premix (up to 4.5%) of the diet. Cost is approximately US$1/kg of this product. Mootral has a program of carbon credits from enteric methane reduction from cattle (CowCredit™). CowCredit (1 CowCredit = 1 t CO_2e reduction) can co-finance the cost for Mootral for ruminants via selling the credits on voluntary carbon markets and serves as certification for GHG reductions.

Constraints to use	The volatility and instability of EO during feed processing and storage may constrain their use. Similarly, the cost relative to expected return may also constrain use and this is made worse by the variety of formulations available making it difficult to consistently predict economic benefits. There is growing recognition that the bioactive compounds in EO may be produced by synthetic chemistry or by biotechnology[214,] rather than relying on extraction from plants which may reduce cost and availability constraints in future.
Residue in animal product	Due to the aromatic properties of EO, there is a concern that essential oils compounds may be incorporated in milk and meat resulting in unfavourable organoleptic characteristics. However, some recent sensorial studies revealed non-effect[84] or higher acceptability of meat from animals fed essential oils[86,87]. The highly aromatic and volatile EO can have negative and positive effects on oxidation and attributes of ruminant meat with Simitzis et al.[88] finding "oregano essential oil exerted strong antioxidant effects retarding lipid oxidation in the carcass of lambs." Inclusion of garlic in the diet of dairy cows has also been shown to result in off-flavours in milk[213.]
Impacts on manure	There is extensive literature on the effects of soil manures and fertilizers on the essential oil content of various herbs, but little information on the effects of residual EOs that may be excreted in livestock manures on the environment. When added directly to manures, EO are highly effective in suppressing pathogens[89], but the economic viability of this practice is questionable. As most EO are on the GRAS list, there is little concern with regard to adverse environmental effects.
Potential regional distribution	EOs are available worldwide. They are incorporated in diets for their protective properties (antioxidant and antimicrobial capacity) and possible effects on animal performance (DMI, feed efficiency, etc.). Several commercial EO products make claims as methane mitigators (Agolin and Mootral) which are mainly available in Europe and North and South America. Regional plants with lower cost may be an alternative to commercialise rather than use of extracted oils (thyme, oregano, rosemary, cinnamon, clove, etc.). Studies continue to seek out additional sources of EO from regionally produced plant species.
Life-cycle Assessment (LCA)	LCA have been conducted for the extraction of EO from various plant sources[90,91], but the impact of EO on the carbon economy of ruminant production systems have not been assessed from an LCA perspective. Given the diversity of EO, a LCA of their impact on ruminant production would likely need to be EO-specific.

Actions needed to accelerate technology roll-out	Farmers are reluctant to adopt new feed additives without evidence of robust economic benefits. For a large number of laboratory-based publications, there are relatively few animal studies and even fewer at the scale needed to detect a significant reduction in emissions or production of <5%. The primary need is for large, properly designed and replicated studies to assess the efficacy of the existing EO blends and their bioactives to reduce methane emissions, using appropriate methane measurement methods.
Assessment Summary	EOs and their blends have been extensively researched in laboratories but far less so in animals. The variability across treatments (and product formulations) is high. The most consistent results for methane mitigation were observed using thyme, oregano, cinnamon, and garlic or their principal components (thymol, carvacrol, cinnamaldehyde, and allicin, respectively)[77]. There is **low agreement** and **medium evidence** of a EO causing a low level of mitigation of methane emissions in peer reviewed research conducted in animals. EO efficacy is highly dependent on several factors (plant species, harvest time, method of extraction or compound synthesis, and dose) making it difficult to compare studies and clearly define effective formulations and dosages.

Saponins

Saponins are a class of plant secondary metabolites with a great diversity of structure and biological activity[92]. Their chemical structure consists of a sugar base (e.g., glucose, galactose, glucuronic acid) linked to a hydrophobic aglycone or sapogenin. The biological activity of saponins depends on the nature, number and sequence of the sugars in the structures. The most studied saponins in the literature have been sourced from quillaia, tea and yucca plants.

See the reference list

Evidence of efficacy	A meta-analysis by Jayanegara et al.[93], with 23 laboratory studies that directly measured methane emissions, revealed that the addition of increasing levels (up to 500 mg/g of substrate) of a saponin-rich source (quillaia, tea or yucca) decreased methane emission per unit of substrate by (7.9%, 13.0%, or 22.3% respectively) as well as per unit of total gas produced (9.5%, 13.2%, or 23.3%, respectively). A decrease in acetate proportion and an increase in propionate proportion were also observed, while protozoal counts decreased with increasing levels of saponins. Ridla et al.[94] recently reviewed 17 animal studies and reported a significant 8.6% reduction in methane/kg DMI for doses ≤0.5% saponin. Darabighane et al.[95] likewise reported a significant (0.85 g CH_4/kg DMI) reduction in methane yield in sheep, but Honan et al.[96] highlighted the variability across studies. If reductions in methane are solely related to loss of rumen protozoa from saponin intake, reductions in methane may be transient, as increased activity by other members of the methanogen community not associated with protozoa may return emissions to pre-treatment levels.
Mode of action	Saponins are known to be the "natural detergents" with membrane degrading groups that complex with sterols in protozoal cell membranes, causing cell lysis. They modify ruminal fermentation largely by suppressing ruminal protozoa and selectively inhibiting some bacteria[97]. However, there is some ambiguity in the literature concerning the action of saponins to reduce methanogen populations. Guo et al.[98] observed a decreased activity of the mcrA gene (an indicator of the methanogenic activity of the methanogen population), without changing the total methanogen numbers, while other studies reported that saponins decreased methane emission due to a lower relative abundance of methanogens[93].
Dose	Inclusion levels of ≤0.5% are recommended for maximum methane mitigation and to avoid adverse impacts on digestibility[94].

Manufacturer	There is strong global supply of saponins, with multiple plant extracts generated in Asia where they are used as natural pesticides for soil invertebrates and in the US (e.g., www.desertking.com).
Availability	Widely available.
Impacts on animal production	The meta-analysis of Ridla et al.[94] showed DM digestibility was increased by a low level of saponin (≤0.5% in DM) but reduced at a higher level. In the review by Wina et al.[99], 3 studies with yucca extracts showed no changes in urea concentration in milk and 5 out of 9 studies reported improvements in animal performance (average daily gain) of cattle and sheep fed saponins sources, especially when animals were fed high roughage diets. This could be associated with a better utilization of N and microbial synthesis due to elimination of rumen protozoa. No information regarding the effect of saponin on animal reproduction was found in the literature.
Applicability	Saponin could be incorporated into mineral supplements for farmers in developing countries that adopt extensive pasture systems to improve N retention. Saponin extracts could also be incorporated in diets by firstly mixing with concentrate and then adding to total mixed rations for more intensive production systems (up to 0.5% of the diet) to reduce CH_4 emissions and achieve other potential benefits from protozoal defaunation.
Expected market trajectory	Yucca and Quillaia saponins are already commercially available products that have been used not only as feed additives but for other purposes like foaming and emulsifying agents in beverages and cosmetics.
Expected cost	A yucca saponin extract powder containing approximately 40% Yucca saponin costs approximately US$26/kg saponin.
Constraints to use	Some plant saponins may be toxic to ruminants, causing photosensitization. This could lead to liver and kidney degeneration and gut problems such as gastroenteritis and diarrhoea[99].
Residue in animal product	Saponins can be degraded in the rumen to sapogenins, and then excreted in faeces or absorbed in the duodenum and transported to the liver where they may be conjugated with glucuronide and excreted in bile. It is unlikely that saponins will be found in meat or milk, but even if they are retained, saponins show no or little toxicity and do not seem to be of hazard for consumers[100].

Impacts on manure	Saponins have been used to remove heavy metals and hydrophobic organic compounds from contaminated soils[101] but there is no available literature on effect of dietary saponins on the properties or usefulness (e.g., fertilizer, biogas production) of manures.
Potential regional distribution	Saponins can be found worldwide in a large variety of plants and plant tissues, so they can be a part of the forage stand during grazing. Extracted saponins, however, are far less common and principally available in the Americas and Europe.
Life-cycle Assessment (LCA)	No lifecycle analyses for tea, quillaia or yucca appear to have been completed.
Actions needed to accelerate technology roll-out	The principle uses for extracted plant saponins are in the human health sphere. Given their modest efficacy of mitigating enteric methane from ruminants, it would seem unlikely that this would be a market that will grow with additional research investment.
Assessment Summary	Recent review indicates **low agreement** and **medium evidence** of saponins causing low to medium mitigation of methane emissions in animals**,** as had been found in the laboratory. Modest doses supporting reduced methane production can also improve DM digestibility of supplemented ruminants, and low doses may support increased animal growth, but this is highly variable. Saponins are a heterogenous group of compounds with diverse biological activities, so any observed benefits are likely to be source specific.

Tannins

Tannins are plant secondary compounds that are rich in phenols and have a strong tendency to bind to proteins, often making them unavailable for digestion and absorption. As such they are generally regarded as 'anti-nutritive' factors in forages used by livestock and the effects of both hydrolysable and condensed tannins have been reviewed[102,103]. Naturally occurring tannins are thought to be the main cause of low methane yields (CH_4/kg DM) in legumes such as *Lotus* spp.[104], Leucaena[105] and Desmanthus[106]. Condensed tannins are commercially extracted and prepared for tanning leather, and it is these dried tannin extracts from acacia species as well as extracted quebracho and chestnut tannins that have been typically evaluated as feed additives.

