

Pure

Scotland's Rural College

Nutritional strategies to reduce enteric methane emissions

Rooke, JA; Miller, G A; Flockhart, JF; McDowell, MM; MacLeod, M

Print publication: 01/01/2016

[Link to publication](#)

Citation for published version (APA):

Rooke, JA., Miller, G. A., Flockhart, JF., McDowell, MM., & MacLeod, M. (2016). *Nutritional strategies to reduce enteric methane emissions*. Scotland's Rural College (SRUC).
https://www.climatexchange.org.uk/media/2033/nutritional_strategies_to_reduce_enteric_methane_emissions.pdf

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Nutritional strategies to reduce enteric methane emissions

J. A. Rooke, G. Miller, J Flockhart, M. McDowell & M. MacLeod
Scotland's Rural College, Kings Buildings, West Mains Road, Edinburgh EH9 3JG
Enquiry received 5th July 2016 for response by end September 2016

Enquirer: Gordon Struth & Keith McWhinnie,
Scottish Government, Climate Change & Agriculture

Executive Summary

Background

Methane (CH₄) emissions from ruminants are responsible for approximately 50% of the greenhouse gas (GHG) emissions associated with agriculture in Scotland. Reducing the emissions intensity (EI; the amount of GHG emitted per unit of meat or milk produced) of ruminants is, therefore, key to reducing agricultural emissions in Scotland.

Scottish Government commissioned ClimateXChange to carry out a rapid evidence assessment for the effectiveness of probiotics, nitrates and high fat diets in addressing enteric fermentation as a source of GHG emissions.

Key findings

- Three of the twelve nutritional strategies evaluated showed convincing evidence for efficacy in reducing enteric CH₄ emissions – lipids, nitrate and 3-nitro-oxypropanol (3NOP). Of these three, increasing the lipid content of diets is the most immediately applicable.
- The potential reduction in CH₄ emissions achievable ranged from 11 to 21 % when reductions were expressed per unit feed intake (CH₄ yield).
- Barriers to uptake of these strategies were identified
 - difficulties in administration to grazing animals,
 - likely requirement for two strategies to be approved under EU Feed Additives legislation
- The effects on the GHG emissions associated with the production and transport of feeds were small, as was the impact of the strategies on indicative daily diet cost.

Discussion

This report delivers a rapid evidence assessment of the potential contribution that can be made towards reducing enteric CH₄ emissions from Scottish animal agriculture by manipulating the diet. This assessment provides an analysis of the available evidence for reduction in enteric CH₄ emissions by twelve candidate nutritional strategies. Evidence was based on a quantitative analysis of the published literature over a 10-year period to update the conclusions of an authoritative FAO report. Three strategies were identified as possessing the necessary evidence base for more detailed investigation of their suitability for use under Scottish conditions. This assessment was supplemented by estimating the impact upon diet cost of using these strategies and upon GHG emissions associated with production of materials.

Overall, the evidence suggests that reductions in CH₄ yield of approximately 10% (lipids) and 20% (nitrate or 3NOP) were possible. Reductions in CH₄ EI (milk or meat) achieved through the implementation of these strategies would be similar as animal performance was largely unaffected by the strategies. However, the overall benefit of these strategies would be diluted because of difficulties in implementation in grazing cattle and sheep.

Implementation of each of the three effective strategies is faced with difficulties. 3NOP is still under development and requires registration as a zootechnical feed additive under European Union legislation. The same is likely true for nitrate; in addition, there is a requirement for nitrate to be added gradually and carefully to the diet to minimize the ever present risk of nitrate toxicity. For lipids, there is an upper limit to

dietary inclusion (70 g total lipid /kg feed dry matter, DM) which limits mitigation potential. The 10% reduction in CH₄ yield above is that obtained by increasing lipid up to 70 g/kg DM from a baseline concentration of 30 g lipid / kg DM. When lipid is supplied from by-product feeds, the maximum dietary inclusion is also limited by the need to avoid feeding excess protein.

