

TRL-niveau for nye, metan-reducerende fodertyper og fodertilsætningsstoffer

Rådgivningsnotat fra DCA – Nationalt Center for Fødevarer og Jordbrug, Aarhus Universitet

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Baggrund

Fødevarestyrelsen har i en bestilling fremsendt den 29. januar 2025 til DCA – Nationalt Center for Fødevarer og Jordbrug – ved Aarhus Universitet (AU) bedt om en vurdering af Technology Readiness Level – TRL – og metan-reducerende effekt for andre foderklimavirkemidler end dem, som fødevarestyrelsen for tiden har "i brug" i lovgivningen (Bovaer og fedt). Der ønskes en kort, overordnet, skematisk fremstilling der inkluderer de virkemidler, der er nævnt i AU's vidensyntese om metan-reducerende foder fra 2023, under betingelse af, at de fortsat er relevante. Det vil sige:

- SilvAir/nitrat
- Asparagopsis Taxiformis
- Andre alger/tang end A. taxiformis
- Stof X2
- Sukkerroer
- Tanniner, saponiner og essentielle olier - evt. opdelt i enkeltprodukter, hvis det giver mening
- Biokul – hvis det fortsat er relevant at tage i betragtning.

Dertil kommer afgræsning.

Der ønskes også en effektvurdering, hvis den er anderledes end i vidensyntesen, eller hvis den kan konkretiseres mere, end den er i vidensyntesen. Hvis der er kommet nye potentielle virkemidler til, må de også gerne indgå.

Besvarelse

I tabel 1 er der taget udgangspunkt i de TRL-niveauer, som anvendes af GUDP:

TRL 1. Grundforskning - aktiviteter, der vedrører teoretisk og forsøgsmæssigt arbejde med henblik på erhvervelse af ny viden og grundlæggende elementer uden specifik anvendelse for øje, men som kan føre til identifikation af nye koncepter.

TRL 2 - Formulering af teknologisk koncept – Opfindelsen er i sin begyndelse og det nye koncept undersøges nærmere. Antagelser er spekulative, og der er muligvis ingen beviser eller detaljerede analyser, der understøtter antagelserne.

TRL 3 - Eksperimentel eftervisning af koncept – Aktiv forskning og udvikling igangsættes. Dette omfatter analytiske undersøgelser og laboratorieundersøgelser til fysisk at validere analytiske forudsigelser af separate elementer af teknologien.

TRL 4 – Teknologi er testet og valideret i et laboratorie-miljø – Basiskomponenter sættes f.eks. sammen for at teste, hvordan de virker sammen. Der er relativ lav nøjagtighed (low fidelity) i forhold til det endelige design. Skærpede numeriske/kvantitative analyser til validering af teknologien.

TRL 5 - Teknologi er testet og valideret i et relevant miljø - Nøjagtigheden af teknologien øges markant. De grundlæggende teknologiske komponenter er integreret med rimeligt realistiske støtteelementer, så teknologien kan testes i et simuleret miljø. Stabil og robust funktion demonstreres i det simulerede miljø.

TRL 6 - Teknologi er demonstreret i et relevant miljø - En repræsentativ model eller et prototypesystem, som er langt ud over TRL 5, er testet i et relevant miljø med en variation af driftsvilkår. Demonstration af funktion i kombination med andre teknologier. Repræsenterer et stort skridt op i en teknologisk demonstreret parathed.

TRL 7 - Prototype er demonstreret i et drifts/produktionsmiljø - Tæt på eller færdigudviklet prototype. Repræsenterer et stort skridt op fra TRL 6, og kræver demonstration af en egentlig prototype i et driftsmiljø, hvor der kan demonstreres overholdelse af eksempelvis miljømæssige forhold eller nationale standarder. Fremstillingsmetode defineres og integrationer med opstrøms eller nedstrøms teknologier valideres.

TRL 8 - Teknologien/systemet er komplet og færdigudviklet på kommercielt niveau - Funktionen af det endelige produkt er med succes demonstreret gennem test og analyse i driftsmiljøet (real world). Der er fuld overholdelse af forpligtelser og standarder for det adresserede marked.

TRL 9 - Teknologien/systemet er klar til produktion/drift på fuld skala - Det endelige produkt er anvendt med succes i driftsmiljø og klar til markedsføring

I tabel 1 er der angivet et reduktionspotentiale for udledningen af enterisk metan (CH₄). Dette reduktionspotentiale (%-reduktion i g CH₄/kg fodertørstof/d) er baseret på reduktion i enterisk metan på en given dag, hvor virkemidlet anvendes, dvs. at der ikke er taget hensyn til f.eks. produktionsform (økologisk versus konventionel produktion), afgræsning eller lignende managementstrategier, som kan begrænse dels muligheden for udbredelse til alle bedrifter og dels anvendelse indenfor et kalenderår på en given bedrift.

Qua den korte tidsfrist er besvarelsen ikke baseret på en systematisk gennemgang af den relevante litteratur indenfor et givent virkemiddel, men er primært en faglig vurdering for nuværende status for de enkelte virkemidler samt data præsenteret af Hegarty et al. (2021) (bilag 1) samt af Arndt et al. (2022), Beauchemin et al. (2022), Kjeldsen et al. (2022), Roques et al. (2023), Hristov (2024) og Martins et al. (2024).