See the reference list

Evidence of efficacy	Much of the research on tannins and methane has been based on tannins present in forage rather than tannins used as a feed additive, so the evidence is differentiated on this basis below (innate forage vs added extracted tannins). **Tannins in the main diet or forage:** Jayanegara et al.[107], in an analysis of 15 experiments (11 of them with tannin of non-extract form) with 41 comparisons found a weakly negative linear relationship between methane yield and dietary tannin level (g/kg DM), such for every 1% tannin in the diet, MY declined by approximately 0.8 g CH_4/kgDMI. Patra and Saxena[108] ascribed this to (1) reduced carbohydrate fermentation in the rumen, (2) a direct toxic action on methanogens, and (3) a direct suppressive effect on protozoa. In a meta-analysis of six rumen simulation technique (RUSITEC) experiments, Jayanegara et al.[109] found that methane emission decreased linearly with increasing level of dietary tannin due to a reduction in digestibility of nutrients (especially fibre) and inhibition of methanogens. **Tannin extracts:** Aboagye & Beauchemin[102] found reduced MY in 7 of 9 animal studies, largely based on feeding tannin extracts**.** Meta-analysis of cattle data by Orzuna-Orzuna et al.[110], with up to 32 cattle comparisons in which 26 of them used tannin extracts, observed a reduction in the concentration of ammonia nitrogen in the rumen (5.9%), urinary N excretion (3.0%), and dry matter digestibility (4.5%), without

	affecting animal performance. Methane yield was measured in 10 studies and methane emission/kg DMI was reduced by 5.9%, with the effect increasing in animals fed tannins for longer periods. There is a commercially available tannin extract (Silvafeed) with some documented emission and productivity responses: • Methane production of sheep expressed in g/kg DM intake was reduced by 12%, 30% and 19% for Silvafeed, crude Acacia tannin extract and encapsulated Acacia tannin extract, respectively[111]. • Silvafeed at 30 g/ kg of DM reduced (6%) total CH_4 production (24 h) but increased (5%) production of CH_4 per g of degraded DM. These results suggest that these tannins affected CH_4 mainly by decreasing fibre digestibility[112]. • Silvafeed (67 mg/L of inoculum) had no effect on gas production and methane production in the laboratory[113].
Mode of action	Tannins bind strongly to proteins in the rumen and this is the main reason they reduce proteolysis in the rumen and increase protein outflow to the abomasum and intestine[108]. Exact mechanisms of mitigating enteric methane are likely to vary with tannin source but may include direct inhibition of methanogens, inhibition of protozoa, binding to polysaccharides as well as proteins, and an increase in propionate production[114].
Dose	Tannins are not uniform but Jayanegara et al.[107] found most animal studies used <40 g/kg DM. Higher doses can inhibit feed intake and DM digestibility.
Manufacturer	Global producers exist for tannins, principally marketed to preserve skins and hides as 'veg-tan' hides. Products specifically produced for feed applications are made, but none have a claim for reduced methane production. Silvafeed is a commercial additive composed of a mixture (60:40) of hydrolysed tannin from chestnut (*Castanea sativa* Mill) and condensed tannin from quebracho (*Schinopsis lorentzii* Engl) produced by SilvaTeam. This limited supply of dietary tannin extracts contrasts with a very strong research and commercial interest and investment in evaluating and developing tanniniferous shrubs and forages, especially legumes[115] (https://www.progardes.com.au/research)

Impacts on animal production	The meta-analysis of cattle studies by Orzuna-Orzuna et al.[110] summarized the following effects in cattle based on up to 32 peer reviewed publications. Animal Performance: Feed intake, cattle growth rate and final liveweight were unaffected by tannins. Feed Digestibility:Reduced feed digestibility at doses above 12 g/kg DM[110], and a tendency for reduced feed efficiency. Nitrogen Utilization: An intake of below 50 g condensed tannin/kg DM may contribute to greater amino acid absorption. For animals with higher protein requirements (capable of responding to an increase of dietary protein) forages containing condensed tannins had the potential to increase average daily gain by 8 to 38%, and milk production by 10 to 21%[116]. Tannin supplementation has reduced ruminal N concentration but did not affect the efficiency of nitrogen use[110]. Environment: Tannin supplementation reduced N excretion in urine and increased N in faeces. The shift from urinary to faecal N may be beneficial from an environmental perspective, as urinary N is volatilized to ammonia which can lower air quality, whereas slower release faecal N is more likely to contribute to soil health[114]. Animal Health: Tannin extracts have the potential to reduce bloat incidence[117]. Bloat is caused by very high solubility of forage proteins which produce stable foam in the rumen that traps rumen gases. Concentrations >5 g/kg of condensed tannins in DM is needed to make forages bloat-safe[118].
Applicability	Tannins can be consumed naturally as ingredients in forages or added to prepared feeds and supplements as extracts mixed into the diet.
Expected market trajectory	The market trajectory for tannin-rich forages and browse species is strong but the prospects for delivery of extracted tannins in supplementary feed is weak due to the astringency of the tannins and lack of supply.
Expected cost	Tannin extracts cost US$3-4/kg for a product that is approximately 95%DM and 60-70% tannin in DM. Silvafeed in Brazil is marketed to the feed industry and farmers in packages of 25 kg to be mixed into the diet (0.03-0.08% of DMI) with cost around US$3/kg of additive.

Constraints to use	Kumar and Singh[119] document the constraints of tannins in natural feedstuffs, including (1) reduction in feed intake if >2% in forage, and (2) reduction in digestibility. Therefore, while rumen protection of proteins may enhance productivity in protein-deficient grazing situations and stimulate animal performance, effects are likely to be minimal with protein rich diets. Depending on the species, tannin-rich forages may also be difficult to establish and have yields that are lower in some environments than some alternative forages. Further, if tannins are provided as an additive in dietary supplements, they will need to be in a high concentration in the supplement and their high astringency may render the supplement unpalatable.
Residue in animal product	As tannins are high molecular weight complex molecules that are largely undegradable and not absorbed from the digestive tract, they are unlikely to generate residues in either meat or milk.
Impacts on manure	While diversity in tannin effects on feed digestion have been summarised above, in general tannins reduce the proportion of N excreted in urine[120] and increase N excretion in faeces. The presence of the tannin in faeces has a persistent effect and reduces the overall greenhouse gas emissions from the manure (CO_2, N_2O, CH_4)[121]. However, this effect may be lost over time as there was no difference in total gas or methane production over 90 d in bench scale incubations[122].
Potential regional distribution	As tannins are most common in the forages and browses of tropical countries, they are widely accessible. Similarly, extracted tannins for tanning hides are globally available, although that does not mean they can legally be used in supplements or TMR. Their approval as a feed additive depends on the requirements of the regulatory agencies in each jurisdiction. To the best of our knowledge there is only one commercially available tannin extract in the market for animal use. SilvaTeam is an Italian company, with factories located in Argentina, Brazil, Peru, USA, and China, and reputedly currently sells product in over 60 countries.
Life-cycle Assessment (LCA)	Life cycle assessment of different tannin extraction methods from spruce bark revealed that the evaporation process is the primary contributor to the environmental impact of tannin production. The use of preliminary cold-water extraction or multiple extractions can result in a higher tannin yield but has higher environmental

	impact than a single hot water extraction[123]. A LCA from the perspective of the impacts of tannins on ruminant production has not been conducted.
Actions needed to accelerate technology roll-out	Given the low efficacy of tannins at reducing methane emissions and associated negative effects on dry matter digestibility (DMD), the co-benefit of altering nitrogen utilization and generally reducing urinary N excretion may offer motivation for tannin inclusion. There are also strong reasons for promoting tannin containing, nitrogen fixing legumes in the pasture/browse available to livestock grazing or fed with cut and carry systems. The combination of tannin and additional nitrogen may enhance forage yield and animal performance, and lower emissions/unit animal product.
Assessment Summary	Much of the data for tannins come from tannins eaten in forages, not provided as a feed additive. When fed as additives to animals there is **medium agreement** and **medium evidence** that dietary tannins result in a low reduction in CH_4 yield in ruminants, both directly by inhibiting methanogen growth, and indirectly by decreasing nutrient digestibility. Mean reduction in methane yield (5.9%) is less than the reduction in total methane output (9.9%) because tannin additives reduce DM intake, but scale of effect is very dependent on tannin source. Thus, there is low agreement that these effects will result in improvements of animal performance and feed efficiency, but high agreement that tannins shift nitrogen excretion from urine to faeces that may affect manure GHG emissions.

Microalgae

Microalgae have been principally evaluated as a renewable biomass substrate for producing algal oils for biodiesel or for gasification to methane in biodigesters[124,125]. They are valued for nutrient removal from wastewater and so contribute to the vision of cyclical agriculture. Microalgae, such as *Chlorella* spp., are likely to require cell disruption or solubilization before use as feedstock, and this has limited their use in biogas production[126]. Microalgaes have been evaluated as substrates for fermentation and biogas production[127,128], but more recently their potential as feed additives to provide protein[129] and suppress methane in ruminants has been assessed[130].

See the reference list

Evidence of efficacy	McCauley et al.[130] concluded that the "effects of microalgae as a ruminant feed supplement on CH_4 production and other fermentation parameters have not been fully understood or investigated". Kiani et al.[131] found no effect of three microalgae on methane production in the laboratory, despite a general suppression in fermentation. To date, no microalgal species has been found to mitigate CH_4 in a manner similar to the red macroalgae, *Asparagopsis*. Among the few animal-feeding studies where microalgae or docosahexaenoic acid (DHA, an algal metabolite) has been fed to animals, as opposed to laboratory studies, Moate et al.[132] and Klop et al.[133] found no effect on methane production.
Mode of action	While the long chained fatty acid, DHA, has been proposed as an anti-methanogenic component of microalgae through its biohydrogenation[134] and potential direct effects on ruminal CH_4 production, McCauley et al.[130] concluded that they do not always reduce emissions. Other bioactives may also play a role in CH_4 mitigation as lipid-free microalgae extracts have also been shown to suppress emissions. Freshwater microalgae typically have less or lack the bromoforms associated with marine red macroalgae. Where reductions in CH_4 emissions are seen, they are likely most often attributable to the high lipid content of some microalgae species.
Dose	The inclusion level of microalgae in the diet depends on whether the product is fat extracted or is sold 'as grown', and whether it is blended with other non-algal components in a premix. High levels of microalgae in the diet can suppress feed intake in ruminants.

Manufacturer	Algal meals are available and are now being used as source of omega three fatty acids for farmed fish.
Availability	DHA in a purified form is readily available as nutraceutical & algal extracts are available.
Impacts on animal production	Algal meals have primarily been evaluated as a protein source and as a source of DHA. Altomonte et al.[135], in reviewing effects on milk quality, found in most cases there was no effect on milk volume (13 of 16 studies) or protein content (11 of 15 studies), with diverse effects on milk fat level. The primary response is an increase in polyunsaturated fatty acids, particularly omega-3 fatty acids in milk. Regarding liveweight gain, a review shows little evidence of improved production from inclusion of microalgae in the diet[136], although it has been shown to increase the omega-3 fatty acid content of meat.
Applicability	While microalgae were expected to be a major protein source for humanity and are currently used as a protein and fatty acid source in fish-farming, to date there is no ruminant feeding application that would warrant their use to mitigate CH_4 emissions. Other feeds such as flaxseed or rumen protected omega-3 fatty acids are more commonly used as a means of increasing the omega-3 fatty acid content of milk and meat.
Expected market trajectory	Algal meal availability will depend on whether microalgae develop as a feedstock for renewable energy.
Expected cost	Both algal meal and algal derived DHA are available for approximately US$20/kg in bulk
Constraints to use	Supply and few demonstrated improvements in production efficiency limits the use of microalgae as a ruminant feed. Nutritional strengths and constraints of algal nutrients in monogastric diets have been reviewed[137].
Residue in animal product	Microalgae have been developed for heavy metal removal from contaminated environments[138]. Therefore, there is a risk they could accumulate heavy metals during culture, although they are principally grown in controlled aquatic environments that avoid heavy metals in culture systems. The lack of heavy metals or biotoxins would need to be confirmed in microalgae that was not grown under controlled culture conditions. As suppliers of PUFA, microalgae will affect the fatty acid profile of milk and meat.