The benefits of nutritional approaches must be weighed against other approaches which reduce EI by improving animal performance.

Approach

We identified recent evidence (10 years) for the use of nutritional mitigation strategies and assessed the efficacy of twelve strategies using as a baseline the conclusions of an authoritative FAO report. Three strategies with proven efficacy were selected for further examination. Efficacy was demonstrated by consistent reductions in CH₄ yield across a range of diet types including long term studies which assessed animal performance. The practicalities with implementing lipids, nitrates and 3NOP in Scottish agriculture were then addressed. The issues addressed were (i) availability of materials and mitigation potential (together with uncertainty), (ii) the cost implications of incorporating materials into typical dairy and beef diets, (iii) problems associated with on-farm feeding and (iv) GHG emissions associated with production of materials.

Contents

Executive Summary	Error! Bookmark not defined.
Background.....	2
Key findings	2
Discussion	2
Approach	3
Contents	4
List of Abbreviations.....	5
Acknowledgements.....	5
1. Introduction and Background.....	6
1.1 Methane emissions from livestock.....	6
2. Methodologies.....	7
3. Results	8
Effective strategies – Lipid.....	11
Effective strategies – Nitrate	12
Effective strategies 3-nitro oxypropanol (3NOP)	13
4. Discussion	14
5. Policy implications	16
6. Recommendations.....	17
7. References	18
Annex 1: Updated nutritional mitigation strategies as classified by FAO (Hristov et al. 2013) updated.....	19
Annex 2: Strategy specific evidence	20
2.1 3NOP (3-nitro oxypropanol)	21
2.2 Nitrate.....	23
2.3 Fumaric acid	26
2.4 Monensin.....	27
2.5 Tannins	29
2.6 Saponins	31
2.7 Essential Oils.....	32
2.8 Dietary lipid	33
2.9 Direct-fed microbials	37
Annex 3: Costs associated with use of nitrate and lipids	39
Annex 4: Environmental impacts of amending rations	41

List of Abbreviations

3NOP – 3-nitro-oxypropanol

CH₄ – methane

CP – crude protein

DDGS – Distiller's dark grains with solubles

DE – digestible energy

DM – dry matter

DMI – dry matter intake

EI – emissions intensity; CH₄ produced per unit product (meat or milk)

EIF – emissions associated with production of feeds

EU – European Union

FAO – Food and Agricultural Organisation of the United Nations

GHG – Greenhouse gases

LUC – land use change

Acknowledgements

The authors wish to thank the advice and opinion received from Karen Stewart, SAC Consulting Ltd and members of the UK Feed Industry: Adrian Packington, DSM Nutritional Products; David McLelland, Norvite Animal Nutrition Company Ltd and John Newbold, Volac International Ltd.

1. Introduction and Background

Cattle and sheep consume cellulosic non-human edible food sources such as grazed grass and produce, in milk and meat, sources of protein of high nutritional quality. However, this comes at a cost. Ruminant production produces GHGs primarily CH₄ and so cattle and sheep contribute significantly to the carbon footprint of farming. The Climate Change (Scotland) Act 2009 sets ambitious emission reduction targets for Scotland, requiring all sectors, including agriculture, to reduce GHG emissions to mitigate anthropogenic climate change.

1.1 Methane emissions from livestock

In 2014, agriculture was responsible for 10.7 Mt CO₂e or 17% of Scotland's total GHG emissions (Scotland Greenhouse Gas Emissions 2014). Nearly half of GHG emissions were attributed to CH₄ (4.7 Mt CO₂e or 44%). The major proportion of CH₄ was derived from enteric fermentation. Non-dairy cattle contributed the greatest share (Figure 1). Reducing CH₄ emissions from enteric fermentation is therefore key to reducing agricultural emissions in Scotland.