For yderligere information om nogle af de enkelte virkemidler henvises der til Hegarty et al. (2021), som har en meget skematisk beskrivelse af nogle af virkemidlerne mht. evidens for effektivitet, mode of action, dosis, fremstillere, tilgængelighed, effekt på dyrets produktion, anvendelse, marked, omkostning, begrænsninger for brug, rester i produkter, effekt på gødningsværdi, LCA, implementering og sammendrag (bilag 2).

Udover Bovaer og øget tildeling af fedt forventes det at nye virkemidler som f.eks. nitrat og tang vil være kommercielt tilgængelige indenfor en kort årrække. For nuværende og nye virkemidler er der derfor behov for viden om: 1) hvorvidt de metan-reducerende effekter af disse virkemidler er additive; 2) hvorvidt effekten af de enkelte virkemidler varierer på tværs af besætninger som følge af forskelle i f.eks. race, ydelsesniveau, rationssammensætning mm; og 3) eventuelle sideeffekter i relation til f.eks. dyrevelfærd, klimatilpasning, miljø og biodiversitet.

1 Tabel 1. Oversigt over virkemidler samt TRL-niveau og reduktionspotentiale.

Virkemiddel	TRL-niveau	Reduktionspotentiale, %	Bemærkninger
SilvAir/nitrat	8	10-20	Nitrat er undersøgt i en række forsøg, og det forventes at indgå i AU's klimavirkemiddelkatalog 2025. Uklart om fuld skala produktion af det endelige produkt er mulig. Manglende afklaring af samlet LCA-aftryk. Høje reduktionspotentiale er sandsynligvis ledsaget af reduktion i foderoptagelse. Effekt på udledning af lattergas i vommen bør undersøges nærmere. Hvis der tilsættes nitrat bør rationens indhold af kvælstof som udgangspunkt tilpasses således at kvælstofudnyttelsen ikke forringes. Overdosering kan lede til methæmoglobinæmi, men dette har ikke været en væsentlig risikofaktor i de af AU afprøvede dosis.
Asparagopsis Taxiformis	6	20-60	Reduktionspotentialet er meget højt i nogle dyreforsøg. Asparagopsis Taxiformis er undersøgt i forsøg primært i USA og Australien. Data fra NV-europæiske produktionsforhold er begrænset, men alle hidtidige har fundet reduktion i foderoptagelse og/eller mælkeproduktion. Forsøgene er med et forholdsvis lille antal dyr. Brugen af tangen skal sandsynligvis ikke godkendes i EFSA, da det er et fodermiddel. Skal sandsynligvis godkendes hvis det er i form af ekstrakter. Det primære aktive stof er bromoform. Indhold af jod og andre indholdsstoffer kan være et problem, men er ikke afklaret. Fremtid regulering af bl.a. bromindhold i foderrationer kan også medføre restriktioner af tilladt inklusionsniveau. Effekt på dyrets sundhed og velfærd og produktkvalitet skal afklares. Ændringer i vomepithel er set i studier. Fastlæggelse af dosis og effekt på foderoptagelse og produktion udestår. Produkter (ekstrakter og tørrede varer) kan være på markedet meget hurtigt. Produktets eventuelle forarbejdning forinden opfodring bør tages i betragtning for evaluering af den samlede klimaeffekt (LCA). Validering under danske forhold af et konkret produkt udestår.
Andre alger/tang end A. Taxiformis	4/5	10-20	Respons i metan er mindre end for Asparagopsis Taxiformis. Der er igangsat forskningsprojekter i Danmark med andre tangarter (f.eks. Asparagopsis Armata og Bonnemaisonia Hamifera). Skal sandsynligvis ikke godkendes i EFSA da tang er et fodermiddel. Fastlæggelse af dosis og effekt på foderop-

			tagelse og produktion udestår. Nuværende og eventuelle fremtidige restriktioner af indholdsstoffer i foderrationer vil påvirke inklusionsraten og derfor reduktionspotentialer.
Stof X2	4/5	10-30	Der er igangsat dyreforsøg som skal fastlægge sammenhængen mellem dosis og effekt på metan. Skal godkendes i EFSA. Fastlæggelse af dosis og effekt på foderoptagelse og produktion udestår. Nuværende og eventuelle fremtidige restriktioner af indholdsstoffer i foderrationer kan eventuel påvirke inklusionsraten og derfor reduktionspotentialer.
Sukkerroer	5	0-10	Reduktionspotentialer er meget usikkert og vil sandsynligvis også være forskelligt afhængig af om det er friske roer eller roensilage med et højt indhold af ethanol samt afhængig af hvilke fodermidler roer erstatter i rationen. Der er behov for mere viden om effekt på enterisk metan og især på den samlede effekt af dyrkning af sukkerroer, inkl. brug af roetop, både i forhold til klima og kvælstof.
Tanniner	3/4	0-15	Findes i planter og består af diverse gruppe af stoffer der indeholder phenol grupper, der varierer mellem planter. De afrapporterede reduktionspotentialer er meget forskellige, og der er ikke et specifikt produkt på vej. Testet i meget begrænset omfang i NV Europa. In vitro forsøg viser markante reduktioner ved brug af rene bioaktive stoffer dog ved inklusionsrater langt højere end muligt in vivo. Brug af tanninrige grovfodermidler kan være ledsaget af reduktion i fordøjelighed og foderoptagelse og lavere N-udnyttelse.
Saponiner	3	0-15	Saponiner er en divers gruppe af plantestoffer, som har forskellige sammensætning og biologisk aktivitet. De afrapporterede reduktionspotentialer er meget forskellige, og der er ikke et specifikt produkt på vej. Ikke testet i NV Europa.
Essentielle olier	3/4	0-15	Kommercielle produkter er på markedet i USA, men reduktionspotentialer er meget usikkert og varierende.
Andre bioaktive stoffer	2-5	0-20	Nyt forskningsområde med betydelig variation indenfor stofgruppe og mellem stofgrupper. Det kan f.eks. være ekstrakt af cashew skaller, hamp, pil mm. Kan være et virkemiddel, som kan bruges i den økologiske produktion.
Biokul	2	0-10	Biokul anvendes som toksinbinder i husdyrproduktion og deres egenskaber er meget afhængige af kemiske grupper på overfladen. Vurderes ikke at