Impacts on manure	Like the macroalgae *Asparagopsis*, microalgae have been used as fertiliser[46,139] and their biochar as a soil conditioner. Increasingly, microalgae biomass is seen as playing a key role in future bioenergy production and as part of circular agricultural systems[140].
Potential regional distribution	The greatest growth in the microalgae industry will occur as result of their use as a 3rd generation bioenergy source and any livestock use will depend on this industry development.
Life-cycle Assessment (LCA)	Microalgae LCAs are primarily based on their use in bioenergy, and depending on species and growing conditions they can be used as a renewable substitute for fossil fuels to lower the carbon footprint[141].
Actions needed to accelerate technology roll-out	A very low mitigation efficacy places microalgae at a competitive disadvantage relative to *Asparagopsis* and no immediate development of a microalgae product for methane mitigation is apparent. However, if microalgae are grown for carbon capture and for bioenergy production, greater supply may increase their direct use or use of associated by-products as livestock feed.
Assessment Summary	Microalgae meals are available in raw or defatted form and in pure or diluted forms. Their key nutritional metabolites (PUFAs) are also available as extracts. Generically, microalgae studies have shown there is **little evidence** and **little agreement** that microalgae offer even a low level of sustained methane mitigation. Correspondingly there is little evidence and little agreement that adding microalgae into a balanced dairy diet will increase milk production. Consequently, while individual algal bioactives may yet prove to mitigate methane emissions, there is currently no basis to recommend microalgae as a means of mitigating methane.

Biochar

Biochar is a carbon based (charcoal-like) product arising from combustion in an O_2 depleted environment (pyrolysis) of purpose-grown or waste vegetation. Its production can generate syngas and heat as co-products and it is finding wide application in agriculture, both as a long-term stable carbon store and as a soil ameliorant[142]. In livestock feeding biochars have been used to adsorb toxins and alter manures[143]. Biochar has also been shown to facilitate methane oxidation (destruction) in soils[144], largely where soils become aerobic, but anaerobic methane oxidation has been hypothesized to occur in the biofilms in the rumen[145,146]. However, it is a minor process in the rumen[147] and requires another electron receiver (such as sulphate) to be present.

See the reference list

Evidence of efficacy	There is only limited evidence that biochars affect rumen methane production with partial data to 2018 collated by Schmidt et al.[143]. Effects on methane are not consistent across trials or chars[148,149]. Recently the need to acidify chars for improved methane mitigation has been found[150] but little data on acidified chars is available and initial results show no advantage[215]. Biochars can affect the rumen biome in batch[151] and continuous culture[152], but these studies and others across a range of biochars have found no effect on methane emissions[153] and a further 6 chars showed no effect on methane as % of gas produced[149]. In animal studies, methane production has shown inconsistent response[154], and for these reasons Black et al.[34] ascribed 0% mitigation to biochar in Australia.
Mode of action	Given that mitigation is small and sporadic it is hard to ascribe a mechanism to the mitigation. If mitigation occurs it is likely to reflect changes in one or more of the following: 1) reduced rumen protozoa populations[151] 2) the pore structure of the biochar allowing a different microbial biome (*ib-id*) and 3) the biochar acting as an electron shuttle moving electrons between microbes or between microbes and chemical acceptors[155]
Dose	0.5 – 1.0% in feed DM. Intake has been suppressed at 1.5% biochar[156] in cattle.
Manufacturer	Biochars can be generated from 1st (crop based), 2nd (crop waste based) or 3rd generation (algal based) feedstocks. There are over 130 biochar producers in the USA but the majority of global production occurs in China[155].

Availability	Industrial scale agronomic use in China but only pilot scale usage in most of world, and it is not routinely used as a feed additive in ruminants.
Impacts on animal production	The traditional roles of biochar in animal feeds is for adsorption of mycotoxins, pollutants, heavy metals and the management of ammonia and ion loss from manures; these impacts are summarized in reviews[143,146]. There are direct production benefits when such anti-nutritional factors are in the diet (e.g., tannins). When toxins are not a factor there are some studies in high-tannin tropical diets, where growth rate was improved.
Applicability	As shelf-stable products, biochars would readily fit into supplementation programs. However, chars specifically treated to provide methane inhibiting activity will be required and these have yet to be developed.
Expected market trajectory	Biochar production will be principally driven by the biofuels industry and a role in soil amelioration. Global production is approaching 500,000 t/y with China currently the largest producer, and other countries yielding <50,000 t[157,158]. To be widely effective in enteric methane mitigation, chars will need to be custom made with acidification and probably nanoparticle inclusions. Appropriate feedstock availability could also influence market development.
Expected cost	Non-treated char is currently available for approximately US$1000/t[155]. However, this is when capacity to supply is very low, so lower prices can be expected as supply increases and supply of specialist chars also becomes feasible.
Constraints to use	1) Low efficacy in mitigation by available untreated chars 2) Limited supply in most countries 3) Need for specialist treatment and preparation for methane efficacy 4) Can be difficult to handle within mechanized feeding systems
Residue in animal product	Dietary biochar has been seen as a way of preventing toxins being absorbed by livestock, as the toxins are trapped on the biochar and excreted in manure. Reduced bioavailability of heavy metals in biochar treated soils may well also offer protection from heavy metal absorption by plants and the same may apply in animals (research needed). Care should be taken to not bring heavy metals into diets in biochars produced from plants grown on contaminated land. Similarly, marine algae should be checked for high levels of salts (sodium,

	iodine) and bromine if used as biochar feedstock. European standards for feed grade biochar are set by the European Biochar Certificate Standard (https://www.european-biochar.org/en/certificate).
Impacts on manure	The principal biochar market is as a soil ameliorant or additive in high value crops[142]. There are no indications of any adverse effects but there are many positive effects of biochar on soil physical properties and biology. While only small quantities will be fed to livestock, this is seen as a means of distributing biochar via faeces and thereby improving soil fertility[143]. At higher doses than would occur in manure from biochar fed cattle, manure has shown increased gas production in a simulated biogas system when biochar was present[159]. Biochar also affects the redox state of the soil to which it is applied[158].
Potential regional distribution	China is the main global production site[157], but biochar is a transportable bulk commodity and can be produced wherever plant waste is available.
Life-cycle assessment (LCA)	Biochars are part of the bioenergy system and increasingly seen to have advantage in adsorption of undesirable organics and metals from the environment. Ibarrola et al.[160] assessed a range of pyrolysis mechanisms for virgin feedstock and showed all processes lead to emissions abatement, so some of that abatement can rightly be attributed to the biochar arising, among other co-products.
Actions needed to accelerate technology roll-out	Research and development of biochar preparation to deliver a char supporting consistent methane mitigation is required. More research could be conducted by evaluating various feedstock sources. Reliance on crude pyrolysis products that have not been acidified or treated is unlikely to be a solution to lowering livestock methane emissions.
Assessment Summary	Biochars are a highly heterogeneous group of pyrolysis products, and this contributes to difficulty drawing a consistent assessment of efficacy in enteric methane abatement. Currently there is a **low evidence**, based largely on in laboratory incubations, which provide **low agreement** that currently available biochars deliver even a low (<5%) level of rumen methane mitigation. A treated acidic biochar may be specifically developed for methanogenesis but does not yet exist. Biochars may also be considered in animal feeds for heavy metal/toxin management, as a means of distributing biochar over the landscape as a soil ameliorant, and as part of a circular agricultural enterprise.

Bacterial DFM

Direct-fed microbials (DFM) are products that contain live (viable) microorganisms including bacteria and/or fungus. Bacterial DFM are most often composed of propionate-forming bacteria (especially *Propionibacterium* spp.) and lactic acid producing bacteria (LAB) from a range of genera. The primary goal of bacterial DFMs is to enhance animal health and improve feed efficiency in ruminants. Very few DFMs have been assessed specifically for their ability to lower enteric methane emissions. Other less common bacterial DFMs that could specifically alter hydrogen flow in the rumen such as homoacetogens (*Acetitomaculum ruminis, Eubacterium limosum, Ruminococcus productus*), fumarate-reducing bacteria (*Enterococcus faecalis, Mitsuokella jalaludinii*) and nitrate/nitrite-reducing bacteria (*Denitrobacterium detoxificans, Propionibacterium acidipropionici, W. succinogenes, S. ruminantium, V. parvula)* have been assessed for their ability to lower methane emission mainly in the laboratory, with few studies in animals. Only those bacterial DFMs that have been specifically assessed in animals will be discussed further.

See the reference list

Evidence of efficacy	**Propionibacteria:** The effect of various species of propionibacteria on ruminants has been studied for more than 20 years, with the majority of the research focusing on growth and feed efficiency. Although laboratory incubations of various *Propionibacterium* strains have been shown to reduce CH_4 production, research in animals is limited. Among 31 animal studies reported in the literature in the last 20 years, only 6 assessed the effects of propionibacteria on enteric methane emissions. These studies involved different strains (*P. acidipropionici* P169, *P. acidipropionici* P5, *P. jensenii* P54, *P. freudenreichii* T114, *P. thoenii* T159, *P. freudenreichii* T54, *P. freudenreichii* 53-W), different hosts (beef cattle, dairy cow and sheep), and fed different diets (high forage, high concentrate). The dosage of bacteria provided ranged from 5×10^9 – 11.5×10^{11} CFU/head/d. None of these studies found that propionibacteria reduced enteric methane production. In contrast, Jeyanathan et al.[161] reported that *P. freudenreichii* 53-W actually increased the intensity of CH_4 by 27% (g CH_4/kg milk) in cows fed a high starch diet. Similarly, Vyas et al.[162] also showed that supplementation of *P. freudenreichii* T114, *P. thoenii* T159, *P. freudenreichii* T54 to beef cattle fed a high forage diet tended to increase methane yield per unit of DM or GE intake. The limited information obtained so far suggests that despite the ability of propionibacteria to produce propionate as a hydrogen sink during fermentation, it does not lower enteric CH_4 emissions.