			være relevant for nærværende, da litteraturen baseret på dyreforsøg er meget sparsomt. Behov for udvikling af nye typer af biokul og mere forskning indenfor området før virkemidlet kan endeligt vurderes.
Afgræsning	6	10-25	Afgræsning er især vigtig for den økologiske produktion. Data fra NV Europa viser en markant effekt om foråret og i forsommeren, men der mangler afklaring generelt af reduktionspotentialet pr. årsko. Det angivne reduktionspotential kan derfor ikke direkte sammenlignes med virkemidler som kan bruges en større del af året som f.eks. foderadditiver og ændringer i fodringen. Derudover mangler beskrivelse af virkningsmekanisme, f.eks. sammenhæng til græskvalitet, sukkerindhold, nitratindhold, afgræsningssystem mm. Emission af lattergas fra marken ved nedbrydning af grønne planterester fra afpudning, nedtrædning samt urinpletter er uafklaret.
Andre kemiske foderadditiver	2-6	25-35	Der er en række nye foderadditiver på vej, som har vist et markant reduktionspotential i laboratorieforsøg eller i de første dyreforsøg. Nogle af disse virkemidler har samme virkningsmekanisme som Bovaer, mens andre har andre virkningsmekanismer som f.eks. er relateret til omsætning af nikkel. Virkemidlerne skal sandsynligvis godkendes i EFSA. Fastlæggelse af dosis, reduktionspotential og afledte effekter udestår.
Brintbindere	2/3	10-20	Kan f.eks. være fumarat og malat. Nyt meget lovende forskningsområde som udover den direkte konkurrence med metanogener om brint, måske også kan have en positiv effekt på omsætning af næringsstoffer i vommen, foderoptagelse og produktion, hvis de tildeles sammen med foderadditiver, som i sig selv giver højere koncentration af brint i vommen.
Ændret fodring	8/9	5-10	Forbedret grovfoderkvalitet, øget kraftfoder indhold og generel ændret kulhydrat sammensætning (mere stivelse på bekostning af NDF) kan reducere enterisk metan. En øgning af kraftfoderandelen fra 40% til 50% er sandsynligvis det realistiske under danske forhold. Manglende afklaring af sideeffekter i forhold rationens klimaaftryk (dyrkning), miljø og produktionsøkonomi.
Halogener	2-6	10-40	Der er igangsat forskning i den direkte effekt af forskellige halogenerede forbindelser, dvs. kemiske stoffer, hvor brint er erstattet af halogener som f.eks. brom og jod. Den metan reducerende effekt er i nogle forsøg meget markant, men den afledte effekt på produktion, sundhed og produktkvalitet er

			sandsynligvis en udfordring, hvilket sætter en begrænsning på dosis og dermed reduktionspotentialer. Der forskes i både naturlige og syntetiske halogenerede forbindelser som f.eks. bromoform fra tang, som også er undersøgt som det rene kemikalie. Nuværende og eventuelle fremtidige restriktioner på f.eks. jod- og bromindhold i foderrationen vil påvirke dosis og dermed reduktionspotentialer.
Tildeling af mikroorganismer	2-4	0-10	Tilsætning af bakteriekulturer som f.eks. mælkesyrebakterier og gær har i nogle dyreforsøg reduceret emission af metan som følge af en ændret VFA-profil i vommen og især en højere produktion af propionsyre. Tilsætning af acetogener, som kan bruge brint til produktion af eddikesyre er et nyt og spændende forskningsområde. De første danske forsøg har vist begrænset effekt af tildeling af forskellige typer af mikroorganismer. Tilsætning af phager (virus) der specifikt angriber metanogenerne er et nyt forskningsområde. Der er behov for en bedre forståelse af samspillet mellem foderadditiver og omsætning af brint og kulstof i vommen.
Tidlig prægning	2-4	0-20	Tildeling af foderadditiver som påvirker metanogernes etablering i vommen tidligt i livet og en generel manipulering af vommikrobiomets etablering er et nyt forskningsområde. Nogle forsøg har vist en persistent reduktion i metan senere i dyrets liv. Der er igangsat forskning ved AU indenfor dette område.
Vaccination	1-3	10-20	Nyt forskningsområde, hvor der ikke er meget litteratur.