	Lactic acid producing bacteria (LAB): Information on the efficacy of LAB on enteric methane production is very limited. Only 5 research publications evaluated various strains as either DFM or as silage inoculants on enteric methane production from dairy cows or sheep. Jeyanathan et al.[161] showed that *L. pentosus* D31 (3.6×10^{11} CFU/cow/day) and *L. bulgaricus* D1 (4.6×10^{10} CFU/cow/day) did not affect CH_4 emission from cows fed either high starch or high fibre diets. Ellis et al.[163] reported that *L. plantarum* (6072), *L. lactis* (O-224), *L. buchneri* (LB1819), *L. lactis* (SR3.54) as silage inoculants (1.5×10^5 CFU/g of grass) or supplemented 16 h before morning feeding (5×10^9 CFU/cow/day) did not affect CH_4 emission regardless of the unit of expression, but tended to increase CH_4 produced per unit of metabolic BW. Philippeau et al.[164] examined combinations of *Propionibacterium* P63 with *L. plantarum* 115 or with *L. rhamnosus* 32 administered at 10^{10} CFU/d on methane production of dairy cows fed either high- or low-starch diets. Cows fed low starch diet with P63+ *L. rhamnosus* 32 tended to have decreased CH_4 emissions when expressed per kilogram of milk or 4% fat-corrected milk, but P63 alone did not. However, Cao et al.[165] found that compared to sheep fed unfermented TMR, sheep fed fermented TMR together with *L. plantarum* Chikuso-1 had lower daily methane emissions and energy losses resulting from increased conversion of lactic acid to propionic acid in the rumen. Mwenya et al.[166] assessed the effect of feeding *Leuconostoc mesenteroides* subsp. *Mesenteroides* (AOK1789) to sheep at $1.5–1.8 \times 10^9$ CFU/head/d and found no effect on methane emissions. In general, to date, bacterial DFMs have failed to reduce enteric methane emissions. Further development of acetogenic or methanotrophic DFMs may offer future possibilities as they have the potential to directly impact hydrogen flow and the half-life of CH_4 in the rumen.
Mode of action	In general, the potential mode of action by which DFM favourably alter feed digestibility includes altering ruminal acid production, establishing desirable microflora, and increasing fibre digestion[167]. The mode of action of DFM depends on many factors, such as dosage, feeding times and frequencies, diet, and strains of DFM. Some DFMs act within the rumen while others impact the lower gastrointestinal tract. However, mechanisms by which bacterial DFM may alter enteric methane production are unclear. Results from laboratory incubations and limited animal research offer the following potential explanations: **Propionibacteria**:

	1). Propionibacteria are natural propionate producers in the rumen and convert carbohydrates to propionic acid, leading to increased propionic acid concentration in the rumen. Propionate is a H sink in the rumen and its production can reduce the availability of H for the reduction of CO_2 to CH_4 by methanogens. Indeed, some researchers have shown increased ruminal propionate with *Propionibacterium* P169 in cattle[168,169]. 2). Another possibility for the lower intensity of CH_4 emissions could be the antimicrobial activity of some *Propionibacterium* strains, which produce inhibitory metabolites such as short-chain fatty acids and antimicrobial peptides known as bacteriocins. It has been shown some strains of *Propionibacterium* have inhibitory activity against ruminal bacteria, thus reducing CH_4 and total gas production in the laboratory[170,171]. However, this response could reflect an overall reduction in fermentation and to date it has not been shown that propionibacteria produce inhibitors that are specific to rumen methanogens. **Lactic acid producing bacteria (LAB)**: Doyle et al.[172] summarized the possible mechanisms by which LAB decreased methane production in ruminants: 1). Addition of LAB may stimulate the production of lactic acid and the growth of lactic utilizing bacteria that convert lactic acid to propionate[173]. 2). Metabolites such as bacteriocins (e.g., nisin from *Lactococcus lactis*, PRA1 from *L. plantarum* TUA1490L and pediocin produced by *P. pentosaceus* 34) may inhibit the growth of hydrogen producing microorganisms and thereby reduce substrate availability to methanogens. However, if these mechanisms exist, they have yet to be elucidated.
Dose	DFMs are typically administered directly through the diet. Some preparations may be encapsulated so as to promote their passage through the rumen to the lower digestive tract. Most preparations are administered at rates ranging from $1 \times 10^6 - 1 \times 10^{12}$ CFU/head/d. LAB are also extensively used as silage inoculants.
Manufacturer	International and local manufacturers.
Availability	Commercial products are readily available globally, but are registered for improvements in feed efficiency, not for reduction in enteric CH_4 emissions.

Impacts on animal production	The impact of bacterial DFMs on ruminant growth and feed efficiency has been recently reviewed[172,174,175]. In general, effects of bacterial DFMs on animal performance are inconsistent, a response attributable to differences among strains, strain viability, dosage, diet composition and host genetic and physiological traits. **Propionibacteria:** Azzaz et al.[175] reviewed 26 reports on the use of propionibacteria strains in ruminants, showing 3 with increased, 4 with decreased and 19 with no effect on feed intake. Similarly, among the 12 publications assessing effect of propionibacteria on milk production, only 3 that used a combination of propionibacteria and LAB or yeast resulted in increased milk production, with the remaining 9 having no effect. Three reports showed no effect on body weight gain, but one showed increased wool production in sheep. Similar trends were also found for the effect of LAB on feed intake and animal performance. **LAB:** Doyle et al.[172] generated a critical review on the use of LAB in ruminants. Meta-analysis of LAB supplementation in young calves has shown that LAB can exert a protective effect and reduce the incidence of diarrhoea and improve weight gain and efficiency[176] when one particular strain was administered in whole milk or as an inoculum[177]. When LAB were administered to mature ruminants, the prevalence of *Escherichia coli* O157 in beef cattle was reduced[178], suggesting that some DFM may promote food safety. LAB DFMs have also been shown to reduce the risk of ruminal acidosis in some instances[179,180]. Milk production was increased in 2 out of 8 studies and the remaining 6 were not affected by various LAB strains. The reported two studies involving beef cattle showed increased growth in one study whilst there was no effect in another. Often, propionibacteria and LAB were mixed within DFMs and occasionally even yeasts are included in the product. Consequently, it is difficult to separate observed responses and attribute them to any single component within mixed products.
Applicability	Readily delivered either through direct inclusion in feed or through silage. Occasionally, also administered to individual animals in the form of a bolus.
Expected market trajectory	Products are already available on the market, depending on the organism. If a successful DFM mitigant could be identified, commercialization, regulatory requirements, production, distribution and adoption streams could already be in place.

Expected cost	Bacterial direct-fed microbials typically range in price from US$0.05-0.25 per head per day. Cost depends on fermentation conditions, cell yield and product stability.
Constraints to use	Easiest if dealing with microorganism on GRAS list. Other microbial candidates would have to be submitted through the appropriate regulatory pathways. Lack of DFMs that have proven efficacy in terms of CH_4 mitigation.
Residue in animal product	No concerns over residues in animal products. Increasing requirement for bacterial DFM to be genetically sequenced and assessed for undesirable genes that may be associated with factors such a virulence or antimicrobial resistance. Bacterial species with pathogenic properties are deemed unsuitable as DFMs.
Impacts on manure	The main role of DFM in manure properties has been the frequent (but not consistent) reduction in the level of pathogens (*Escherichia coli* and *Salmonella* strains) as summarised by McAllister et al.[167]. Changes in the quantity of manure produced (reflecting DM Digestibility) and composition are small and variable.
Potential regional distribution	Global distribution.
Life-cycle Assessment (LCA)	An LCA of probiotics used in fish farming showed a modest carbon cost (1,153kg CO_2e/ batch), with 90-97% of the energy cost coming from the lyophilization of the product, so a similar cost could be assumed for ruminant probiotics[181]. As no bacterial DFM with clear mitigation responses have been identified, LCA from the perspective or ruminant production systems have not been conducted.
Actions needed to accelerate technology roll-out	More work is needed to identify bacterial DFMs that clearly result in a reduction in methane emissions. The persistence of effective strains within the ruminant environment also requires investigation.
Assessment Summary	Bacterial direct fed microbials are increasingly being included in high grain diets to reduce the risk of lactic acidosis in the absence of antibiotic rumen modifiers. There is **low evidence** from animal studies, and **low agreement** that bacterial DFM will mitigate enteric methane production by up to 5%. There is also medium evidence and medium agreement that bacterial DFM can have positive effects on animal health.

Fungal DFM

Fungal DFM mainly consist of *Saccharomyces cerevisiae* (yeast) and *Aspergillus oryzae*, with *S. cerevisiae* being the most common for ruminants. Other fungi including *Trichosporom sericeum*, *S. lipolytica, A. terreus* and *A. niger* have also been explored, but to a far lesser extent.

See the reference list

Evidence of efficacy	Darabighane et al.[182], conducted a meta-analysis to assess the impact of *S. cerevisiae* on methane production in dairy and beef cattle. The fact there were only 7 papers published between 1990 and 2016 reflects the lack of data. Using three datasets (all cattle, dairy cattle, or beef cattle) they concluded that yeast did not affect either daily CH_4 production or CH_4 production per dry matter intake (CH_4/DMI). Since 2017, one study showed that *S. cerevisiae* CNCM I-1077, at a dosage of 1 x 10^{10} CFU/head/d, tended to increase CH_4 per unit of feed intake in lactating dairy cows . In another study, *S. cerevisiae* supplemented at 2, 4 and 6 x 10^{10} CFU/head/d also did not affect CH_4 production regardless of whether it was expressed as grams per day or per unit of milk yield, dry matter intake, digested organic matter, or digested non-fibre carbohydrate[184]. Similarly, Oh et al.[185] reported that supplementation of a DFM product containing *S. cerevisiae* (5 x 10^{10} CFU/head/d and 1.1 × 10^8 CFU/g of a mixture (3 x 10^9 CFU/head/d) of *L. lactis, Bacillus subtilis, E. faecium,* and *L. casei* had no effect on enteric methane production, yield (methane per kg of dry matter intake, DMI), or emissions intensity (methane per kg of energy-corrected milk yield). Furthermore, Meller et al.[186] supplemented *S. cerevisiae* (from Yea-Sacc 1026) at a dose of 5 x 10^9 CFU/head/d to lactating Jersey cows and found no effect on CH_4 production when the diet contained either urea or NO_3^- . In contrast, Cagle et al.[187] showed that supplementing S. cerevisiae Sc47 CNCM I-4407 at the levels of 2.5, 5 and 10 x 10^{10} CFU/head/d to steers and heifers fed a growing, transition and finishing diet decreased CH_4 only when it was administered at the highest level in a grower diet. Research to date has failed to show that fungal based DFMs result in a predictable reduction in enteric methane emissions.
Mode of action	Proposed modes of action for fungal DFM include: • Reduction of oxygen in the rumen

	• Prevention of excess lactic acid in the rumen • Provision of growth factors such as organic acid and vitamin B • Increase of rumen microbial activity and numbers • Improved balance of ruminal end products (e.g., VFA, rumen microbial protein) • Increase of ruminal DM digestibility • No clearly defined mechanism in terms of reducing enteric CH_4 emissions. The most commonly reported mode of action of *S. cerevisiae* is the creation of a more anaerobic and stable environment, which promotes the growth of two key classes of ruminal bacteria: fibrolytic and lactate-utilizing bacteria. The increase in lactate-utilizing bacteria leads to the stabilization of pH, prevention of lactate accumulation and an increase in VFA production. Live yeast may also be able to metabolize the lactate itself, further decreasing the concentration within the rumen and enhancing its effects. However, these responses have not been linked to a consistent reduction in ruminal CH_4 production.
Dose	Fungal DFMs are administered in the diet at rates ranging 10^8-10^{11}/head/d depending on strains, animal, diet, etc. Most commonly they are administered at 10^9-10^{10} CFU/head/d.
Manufacturer	International and local manufacturers.
Availability	Commercial products are readily available globally, but are registered for improvements in feed efficiency, not for reduction in enteric CH_4 emissions.
Impacts on animal production	Numerous reviews have been published in the last 20 years to document the effects of fungal microbial products on animal productivity, in particular the use of yeast (*S. cerevisiae*) in dairy cattle[167,188–194]. In general, fairly consistent positive effects of yeast on milk production and composition have been found in lactating dairy cows. In a review of 32 lactation studies conducted between 1986 and 1997, supplementation with yeast increased milk production on average by more than 1.1 kg per day with the response being greater for cows in early lactation[188]. An average increase in milk production of 0.45 kg of milk per day was also documented by summarizing 26 comparisons where fungal extracts from *A. oryzae* were fed to lactating ruminants[188]. In a review by Robinson and Erasmus[190], yeasts increased milk yield by 3.6% on average.

	Fungal cultures have also been fed to calves, sheep, and steers but studies with these livestock are far fewer than with lactating dairy cows. Sales[191] conducted a meta-analysis and the results revealed that yeast did not have any effect on growth, feed conversion, ruminal parameters or fibre digestibility in sheep. Sartori et al.[195] conducted meta-analysis on data from 12 publications reporting 22 trials about the effect of live yeast supplementation on beef cattle performance prior to 2014. The results showed that yeast increased ADG when the forage level in the diet was between 30 and 50% but decreased it when the forage level range from 51 to 75%. When the diet contained 60% NDF, the use of yeast decreased the ADG by 407 g/day. Overall, supplementation with *S. cerevisiae* in the diet of beef cattle decreased DMI, had no effect on ADG, but improved feed conversion due to the reduction in DMI. However, two recent reports found that supplemented live *S. cerevisiae* to beef cattle (4.9 x 10^{12}/head/d) did not affect DMI, ADG or feed efficiency[196], and that beef cattle supplemented with 4 x 10^{10} CFU/head/d exhibited increased average daily gain, improved feed efficiency and enhanced digestibility of neutral detergent fibre and acid detergent fiber[197]. In contrast, Finck et al.[198] showed that supplemented yeast at 5 x 10^{10} CFU/head/d increased DMI, but not ADG, and reduced morbidity in receiving calves. Although yeast have resulted in improvements in productivity in ruminants these responses have not been linked to a reduction in enteric CH_4 emissions.
Applicability	Readily applicable through feeding.
Expected market trajectory	Products are already available on the market, depending on the organism. If a successful DFM mitigant were identified, commercialization, regulatory requirements, production, distribution and adoption streams could already be in place.
Expected cost	Product costs vary but an example may be US$7000/t and fed at 10g/head/d.
Constraints to use	Easiest if dealing with microorganism on GRAS list. Other microbial candidates would have to be submitted through the appropriate regulatory pathways. Lack of DFMs that have proven efficacy in terms of CH_4 mitigation.
Residue in animal product	None expected No evidence

Impacts on manure	No direct impacts on manure other than potential alterations in carbohydrate composition as a result of improved ruminal fibre digestion.
Potential regional distribution	These shelf-stable products are already marketed globally.
Life-cycle Assessment (LCA)	As products have not been shown to consistently lower methane emissions, no LCA have been conducted for ruminant production systems.
Actions needed to accelerate technology roll-out	Need to identify yeast strains that result in a consistent reduction in enteric methane emissions.
Assessment Summary	Fungal DFMs are widely used in grain-based beef and dairy systems. Consequently, extensive reviews and meta-analyses have been conducted confirming there is **low agreement** and **low evidence** that fungal DFMs cause even a low reduction in methane emissions from ruminants. However, fungal DFS do increase milk production in dairy cows and can increase growth rate of beef cattle on low (30-50%) forage diets but not on high forage diets.

Less Advanced Methane Mitigating Feed Additives

Biopremix	A patented probiotic "for reducing methane production in a ruminant animal comprising the step of administering to said ruminant animal an effective amount of at least one strain of bacterium of the genus *Propionibacterium."* This product is patented to DuPont Nutrition Biosciences. Application Number: US20140112889A1
Cinnamaldehyde	Cinnamaldehyde is the principal constituent of the essential oil extracted from Cinnamon bark. Variable results in laboratory and animal studies. Refer to Essential Oils section
Cowbucha **(A microbial additive)**	An early-stage probiotic additive being developed for dairy cattle
Chitosan	Chitosan derived from chitin has been used to control the release of additives in the digestive tract. Its inclusion in ruminant feeds has given minor but inconsistent effects on methane production after 4h[199] or 96h[200] measured in the laboratory,, but no effect on methane emission was evident in cattle on forage or concentrate-based rations[200,201].
Fumerate	An organic acid effective in mitigating emissions but very expensive. See malate (below)
Gut motility controllers	Several pharmaceutical products are effective in decreasing the retention time of feed in the digestive tract and can thus reduce methane production[202]. These include Slaframine produced as a fungal toxin and Thyroid hormone[203]. Reduced retention time could also result in lower DM digestibility depending on diet composition.
Glucosinolates	Purified glucosinolates may affect enteric emissions and this may be mediated by effects on digesta kinetics[205].
Harit Dhara	Harit Dhara is a tannin and saponin plant extract developed by scientists of the Indian Council of Agricultural Research (ICAR) showing early promise in mitigating. However, at 500g/head/d for mature bovines it represents a significant proportion of the diet and should be seen as a feed ingredient (like cotton seed or vegetable oils) rather than an additive[204].

Malate	Malate is an organic acid that is readily utilised by rumen organisms which produce propionate, depriving methanogens of hydrogen. Chemistry suggests 4g of methane will be spared per 134g of malate fed to ruminants[216]. Since malate is approximately US$5/kg this equates to US$168/kg CH_4 abated, so malate is a safe and effective mitigant but unlikely to ever be economic. Fumerate has the same mode of action.
Macroalgae other than Asparagopsis	While *Asparagopsis* is the principal algae being developed for methane mitigation, the green and brown macroalgae are also functional in abatement[206,207]. However, due to their lower bromoform levels they have not as rapidly been taken to animal trials.
Oregano	This flavoursome herb contains essential oils rich in the bioactives thymol and carvacrol[96] and has been tested in the laboratory and animals for methane inhibitory effects, with mixed results. Refer to Essential Oil section.
Propolis	Honeybee propolis is effective in reducing methane production[208] and this may be due to the flavonoids and phenolics it contains[209]. High cost is likely to make it uneconomical as a CH_4 mitigant.
Sop Star Cow	This commercial European product has primarily been marketed as a microbial means of reducing ammonia and methane from housed animals but there is initial evidence of efficacy as a feed additive[210].
Statins	Statins are highly bioactive and central in cholesterol medication for humans. They are also prevalent in plant products and may affect methane production[211] and the gut microbiota[212].
Synthetic bromoform	Since bromoform is the principal methane inhibiting compound in *Asparagopsis* seaweed, there is considerable interest in introducing the bromoform without needing to provide the seaweed. Bromoform is not included on the generally regarded as safe (GRAS) list https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras (refer 21 CFR 182) and there are health impacts at some concentrations that would need to be addressed (refer: https://www.epa.gov/sites/production/files/2016-09/documents/bromoform.pdf)
Tradilin	This commercial extract of linseed oil is rich in alpha linolenic acid and is sold commercially with linkage into a methane evaluation program.

Assessment of industry preparedness for methane-lowering feed additives

Introduction

There has been considerable global investment in researching the ten feed additives described in the attached scientific report, as well as an even wider array of additives that have not yet shown sufficient promise to warrant extensive investigation. Despite this investment, little is known about the preparedness of the delivery pipeline to take feed additives into commercial production, to use them in prepared feeds or supplements, and to have those products purchased and used by commercial livestock managers. In the following assessment, we developed an introductory understanding of the interest and level of preparedness of different actors in the feed additive pipeline (additive-manufacturers, feed or supplement manufacturers and livestock managers) to achieve significant GHG emission mitigation in developed and developing countries.

Methods and materials

Questionnaires were prepared relevant to each actor in the feed additive pipeline as presented following this report (Appendix 1). We sought to reach at least five actors in each category (Table 2). Respondents were not all chosen at random, with some being known contacts or being selected on appearance in published literature or promotional material. We shared the questionaries as a MS Word file and as an on-line form (Google Forms). Table 1 shows the number of questionaries completed and Figure 4 shows the source countries for responses.

Questions were designed to elicit a YES/NO response wherever possible. Where a result was unclear the responding company was approached again, and the answer clarified. Some organisations approached did not complete the survey but provided insights that are included in the discussion.

Table 2. Summary of the number of responses received from participants in each sector, partitioned by stage of development of the country.

Sector	Developed countries	Developing countries
Additive manufacturers	4	4
Feed/supplement manufacturers	6	8
Livestock managers	5	21

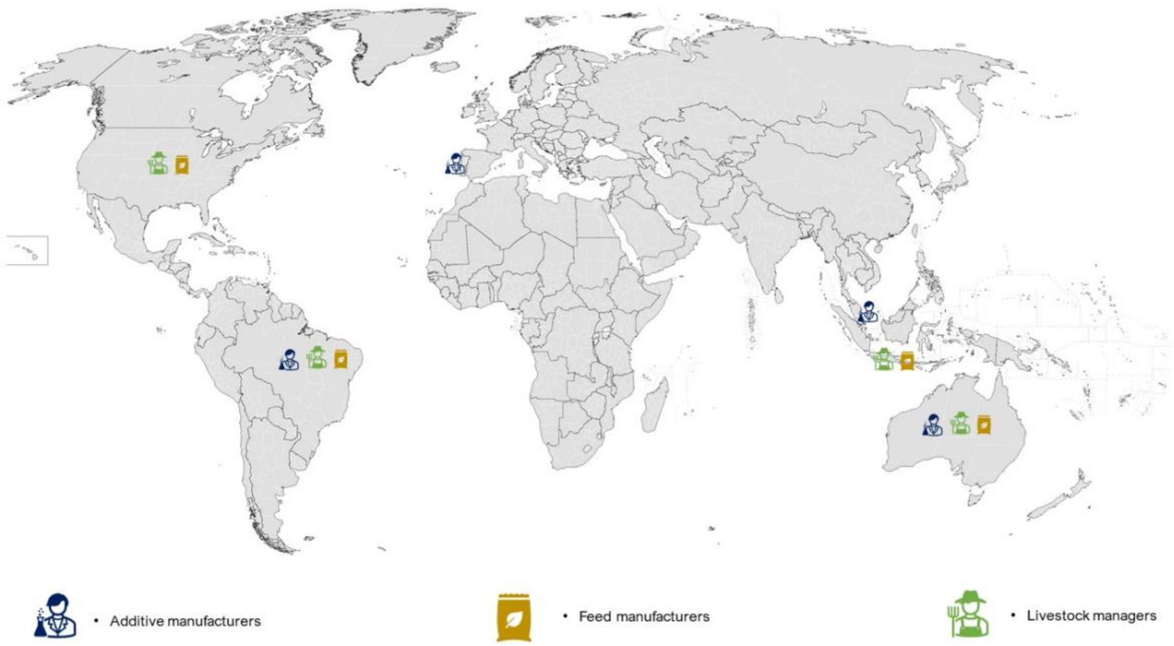


Figure 4. Location of organisations completing questionaries for Additive manufacturers (8), Feed manufacturers (14) and Livestock managers (24)

Results and discussions

Findings are presented in written form below followed by a more visual 'Info-graphic' format on the subsequent pages.