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Bilag 1. Oversigt over forskellige foderadditiver (Hegarty et al., 2021)

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			Potential animal welfare risks	Potential food safety risks	Potential co-benefits	Production system applicability ⁴	Development needs
	CH ₄ reduction potential ¹	No. of academic papers ²	Confidence in efficacy ³					
3-Nitrooxypropanol	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 ₂ O 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.

Bilag 2. Beskrivelse af status for forskellige virkemidler (Hegarty et al., 2021)

Library of ruminant feed additives

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.

Library of ruminant feed additives

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.

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<p>Impacts on animal production</p>	<p>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.</p> <p>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:</p> <p><u>Diet Digestibility</u>: 3-NOP supplementation did not alter fibre digestibility¹⁴.</p> <p><u>Feed Intake</u>: 3-NOP supplementation reduced DMI up to 4.5 % overall¹⁴ (P = 0.02) but showed no effect in many studies and was not significant in Jayanegara's meta-analysis in 2017¹³. Kim et al.⁹ reported a (near significant) decline in DMI with 3-NOP inclusion in beef but no effect in dairy cattle.</p> <p><u>Live weight gain</u>: Significant data on grain-based diets showed no advantage to live weight gain from 3-NOP inclusion¹⁴ and there may be reductions in growth at high levels of 3-NOP inclusion⁸.</p> <p><u>Milk production</u>: 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.</p>
<p>Applicability</p>	<p>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.</p> <p><u>Feedlot</u>: 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.</p> <p><u>Cut and carry</u>: Inclusion of 3-NOP in concentrate or premix for ruminants fed cut pasture is highly possible as has been done in research trials².</p> <p><u>Dairy</u>: 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.</p> <p><u>Grazing</u>: 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 efficacy¹⁰.</p>

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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 ₂ e/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%.

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	<p>Alvarez-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%.</p>
<p>Actions needed to accelerate technology roll-out</p>	<p>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.</p> <p>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 CH₄ emissions return to pre-treatment levels within a few days after the administration of 3-NOP ceases.</p>
<p>Assessment Summary</p>	<p>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.</p>

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Freeze-dried *Asparagopsis* meal

[See the reference list](#)

Evidence of efficacy	<p>The efficacy of freeze-dried <i>Asparagopsis</i> meal has been shown in the laboratory^{27,28,217,219} and in sheep³⁰, 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 <i>Asparagopsis</i> used. A meta-analysis of effects of feeding predominantly <i>Asparagopsis</i> algae to cattle found a reduction in methane yield of 37% or 5.3gCH₄/kg DMI³³ (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 diets³¹.</p> <p>The only study to test the efficacy of <i>Asparagopsis</i> fed to sheep³⁰, 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.</p> <p>A test of the efficacy of <i>Asparagopsis</i> in feedlot steers found up to a 98% reduction in methane⁴⁰ 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 <i>Asparagopsis</i>, and methane was reduced by up to 80% and feed-to-gain efficiencies were increased by 14%. This study also demonstrated the efficacy of <i>Asparagopsis</i> for high-, mid- and low-forage diets, as well as persistent methane reductions for the 21-week period.</p> <p>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% <i>Asparagopsis</i> DM was included in the diet. Stefenoni et al (2021)⁴¹ reported methane reductions as high as 80% using 0.5% DM.</p> <p>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 <i>Asparagopsis</i> studies¹⁴. However, this analysis did not include the two most recent studies to be published, both with methane reductions up to 80%^{31,41}.</p>
Mode of action	<p>The bioactive array of algal chemicals is complex³⁵ 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 cells²¹⁹. In <i>Asparagopsis</i>, bromoform has</p>

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	<p>been shown to be the principal compound suppressing methane emissions²⁹. Bromoform (CHBr₃) 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}.</p>
Inclusion Rate	<p>It has been hypothesized that <i>Asparagopsis</i> 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% <i>Asparagopsis</i> 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 <i>Asparagopsis</i> material.</p>
Manufacturer	<p><i>Asparagopsis</i> is sourced from both wild harvest and from commercial farms with an increasing number of <i>Asparagopsis</i> producers developing in both the northern (e.g., Greener Grazing, Blue Ocean Barns, Symbrosia, Volta Greentech) and southern hemisphere (e.g., Sea Forest, CH₄ 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 <i>Asparagopsis</i> 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.</p>
Availability	<p>It is expected that commercial availability of <i>Asparagopsis</i> will begin in early to mid 2022.</p>
Impacts on animal production	<p>In a meta-analysis of cattle studies, Lean et al.³³ found no effect of (predominantly) <i>Asparagopsis</i> algae on growth rate, nor any effects on DMI. Across these cattle studies, <i>Asparagopsis</i> improved feed conversion efficiency by 7%. However, severe feed refusals at approximately 6% inclusion in DM³⁰ and a reduction in feed intake at 1.84% vs 0.92% in diet DM³² have been observed, and a reduction in intake at 0.5% vs 0.25% in DM⁴¹, suggests inclusion levels should be restricted to <1% in diet DM. Lactating dairy cows appear to be the most sensitive to <i>Asparagopsis</i> 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 consequence³². When incorporated in a TMR, <i>Asparagopsis</i> inclusion should be kept under 1% DM to avoid decreases in intake of the seaweed resulting</p>

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	<p>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 steers³¹. 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 <i>Asparagopsis</i> inclusion on live weight change of 2-year-old sheep. Daily milk production⁴¹ was not reduced by <i>Asparagopsis</i> at 0.25% inclusion in DM⁴¹ but was reduced by 0.5% inclusion, and was reduced at 1.84% <i>Asparagopsis</i> in DM in another study³¹.</p>
<p>Applicability</p>	<p>Preparation and storage conditions are important to minimize bromoform volatilization³⁹. Freeze dried product is currently the gold standard, although other forms of processing, including an <i>Asparagopsis</i> oil suspension, are being investigated⁴.</p> <p>There are co-benefits from <i>Asparagopsis</i> farming because seaweed production can be improved by utilizing waste nutrients (P, N, CO₂) from other forms of aquaculture (finfish, shellfish, prawns), if co-located. <i>Asparagopsis</i> farming can also enhance regional economies by using local labour, and <i>Asparagopsis</i> 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.</p>
<p>Expected market trajectory</p>	<p>There are multiple <i>Asparagopsis</i> 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 <i>Asparagopsis</i> and expand the capability for large scale global supply. Industrial production facilities in temperate latitudes are also under development.</p>
<p>Expected cost</p>	<p>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.</p>
<p>Constraints to use</p>	<p>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 toxicity⁴². However, Kinley et al.⁴⁰ found no bromoform residue in meat or kidneys of cattle fed <i>Asparagopsis</i>. There is currently (October 2021) no literature on the metabolic fate of bromoform in the ruminant digestive system. Reductive dehalogenation, the breakdown of halogenated</p>

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	<p>compounds such as bromoform, has been shown in certain types of bacteria such as <i>Dehalobacter</i> and <i>Desulfovibrio</i> species²²⁴.</p> <p>Bromoform released into the atmosphere from any source can contribute to ozone depletion^{43,225}. Due to the need to preserve <i>Asparagopsis</i> 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 processing⁴⁴ and by managed cultivation.</p>
Residue in animal product	<p>No bromoform residues were found in samples of kidney, liver, faeces, fat, muscle tissue, or milk, taken from sheep³⁰ and beef cattle^{31,40} which had been offered <i>Asparagopsis</i> at the low effective levels. Low levels of bromoform have been found in milk (even from cows not consuming <i>Asparagopsis</i>), although no significant increases in milk bromoform concentrations have been found in cows fed <i>Asparagopsis</i>^{32,41}. In a study with lactating dairy cows the level of safety of inclusion of <i>Asparagopsis</i> in cattle feed was tested under extreme conditions⁴⁵. 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 <i>Asparagopsis</i> on the day prior to morning milk collection. The milk bromoform content was less than half the USEPA drinking water standard for bromoform⁴⁵.</p> <p>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 <i>Asparagopsis</i> for 147 days. Total <i>Asparagopsis</i> iodine fed per day was approximately 199.1 mg/day, which indicates a very low iodine transfer rate between <i>Asparagopsis</i> 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 <i>Asparagopsis</i> fed or the transfer rate between that and milk.</p>
Impacts on manure	<p>Marine and freshwater macroalgae have long been used as soil conditioners, both in raw⁴⁶ and biochar⁴⁷ form. Furthermore, liquified seaweed or its extracts have been widely used as bioactives for soil/plant health⁴⁸. Being of marine origin, <i>Asparagopsis</i> 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 <i>Asparagopsis</i>, although in subsequent urine testing bromoform could not be detected. As livestock have no way of storing bromine or iodine derivatives,</p>

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	<p>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.</p>
Potential regional distribution	<p>There are tropical (<i>A. taxiformis</i>) and temperate (<i>A. armata</i>) <i>Asparagopsis</i> 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.</p>
Life-cycle Assessment (LCA)	<p>Many LCAs have been conducted for growing algae for biofuel production⁴⁹ but these growth systems are quite different to those which will be used for <i>Asparagopsis</i>, for which no LCA is currently available. At the time of this publication, there are three independent articles under review.</p>
Actions needed to accelerate technology roll-out	<p>Currently (2021) most <i>Asparagopsis</i> 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:</p> <ul style="list-style-type: none"> • 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 <i>Asparagopsis</i> 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	<p>There is growing capacity to farm <i>Asparagopsis</i> in natural marine locations or tanks. The feeding of <i>Asparagopsis</i> to ruminants for methane mitigating purposes is patented. <i>Asparagopsis</i> 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 <i>Asparagopsis</i> 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 for more animal studies, <i>Asparagopsis</i> 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% <i>Asparagopsis</i> in a TMR feed will achieve very high mitigation (>25%) of rumen methane output. There is also limited evidence and low agreement that <i>Asparagopsis</i> increases growth rate in ruminants, but it does show a consistent improvement in feed efficiency.</p>

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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.

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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.

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	<p>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.</p> <p>In the manufacturing sector avoidance of potentially explosive nitrates is important to minimize warehousing and processing safety requirements.</p> <p>The feeding of nitrates for methane mitigation is covered by global patents (e.g., WO2011010921A2 originally implemented by Cargill).</p>
Residue in animal product	<p>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 skin⁵⁵. No evidence of potentially toxic nitrosamines was found in tissues of nitrate-fed cattle⁵⁷.</p>
Impacts on manure	<p>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 excretion⁶².</p>
Potential regional distribution	<p>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.</p>
Life-cycle Assessment (LCA)	<p>Calcium nitrate manufacture shows a carbon cost of 650 grams of CO₂/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 urea⁶³.</p>

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<p>Actions needed to accelerate technology roll-out</p>	<p>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.</p>
<p>Assessment Summary</p>	<p>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.</p>

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Antibiotic Rumen Modifiers

Antibiotic rumen modifiers include ionophores (such as monensin, lasalocid and salinomycin) as well as phosphoglycolipid antibiotics such as bambarmycin 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. <ol style="list-style-type: none">1) It reduces feed intake⁶⁴ and2) 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 organisms⁶⁶.
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.