Observations from additive manufacturers/harvesters

A range of national and international manufacturers were approached, whose products or potential products covered many of the additive classes assessed in the attached technical review. Some companies could be expected to have the capacity to service this market, but presently have no known activity in the sector. Potential factors that prohibited their engagement were considered.

Of the eight manufacturers who responded, six identified methane mitigation as the primary claim of the product they would or do produce, and manufacturers most frequently expected that a product would take less than five years to commercialise. Many nature-based products such as probiotics or essential oil components or blends may not require registration in many countries.

Most manufacturers identified developed countries as a high to very high priority market (seven of eight) as compared to developing country markets (three of eight). In addition, they most often identified feedlots (seven of eight) and dairy (five of eight) as high to very high priority. No manufacturers identified the grazing industry as an extremely high priority for implementation.

Two of eight manufacturers expected that the inclusion of feed additive would increase the cost of ration from 0 to 1%, and two manufacturers expected to increase feed price by from 1 to 5%, but four of eight manufacturers did not declare the likely cost increment. Regarding constraints to developing additives, five of the eight respondents identified research (or lack of research) as a high to very high priority constraint to commercializing products. Lesser restrictions included the manufacture of additives (once discovered) and the registration demands (high to very high priority issues for 50% and 38% of respondents, respectively). Views on consumer demand as a constraint were mixed, with 50% indicating it was a low priority or not a priority constraint and 50% it was a high to very high priority. This may have reflected 3 of the 8 organisations responding have businesses based on *Asparagopsis*, and there has been a very high media profile creating strong consumer awareness and interest, even though supply is limited. Fifty seven percent identified the value proposition (economic return) as a high to very high impact constraint to product development.

Observations from feed and supplement manufacturers

The survey primarily addressed local or regional feed manufacturers (71%) and only 2 of 14 responders said they already produce a feed product with a low methane claim. Many respondents thought probiotics (64%) and essential oils (50%) and antibiotic rumen modifiers (ARM: 50%) effectively reduce methane production, with only 4, 2 and 1 of the 14 respondents aware of 3-nitrooxypropanol, *Asparagopsis*, and nitrate as additives, respectively.

Only 14% of respondents thought feeds or supplements to manage enteric GHG emissions was a current high or extreme priority for their company but 43% of respondents expected this priority to change to a high or extreme priority within five years. Feed manufacturer respondents identified product cost (including additive) as the greatest impediment to developing methane suppressing additives, being high or extremely high priority factor for 57%.

Half of the feed manufacturers said consumer demand was an extremely high priority consideration in their moving to low methane feed manufacturing; that is, that consumer demand would be needed for them to develop and deliver a low methane feedstuff. The fact that 71% then said consumer demand (or lack of it) was the main constraint to their developing a commercial methane inhibiting feedstuff indicates that feed manufacturers do not currently perceive a demand for such feeds or supplements by livestock managers.

Observations from livestock managers

The majority of the livestock enterprises who responded to the questionnaire were beef cattle producers (84.6%) and ranged in operation size, from operations having less than 1,000 head (12 operations) to having more than

10,000 head (9 operations). The operations surveyed were primarily in two developing countries (Indonesia and Brazil; 5 and 16 respectively).

Many are already using feeds that contained additives, with more than 60% using probiotics and more than 50% using ARM. The primary reason for using feed additives was increased animal performance (81%), with improved feed efficiency (73%) and health (69%) being the next most important.

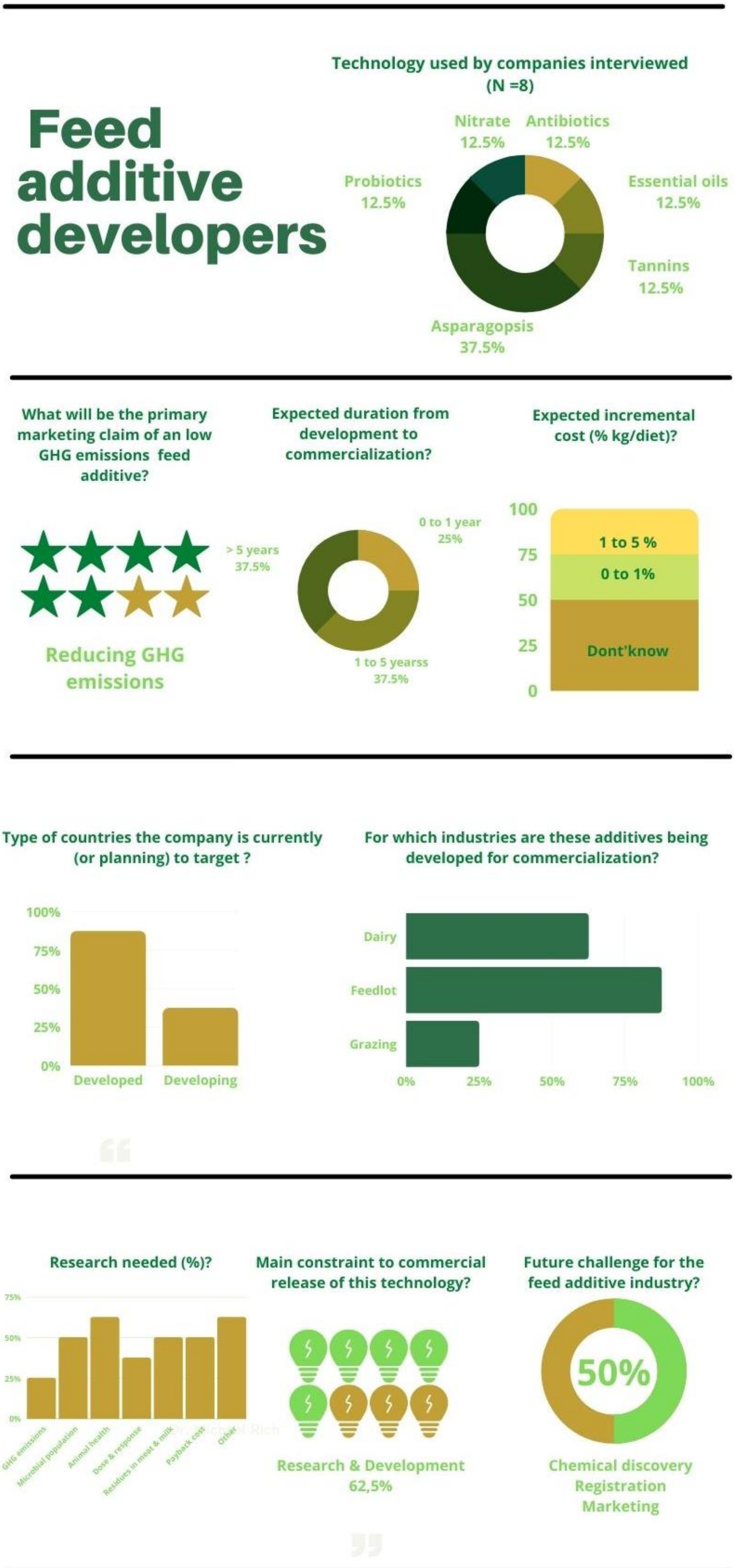
Only one of 26 respondents said GHG management was a current reason for adopting a feed additive, and 81% said GHG management was a low priority, or had no priority. However, respondents expected commercial interest to increase with only 31% of the same respondents expecting additives for GHG mitigation would have low or no priority in 5 years.

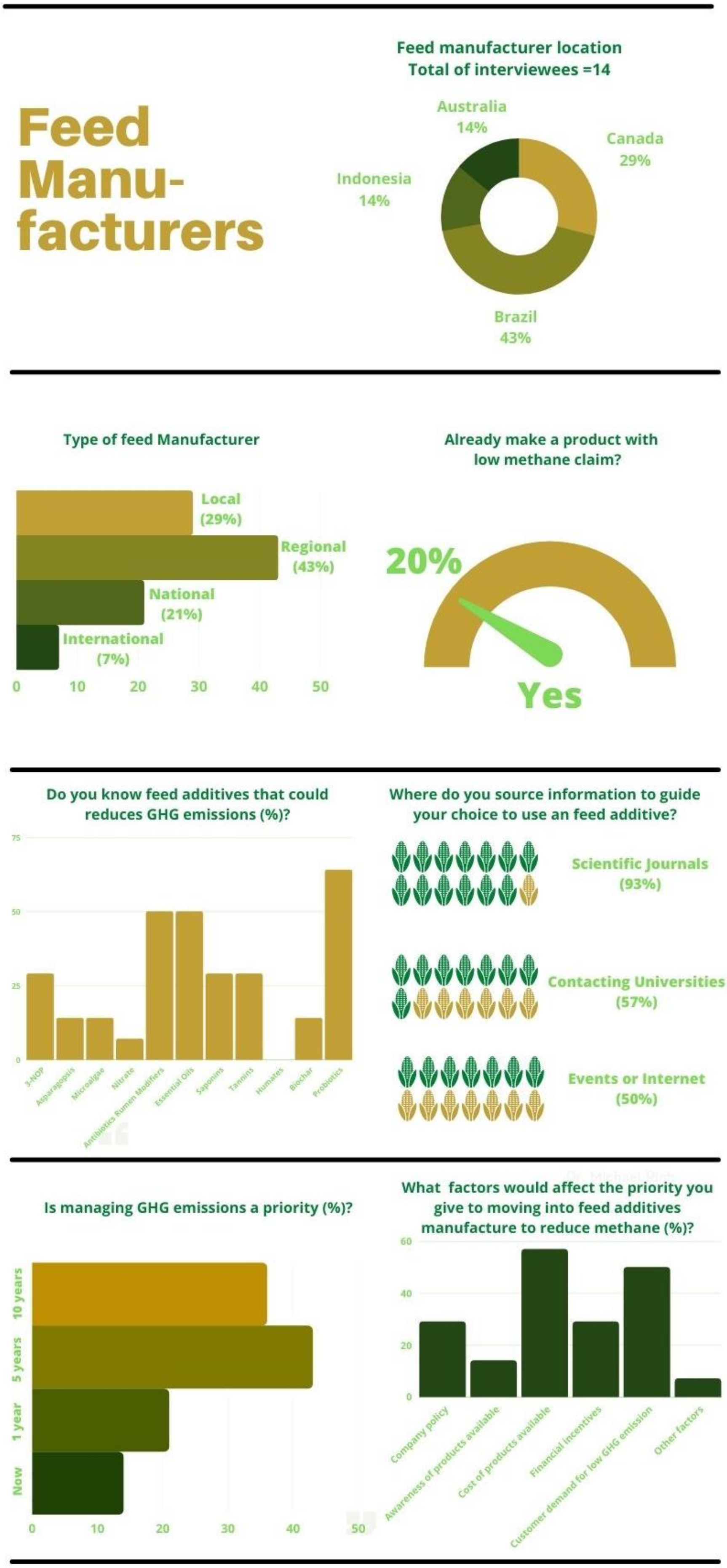
The increased adoption of methane mitigating additives they expected to occur over time was primarily motivated by an expected increase in animal performance and feed efficiency (92% of respondents), with company image and market economics (e.g., C credits) also important motivators (for 73% of respondents).