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Impacts on animal production	<p>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 diets⁶⁷. In dairy cows monensin is highly effective in protecting against bloat⁶⁸. 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.</p> <p>Monensin is also effective in reducing bloat in beef⁶⁸ 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.</p>
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.

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Life-cycle Assessment (LCA)	<p>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.</p> <ul style="list-style-type: none">• 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	<p>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.</p>
Assessment Summary	<p>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%.</p>

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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	<p>There are multiple mechanisms by which EO <u>may</u> influence methane emissions, but these are not independent, and none are robustly evident as reducing CH₄ emissions across published literature.</p> <ol style="list-style-type: none">1) Effects on intake. No significant effects in sheep⁷⁸, 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 papers⁷⁸. In a meta-analysis, protozoal density was reduced in 19 of 24 treatments in laboratory studies, but only in 4 of 17 animal studies⁷⁷.3) 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.

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Dose	<p><u>Agolin</u>: 1 g/d/cow <u>Crina Ruminants</u>: 150-200mg/kg bodyweight <u>Mootral</u>: 10-15 g/cow/d <u>GasolLess</u>: not specified <u>Nutrimix</u>: not specified <u>Garvo</u>: not specified</p>
Manufacturer	<p>There are numerous commercial EO blends available but currently two major blends are marketed specifically for methane reduction.</p> <p><u>Agolin</u> (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.</p> <p><u>Mootral</u> (https://mootral.com/) This allicin (from garlic) and citrus extract is specifically marketed for methane mitigation. It was shown to reduce methane emissions in sheep⁸¹, with preliminary assessments in dairy⁸² but it did not reduce emissions from feedlot cattle³².</p> <p>In addition, there is a suite of regionally available essential oil blends, with some listed below:</p> <p><u>Crina</u> (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.</p> <p><u>Gasolless</u> (https://www.idena.fr/en/products-expertise/ruminants/) <u>Nutrimix</u> (https://www.idena.fr/en/products-expertise/ruminants/) <u>Garvo</u> (https://www.garvo.nl/contact/)</p>
	EOs are available worldwide.

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Impacts on animal production	<p>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%.</p>
Applicability	<p>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.</p> <p>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 periods⁸⁵. 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.</p>
Expected market trajectory	<p>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.</p>
Expected cost	<p>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.</p> <p>Mootral has a program of carbon credits from enteric methane reduction from cattle (CowCredit™). CowCredit (1 CowCredit = 1 t CO₂e 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.</p>

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<p>Constraints to use</p>	<p>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 biotechnology²¹⁴, rather than relying on extraction from plants which may reduce cost and availability constraints in future.</p>
<p>Residue in animal product</p>	<p>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-effect⁸⁴ 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 milk²¹³.</p>
<p>Impacts on manure</p>	<p>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 pathogens⁸⁹, 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.</p>
<p>Potential regional distribution</p>	<p>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.</p> <p>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.</p>
<p>Life-cycle Assessment (LCA)</p>	<p>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.</p>

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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.

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Saponins

Saponins are a class of plant secondary metabolites with a great diversity of structure and biological activity⁹². 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 $\leq 0.5\%$ 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 $\leq 0.5\%$ are recommended for maximum methane mitigation and to avoid adverse impacts on digestibility ⁹⁴ .

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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	<p>The meta-analysis of Ridla et al.⁹⁴ showed DM digestibility was increased by a low level of saponin ($\leq 0.5\%$ in DM) but reduced at a higher level.</p> <p>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.</p> <p>No information regarding the effect of saponin on animal reproduction was found in the literature.</p>
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 Quilliaia 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 .

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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.

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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 (CH₄/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 CH₄/kgDMI. Patra and Saxena¹⁰⁸ 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 & Beauchemin¹⁰² 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

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	<p>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.</p> <p>There is a commercially available tannin extract (Silvafeed) with some documented emission and productivity responses:</p> <ul style="list-style-type: none">• 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, respectively¹¹¹.• Silvafeed at 30 g/ kg of DM reduced (6%) total CH₄ production (24 h) but increased (5%) production of CH₄ per g of degraded DM. These results suggest that these tannins affected CH₄ mainly by decreasing fibre digestibility¹¹².• Silvafeed (67 mg/L of inoculum) had no effect on gas production and methane production in the laboratory¹¹³.
Mode of action	<p>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 intestine¹⁰⁸. 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 production¹¹⁴.</p>
Dose	<p>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.</p>
Manufacturer	<p>Global producers exist for tannins, principally marketed to preserve skins and hides as 'veg-tan' hides. <u>Products specifically produced for feed applications are made</u>, 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 (<i>Castanea sativa</i> Mill) and condensed tannin from quebracho (<i>Schinopsis lorentzii</i> 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 legumes¹¹⁵ (https://www.progardes.com.au/research)</p>