While most livestock managers expected methane-reducing additives to increase productivity and economic gain, 27% of respondents said they would only pay up to 1% above current feed prices, and 27% said they would pay up to 5% more to have the additive included.

All enterprises said they needed additional information on additives to consider their adoption (24 of 24), but where they would source this information varied. Most (58% of respondents) indicated that they would seek information from existing suppliers of existing feed with additives, but 46% said they would search on-line for suppliers or for scientists. Only 39% said they would contact a scientist directly.

The infographics below provide a visual summary of the main observations in each sectoral survey, then the key data and implications are discussed.

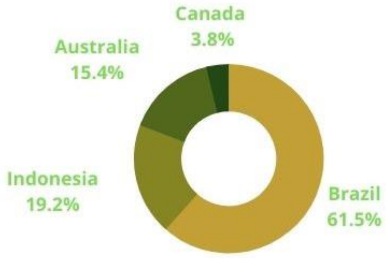

Feed additive developers
Technology used by companies interviewed (N =8)
Nitrate 12.5%
Antibiotics 12.5%
Probiotics 12.5%
Essential oils 12.5%
Tannins 12.5%
Asparagopsis 37.5%
What will be the primary marketing claim of an low GHG emissions feed additive?
Reducing GHG emissions
Expected duration from development to commercialization?
0 to 1 year 25%
> 5 years 37.5%
1 to 5 yearss 37.5%
Expected incremental cost (% kg/diet)?
100
75
50
25
0
1 to 5 %
0 to 1%
Dont'know
Type of countries the company is currently (or planning) to target ?
100%
75%
50%
25%
0%
Developed
Developing
For which industries are these additives being developed for commercialization?
Dairy
Feedlot
Grazing
0%
25%
50%
75%
100%
Research needed (%)?
75%
50%
25%
0%
GHG emissions
Microbial population
Animal health
Dose & response
Residues in meat & milk
Payback cost
Other
Main constraint to commercial release of this technology?
Research & Development 62,5%
Future challenge for the feed additive industry?
50%
Chemical discovery
Registration
Marketing



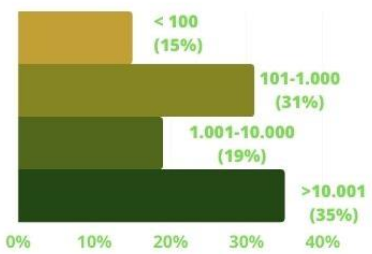
Feed Manu-facturers
Feed manufacturer location
Total of interviewees =14
Australia 14%
Canada 29%
Indonesia 14%
Brazil 43%
Type of feed Manufacturer
Local (29%)
Regional (43%)
National (21%)
International (7%)
0 10 20 30 40 50
Already make a product with low methane claim?
20%
Yes
Do you know feed additives that could reduces GHG emissions (%)?
75
50
25
0
3-NOP
Asparagopsis
Microalgae
Nitrate
Antibiotics Rumen Modifiers
Essential Oils
Saponins
Tannins
Humates
Biochar
Probiotics
Where do you source information to guide your choice to use an feed additive?
Scientific Journals (93%)
Contacting Universities (57%)
Events or Internet (50%)
Is managing GHG emissions a priority (%)?
10 years
5 years
1 year
Now
0 10 20 30 40 50
What factors would affect the priority you give to moving into feed additives manufacture to reduce methane (%)?
60
40
20
0
Company policy
Awareness of products available
Cost of products available
Financial incentives
Customer demand for low GHG emission
Other factors

Cattle Producers and Managers

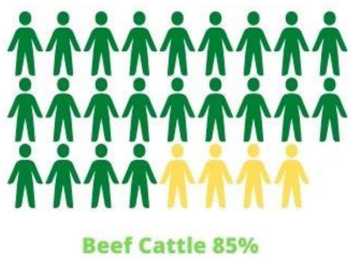
Farm location
Total of interviewees =26



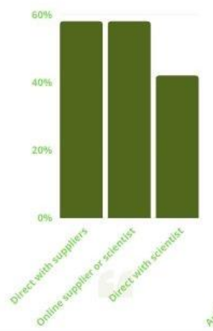
Size of the heard (Nº of heads)



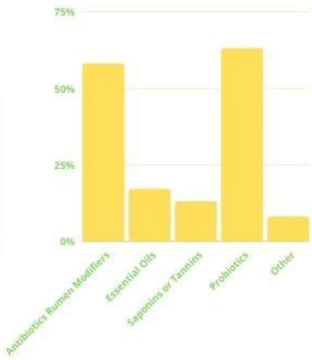
What is the main activity of your farm?



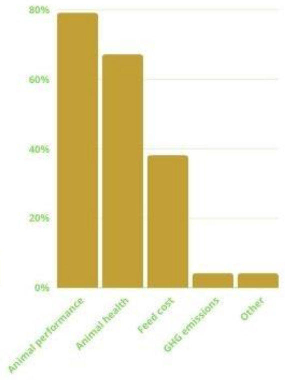
How do you source information about feed additives?



What feed additive do you use ?

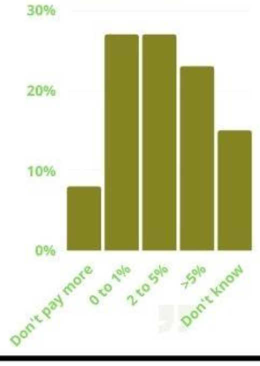


Reasons for adopting a feed additive in the diet (%)



Is managing GHG emissions a priority (%)?

How much more would you pay for a ration with an additive that reduces the emission of GHG ?



What factors would motivate you to use feed additives that reduce the emission of GHG from your livestock enterprise?

Discussion

Although very limited in scope, the data indicate a feed additive pipeline that is poorly prepared to achieve a substantial reduction in global livestock emissions in the next five years. This is because of narrow target markets for additive manufacturers, a poor level of understanding about emerging additives, unrealistic expectation of co-benefits, and lack of clarity regarding potential off-farm financial rewards. Some of these critical points are discussed below.

Towards achieving global mitigation

The survey of additive manufacturers identified that the primary target market for methane mitigating feed additives is the developed countries, and specifically within those, the feedlot and dairy industry. As identified in the main review, feedlots contribute less than 3% of enteric emissions. With none of the seven manufacturers identifying the grazing industry as an extremely high priority market, there is the distinct possibility that the developing world and the grazing industries world-over will be delayed in receiving any new emerging methane suppressing additives. Coupled with this, is low current interest in developing methane suppressing feed products in the feed industry and a poor understanding within the feed industry of available or emerging additives to suppress methane. Rapid global mitigation of livestock methane by feed additives is therefore unlikely to be realized.

Incentives and motivation for methane suppressing additives

The data suggests end-users are mostly expecting the inclusion of a methane additive in processed feeds or supplements to increase the price of that feed by no more than 5%. Both the end-users and the feed manufacturers are expecting an economic return for producing or using feeds containing methane suppressing additives, with that return either via productivity or external economic support. It is a serious concern that of the ten additives evaluated in the main review, none offered robust evidence of productivity or efficiency gains of 10% and most offered far less, if any at all. The large-scale studies required to quantify the scale and variability in productivity responses to mitigation have not been completed. This places the feed industry in a weak position to make a business case for the inclusion of additives.

To further the economic challenge, the cost of the additive was thought by feed manufacturers to be the most important constraint for their developing of low-emission feeds. In general, neither feed mills nor livestock managers would use methane suppressing additives or feeds unless there was an economic advantage or legislated requirement. One observation, from discussion rather than scripted questions, was that livestock producers selling a branded livestock product (a product where the growers name goes with the product through to the final consumer) may absorb the costs of methane mitigation practices, because a low methane claim could favourably affect consumer purchase choices. In contrast, livestock producers selling into a commodity market (effectively losing linkage to their product at the farm gate) would need a financial return from productivity, carbon value or subsidy to justify the use of additives. Several companies that manufacture feed additives are looking for recognition of the carbon value of their products (e.g., Mootral through Verra) or real cash subsidies from industry or government (e.g., SilvAir).

Understanding of methane-lowering additives

More than half of the livestock enterprises were using feeds that contained some natural, synthetic, or probiotic additive, but only one producer (of 26) was using an additive to improve environmental outcomes and over 80% described GHG emissions as a low or non-priority issue for their enterprise. All producers said they needed external information regarding supplements with the highest ranked source for this information being existing industry suppliers of feeds that contain additives.

Unfortunately, the feed manufacturers also lack an in-depth understanding of the methane suppressing feed additives available, as only 4 of the 14 feed manufacturers had any knowledge of the three most efficacious additives (as per main review). So, there is a poor flow of information from scientific research to feed manufacturers and an unrealistic expectation of on-farm co-benefits arising from the use of methane lowering additives.

The future

The data confirm a strong expectation for change among both livestock enterprises and feed manufacturers around the production and use of feeds and supplements containing methane-lowering additives in the next five years. This change can only develop if there is a better exchange of data from additive manufacturers to feed manufacturers and on to the livestock managers. The information flow must also clearly articulate the economic (or regulatory) argument for additive use. The economic case requires rapid completion and dissemination of findings from highly replicated studies of co-benefits which may be associated with each additive product. This large-scale testing is likely to occur for high efficacy additives where there is patent or licencing protection in place, such as 3-nitrooxypropanol, *Asparagopsis* seaweed or nitrate. However, the lack of intellectual property ownership together with the low methane inhibition efficacy of other additives such as tannins, saponins, direct fed microbials, biochars and older antibiotic rumen modifiers means these replicated co-benefit quantification studies are unlikely to be made. Consequently, the commercial argument for these products will remain weak and their defence problematic.

There is also a clear call from the additive manufacturing industry itself for more research to develop new products. From discussion with companies who in some cases opted to not complete this survey, it was apparent that many of the national additive supplying companies operate by commercialising discoveries made by universities or research institutes, and by production of older bio-actives that have fallen out of patent protection. These companies did not have the budget or staff to discover new compounds or pre/pro/postbiotics that may reduce methane emissions. This narrows the field of participants for potential discoveries of new high efficacy methane-inhibiting feed additives. It also identifies the strong need for public sector research for discovery of new additives for this purpose, rather than leave discovery to the commercial sector's limited pool of large international agrichemical companies.

In conclusion, while this survey was limited in scope, it has identified that there is a failing to transfer information about current and emerging methane suppressing feed additives. Communication is needed between publicly funded research providers and potential product manufacturers; between additive manufacturers and feed manufacturers, and through to the livestock managers who ultimately deliver the feed additives. A major barrier to adoption is the lack of assured profitability of methane mitigation additives in the absence of clear animal production impacts on other revenue sources such as improved feed efficiency, growth or meat quality. At this time, incentives such as carbon credits or financial subsidies are likely to be required to encourage widespread adoption of these additives by the ruminant livestock industry.

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Appendix 1. Surveys for livestock producers, feed manufacturers, bioactive manufacturers

To meet the objective of understanding whether the industry delivery pipeline for methane suppressing additives is ahead, behind or in step with discovery of these additives in scientific research, a set of surveys were prepared.

Separate surveys have been developed for:

- Livestock producers/managers
- The processed feed manufacturers and supplement industry
- The feed-additive manufacturing industry

The surveys were either completed on the MS Word documents as presented below or presented as a Google Form.

Survey for livestock producers and managers

Dear participant, the objective of this interview is to document the trajectory, challenges, and future perspective of the feed additive industry in the use of technologies for reducing enteric methane from ruminant livestock.

Note: Can the product specifications files be provided if available please? The views would be anonymous and simply recorded as a set of comments from Livestock Company X.

Name:	**Role:**
Company name:	**Company code:**
QUESTION	**ANSWERS / NOTES**
1) How many ruminants are you responsible for (small/large ruminants)?	Please **mark (X) one** of the options below: (____) 0 – 100 head (____) 101 – 1,000 head (____) 1001 – 10,000 head (____) > 10,000 head Provide partitioning into species (__sheep, __cattle)
2) What feed additive technologies do you already use in your livestock?	Please **mark (X) one or more** of the options below: (____) Monensin/other antibiotic rumen modifiers (____) Probiotics/Direct feed microbials (____) Essential Oil Blends (____) Saponins or tannins (____) Biochar (____) Other______________________

3) What is the reason for adopting this feed additive technology in your livestock?	Please **rank the priority** (1 = none to 5 = extreme priority) in the options below: (____) Animal performance (____) Animal health (____) Feed efficiency (____) Feed cost (____) Environment (____) GHG emissions (____) Other_______________________
4) How important is managing and reducing emissions of GHG from your livestock enterprise now, and going to be in next 1, 5 and 10 years?	Please **rank your priority** (1 = none; 5 = extreme priority) below: (____) Currently (____) In the next 1 year (____) In the next 5 years (____) In the next 10 years
5) What would motivate you to use feed additives that reduce GHG emissions in your diets?	Please **mark (X) one more option** below: (____) Company or legal requirement (____) Desire for a positive company image (____) Market pays me more (e.g., Carbon Credits) (____) Improved animal performance/feed efficiency (____) Improved carcass weight (____) Reduced production cost (____) Improved animal welfare and environment (____) Improved meat or milk quality (____) Other_______________________
6) How much more would you be willing to pay (% of the total diet cost diet) for an additive that reduces GHG emissions?	Please **mark (X) one** of options below: (____) 0 to 1% (____) 1 to 5% (____) 5 to 10% (____) > 10% I don’t know as a percentage, but we expect to pay:…
7) How would you source information to guide your decisions about choosing feed additives to reduce livestock methane production?	Please **mark (X) one or more** of the options below: (____) Don’t need outside information (____) Search online for a scientist or research organisation to ask (____) Search online for companies supplying emission inhibitors

	(____) Search online for products claiming to inhibit livestock methane (____) Contact a known supplier of additives (e.g., Monensin) and ask their advice (____) Contact a local scientist (____) Other__________________
8) Do you have any further comment on your organisation's contribution to producing low-methane livestock, feed or livestock products?	

Survey for feed manufacturers

Dear participant, the objective of this interview is to document the trajectory, challenges, and future perspective of the feed additive industry in the use of technologies for reducing enteric methane from ruminant livestock.

Note: Can the product specifications files be provided if available please? The views would be anonymous and simply recorded as a set of comments from Australian Supplement Company X.

Name:	**Role:**
Company name:	**Company code:**
QUESTION	**ANSWERS / NOTES**
1) Are you a local, regional, national, or international producer of supplements for ruminants?	Please **mark (X) one** of the options below: (____) Local (____) Regional (____) National (____) International
2) Do you already make any products with a low methane claim?	Please **mark (X) one** of options below: (____) Yes (____) No
3) Do you already know feed additives you could look to include to reduce ruminant emissions?	Please **mark (X) one or more** of the options below: (____) 3 Nitrooxypropanol (____) Asparagopsis (____) Microalgae (____) Nitrate (____) Antibiotic Rumen Modifiers (____) Essential Oil Blends (____) Saponins (____) Tannins

	(_____) Humates (_____) Biochar (_____) Probiotics (_____) Other__________________________
4) Is a low methane supplement a desired product for you now, and in the next 1, 5 and 10 years?	Please **<u>rank the priority</u>** (1 = none to 5 = extreme priority) in the options below: (_____) Currently (_____) In the next 1 year (_____) In the next 5 years (_____) In the next 10 years
5) What factors would affect the priority you give to moving into low methane supplement manufacture?	Please **<u>rank the priority</u>** (1 = none to 5 = important factor) in the options below: (_____) Our company policy towards low carbon systems (or carbon neutrality or similar) (_____) Awareness of what products are available (_____) Cost of products available (_____) Financial incentives: (e.g., Government or Carbon Credit) (_____) Customer demand for methane lowering products (GHG friendly) (_____) Other_________________________
6) Where would you or have you sourced information to guide your choice of additive?	Please **<u>mark (X) one or more</u>** of the options below: (_____) Scientific journals (_____) Web pages (Blogs, journals, etc.) (_____) Participation in events (webinar) (_____) University and researchers (_____) Other_________________________
7) What are the main constraints to commercial release of product in these countries and industries?	Please **<u>rank the priority</u>** (1 = none to 5 = extreme constraint) in the options below: (_____) Research and development (_____) Registration/legal approval process (_____) Production scale-up and logistics (_____) Marketing and sales strategy (_____) Consumers' demand (_____) Other _________________________
8) Do you have any further comment on your organisation's contribution to producing low-methane livestock feed or products?	

Appendix 1

Survey for manufacturers of mitigation agents

Dear participant, the objective of this interview is to document the trajectory, challenges, and future perspective of the feed additive industry in the use of technologies for reducing enteric methane from ruminant livestock.

Note: Can the product specifications files be provided if available please? The views would be anonymous and simply recorded as a set of comments from Australian bioactive Company X.

Name:	**Role:**
Company name:	**Company code:**
QUESTION	**ANSWERS / NOTES**
1) What will be the primary marketing claim the company expects to use to launch this methane inhibiting additive into the market?	Please **rank the priority** of any claims likely to be made (1 = none to 5 = principal claim) (____) Inhibits methane production (____) Rumen modifier/enhancer (____) Palatability enhancer (____) Growth promoter (____) Animal health (____) Other__________________________
2) How long was (or will be) the duration from development to commercialization?	Please **mark (X) one** of the options below: (____) 0 to 1 year (____) 1 to 5 years (____) 5 to 10 years (____) > 10 years
3) In which industries (e.g., dairy/ feedlot/ grazing) are these additives currently being anticipated for commercialization?	Please **rank the current priority** (1 = none to 5 = extreme priority target) (____) Dairy (____) Feedlot (____) Grazing
4) What is the expected incremental cost (%) of the diet that farmers will pay for adopting this technology?	Please **mark (X) one** of options below: (____) 0 to 1% more (____) 1 to 5% more (____) 6 to 10% more (____) > 10% more Other measure of cost__________________
5) What countries are the company currently (or planning to) targeting?	Please **rank the priority** of each market (1 = minimal interest to 5 = extreme priority) (____) Developed countries (____) Developing countries

6) What are the current main constraints to commercial release of a methane reducing product in these countries and industries?	Please **rank the priority** (1 = none to 5 = extremely high constraint) in the options below: (____) Research and development (____) Registration/legal approval process (____) Production scale-up and logistics (____) Marketing and sales strategy (____) Consumers' demand (____) Other ________________________
7) What don't we know about this feed additive technology that you think still needs to be researched?	Please **rank the options below** (1 = no need to 5 = extreme research needed) (____) Reduction on GHG emissions (____) Microbial population changes (____) Animal health impacts (____) Dose and chemical composition (____) Residue in meat and milk (____) Cost and payback (____) Other__________________________
8) In your opinion what are the future challenges the industry developing feed additives for GHG emissions will face in the next 5 to 10 years?	Please **rank the options below** (1 = none to 5 = an extreme challenge) (____) Chemical discovery (____) Safety and efficacy testing (____) Registration (____) Market interest (____) Other__________________________
9) Do you have any further comments on your organisation's contribution to producing low-methane livestock feed or products? If you do not/will not manufacture a methane suppressing additive, please explain why not?	

Appendix 2. Project terms of reference

This report was compiled to meet the following contracted obligations:

Scope

The scope of the review should include:

- Technologies that are presently commercially available and those under development that have published data on their impacts.
- Evidence available from developing countries as well as developed countries.
- Impacts on emissions from enteric fermentation, manure, and production or transport of the feed additive if available.

Activities

The assessment will include the following activities:

- Identify known technologies globally, by product type, with examples.
- Compile and evaluate the robustness of the evidence for known technologies' effects on (i) animal health, nutrition, and productivity, (ii) GHG emissions at the animal and lifecycle levels, (iii) food quality or safety, and (iv) any other animal, human, or environmental impacts or risks resulting from the production or use of the technology. Evidence should be drawn from the scientific literature or other reliable and published data sources. Note: Limited information is likely to be available on lifecycle analysis and food quality/safety issue. This information may equate to just one line in any developed reference library depending on the data that can be sourced.
- Summarize the technologies' efficacy based on the evidence compiled.
- Identify conditions for manufacture, distribution, farmer accessibility and use of the technology, including palatability. Identify costs of the technology in developing countries. Use scientific literature where possible, or other sources, including interviews.
- Conduct interviews with feed additive developers (n=10-15) to identify the trajectory of the industry in the next five to ten years and related research needs. Develop a survey template to ensure a consistent approach to the interview process. Results compiled as an addendum to the report.
- Draw conclusions highlighting:
    - Where is the potential for feed additives for greenhouse gas emissions reductions highest, with the least trade-offs?
    - What is needed to further development the feed-additive industry in the next 5-10 years, especially to make additives attractive and accessible in developing countries?

- What additional research is needed to advance the potential of feed additives uptake?

Outputs

- Development of a feed additives reference library (expected n=15-30 technologies). Each technology will be detailed using a specially designed template. This will be a living document, easily updated with time.
- Produce a policy brief summarizing the results of this activity.
- Produce a table of commercially available feed additives, their cost (this may have to be a 5-star scale rather than a dollar value) and their efficacy that can be posted on websites.
- Present results in a GRA-CCAFS webinar.

All activities and outputs would be co-branded with GRA/CCAFS and relevant funding sources and widely promoted in GRA/CCAFS communications.