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Impacts on animal production	<p>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.</p> <p><u>Animal Performance</u>: Feed intake, cattle growth rate and final liveweight were unaffected by tannins.</p> <p><u>Feed Digestibility</u>: Reduced feed digestibility at doses above 12 g/kg DM¹¹⁰, and a tendency for reduced feed efficiency.</p> <p><u>Nitrogen Utilization</u>: 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 use¹¹⁰.</p> <p><u>Environment</u>: 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 health¹¹⁴.</p> <p><u>Animal Health</u>: Tannin extracts have the potential to reduce bloat incidence¹¹⁷. 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-safe¹¹⁸.</p>
Applicability	<p>Tannins can be consumed naturally as ingredients in forages or added to prepared feeds and supplements as extracts mixed into the diet.</p>
Expected market trajectory	<p>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.</p>
Expected cost	<p>Tannin extracts cost US\$3-4/kg for a product that is approximately 95%DM and 60-70% tannin in DM. Silvaeed 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.</p>

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<p>Constraints to use</p>	<p>Kumar and Singh¹¹⁹ 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.</p> <p>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.</p>
<p>Residue in animal product</p>	<p>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.</p>
<p>Impacts on manure</p>	<p>While diversity in tannin effects on feed digestion have been summarised above, in general tannins reduce the proportion of N excreted in urine¹²⁰ 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 (CO₂, N₂O, CH₄)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 incubations¹²².</p>
<p>Potential regional distribution</p>	<p>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.</p> <p>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.</p>
<p>Life-cycle Assessment (LCA)</p>	<p>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</p>

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Actions needed to accelerate technology roll-out	<p>impact than a single hot water extraction¹²³. A LCA from the perspective of the impacts of tannins on ruminant production has not been conducted.</p> <p>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.</p>
Assessment Summary	<p>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 CH₄ 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.</p>

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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 production¹²⁶. Microalgae have been evaluated as substrates for fermentation and biogas production^{127,128}, but more recently their potential as feed additives to provide protein¹²⁹ and suppress methane in ruminants has been assessed¹³⁰.

[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, <i>Asparagopsis</i> . 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.

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Manufacturer	Algal meals are available and are <u>now being used as source of omega three fatty acids for farmed fish.</u>
Availability	DHA in a purified form is <u>readily available as nutraceutical & algal extracts are available.</u>
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.

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Impacts on manure	Like the macroalgae <i>Asparagopsis</i> , 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 3 rd 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 <i>Asparagopsis</i> 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	<p>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.</p> <p>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.</p>

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Biochar

Biochar is a carbon based (charcoal-like) product arising from combustion in an O₂ 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 ameliorant¹⁴². In livestock feeding biochars have been used to adsorb toxins and alter manures¹⁴³. Biochar has also been shown to facilitate methane oxidation (destruction) in soils¹⁴⁴, 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 rumen¹⁴⁷ 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: <ol style="list-style-type: none">1) reduced rumen protozoa populations¹⁵¹2) the pore structure of the biochar allowing a different microbial biome (<i>ib-id</i>) and3) the biochar acting as an electron shuttle moving electrons between microbes or between microbes and chemical acceptors¹⁵⁵
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 1 st (crop based), 2 nd (crop waste based) or 3 rd generation (algal based) feedstocks. There are over 130 biochar producers in the USA but the majority of global production occurs in China ¹⁵⁵ .

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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	<ol style="list-style-type: none"> 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,

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Impacts on manure	<p>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).</p> <p>The principal biochar market is as a soil ameliorant or additive in high value crops¹⁴². 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 fertility¹⁴³. 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 present¹⁵⁹. Biochar also affects the redox state of the soil to which it is applied¹⁵⁸.</p>
Potential regional distribution	<p>China is the main global production site¹⁵⁷, but biochar is a transportable bulk commodity and can be produced wherever plant waste is available.</p>
Life-cycle assessment (LCA)	<p>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.</p>
Actions needed to accelerate technology roll-out	<p>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.</p>
Assessment Summary	<p>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.</p>

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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 CH₄ 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 x 10⁹ – 11.5 x 10¹¹ 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 CH₄ by 27% (g CH₄/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 CH₄ emissions.

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	<p>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 <i>L. pentosus</i> D31 (3.6×10^{11} CFU/cow/day) and <i>L. bulgaricus</i> D1 (4.6×10^{10} CFU/cow/day) did not affect CH₄ emission from cows fed either high starch or high fibre diets. Ellis et al.¹⁶³ reported that <i>L. plantarum</i> (6072), <i>L. lactis</i> (O-224), <i>L. buchneri</i> (LB1819), <i>L. lactis</i> (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 CH₄ emission regardless of the unit of expression, but tended to increase CH₄ produced per unit of metabolic BW. Philippeau et al.¹⁶⁴ examined combinations of <i>Propionibacterium</i> P63 with <i>L. plantarum</i> 115 or with <i>L. rhamnosus</i> 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+ <i>L. rhamnosus</i> 32 tended to have decreased CH₄ 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 <i>L. plantarum</i> 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 <i>Leuconostoc mesenteroides</i> subsp. <i>Mesenteroides</i> (AOK1789) to sheep at $1.5\text{--}1.8 \times 10^9$ 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 CH₄ in the rumen.</p>
Mode of action	<p>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 digestion¹⁶⁷. 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:</p> <p>Propionibacteria:</p>

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	<p>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 CO₂ to CH₄ by methanogens. Indeed, some researchers have shown increased ruminal propionate with <i>Propionibacterium</i> P169 in cattle^{168,169}.</p> <p>2). Another possibility for the lower intensity of CH₄ emissions could be the antimicrobial activity of some <i>Propionibacterium</i> strains, which produce inhibitory metabolites such as short-chain fatty acids and antimicrobial peptides known as bacteriocins. It has been shown some strains of <i>Propionibacterium</i> have inhibitory activity against ruminal bacteria, thus reducing CH₄ 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.</p> <p>Lactic acid producing bacteria (LAB): Doyle et al.¹⁷² summarized the possible mechanisms by which LAB decreased methane production in ruminants:</p> <p>1). Addition of LAB may stimulate the production of lactic acid and the growth of lactic utilizing bacteria that convert lactic acid to propionate¹⁷³.</p> <p>2). Metabolites such as bacteriocins (e.g., nisin from <i>Lactococcus lactis</i>, PRA1 from <i>L. plantarum</i> TUA1490L and pediocin produced by <i>P. pentosaceus</i> 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.</p>
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 x 10 ⁶ – 1 x 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.

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Impacts on animal production	<p>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.</p> <p>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.</p> <p>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 efficiency¹⁷⁶ when one particular strain was administered in whole milk or as an inoculum¹⁷⁷. When LAB were administered to mature ruminants, the prevalence of <i>Escherichia coli</i> O157 in beef cattle was reduced¹⁷⁸, 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.</p> <p>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.</p>
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.

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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 as 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 (<i>Escherichia coli</i> and <i>Salmonella</i> 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 ₂ e/ 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 of 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.

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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	<p>Darabighane et al.¹⁸², conducted a meta-analysis to assess the impact of <i>S. cerevisiae</i> 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 CH₄ production or CH₄ production per dry matter intake (CH₄/DMI). Since 2017, one study showed that <i>S. cerevisiae</i> CNCM I-1077, at a dosage of 1 x 10¹⁰ CFU/head/d, tended to increase CH₄ per unit of feed intake in lactating dairy cows . In another study, <i>S. cerevisiae</i> supplemented at 2, 4 and 6 x 10¹⁰ CFU/head/d also did not affect CH₄ 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 carbohydrate¹⁸⁴. Similarly, Oh et al.¹⁸⁵ reported that supplementation of a DFM product containing <i>S. cerevisiae</i> (5 x 10¹⁰ CFU/head/d and 1.1 x 10⁸ CFU/g of a mixture (3 x 10⁹ CFU/head/d) of <i>L. lactis</i>, <i>Bacillus subtilis</i>, <i>E. faecium</i>, and <i>L. casei</i> 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 <i>S. cerevisiae</i> (from Yea-Sacc 1026), at a dose of 5 x 10⁹ CFU/head/d to lactating Jersey cows and found no effect on CH₄ production when the diet contained either urea or NO₃⁻. In contrast, Cagle et al.¹⁸⁷ showed that supplementing <i>S. cerevisiae</i> Sc47 CNCM I-4407 at the levels of 2.5, 5 and 10 x 10¹⁰ CFU/head/d to steers and heifers fed a growing, transition and finishing diet decreased CH₄ 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.</p>
Mode of action	<p>Proposed modes of action for fungal DFM include:</p> <ul style="list-style-type: none">• Reduction of oxygen in the rumen

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	<ul style="list-style-type: none"> • 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 CH₄ emissions. <p>The most commonly reported mode of action of <i>S. cerevisiae</i> 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 CH₄ production.</p>
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 (<i>S. cerevisiae</i>) 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 <i>A. oryzae</i> were fed to lactating ruminants ¹⁸⁸ . In a review by Robinson and Erasmus ¹⁹⁰ , yeasts increased milk yield by 3.6% on average.

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	<p>Fungal cultures have also been fed to calves, sheep, and steers but studies with these livestock are far fewer than with lactating dairy cows. Sales¹⁹¹ 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.</p> <p>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.</p> <p>Overall, supplementation with <i>S. cerevisiae</i> 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 <i>S. cerevisiae</i> to beef cattle (4.9×10^{12}/head/d) did not affect DMI, ADG or feed efficiency¹⁹⁶, and that beef cattle supplemented with 4×10^{10} CFU/head/d exhibited increased average daily gain, improved feed efficiency and enhanced digestibility of neutral detergent fibre and acid detergent fiber¹⁹⁷. In contrast, Finck et al.¹⁹⁸ showed that supplemented yeast at 5×10^{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 CH₄ emissions.</p>
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	<p>Easiest if dealing with microorganism on GRAS list. Other microbial candidates would have to be submitted through the appropriate regulatory pathways.</p> <p>Lack of DFMs that have proven efficacy in terms of CH₄ mitigation.</p>
Residue in animal product	<p>None expected</p> <p>No evidence</p>

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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.

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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 <i>Propionibacterium</i> .” 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)	<u>An early-stage probiotic additive being developed for dairy cattle</u>
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 .

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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 <i>Asparagopsis</i>	While <i>Asparagopsis</i> 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 <i>Asparagopsis</i> 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 .