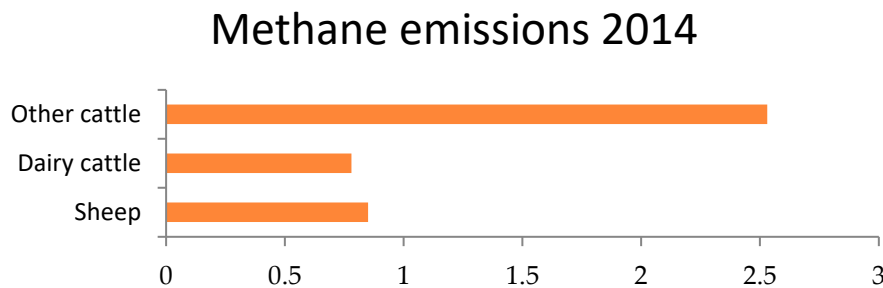


Figure 1. Enteric CH₄ emissions by livestock classes: Scotland, 2014 (Mt CO₂e)

The reticulo-rumen contains a large population of microbes which ferment feed (Figure 2) to CH₄ and volatile fatty acids (VFA). The VFA are absorbed from the rumen and provide the main energy source for the animal.

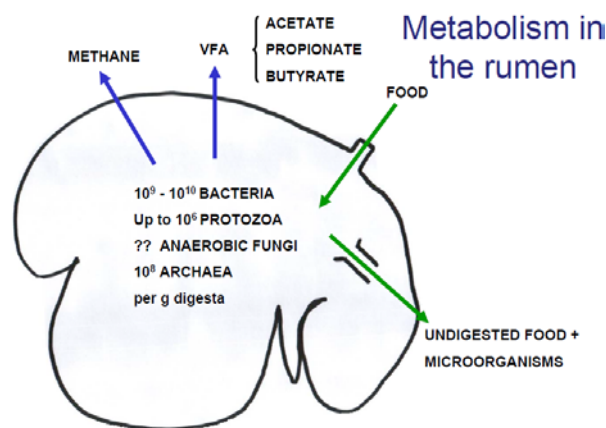


Figure 2. Rumen metabolism

The formation of CH₄ is a two stage process (Figure 3) in which the primary fermentation of feed by bacteria, protozoa and fungi to VFA produces not CH₄ but hydrogen. The archaea then synthesise CH₄ from hydrogen.

Methane formation is necessary in order to prevent hydrogen accumulation which otherwise would inhibit the fermentation process.

Methane production in ruminants

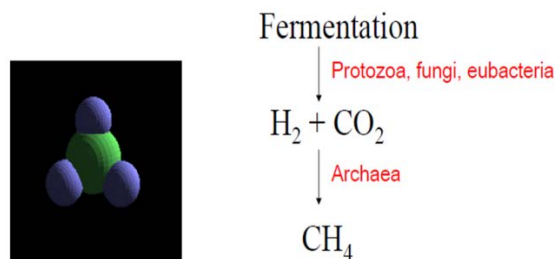


Figure 3 Two stage process of CH₄ formation

Because the formation of CH₄ is a two-stage process, several opportunities exist for modifying the process to reduce CH₄ emissions.

- Changing the composition of the diet to reduce hydrogen formation.
- Inhibiting or manipulating the microbial community to reduce hydrogen formation
- Utilising hydrogen by alternative pathways to CH₄ formation.
- Inhibiting archaea or the synthesis of CH₄ by archaea.

As between 2 and 12% of the energy consumed by ruminants is converted to CH₄ and therefore lost to the animal, there has been a long standing interest in reducing CH₄ emissions (Johnson & Johnson, 1995). In 1980 (Czerkawski, 1986), the annual cost of feed lost as CH₄ was estimated to be £300 - £350 million in the UK. Because of a lack of comprehensive, science-based, information on existing GHG mitigation practices for livestock, FAO commissioned a review of current knowledge which was published in 2013 (Hristov et al. 2013). Most of the studies cited are now more than 5 years old and the conclusions require updating.

This report aims to deliver a rapid evidence assessment of the practical feasibility of widespread on-farm use of nutritional strategies for reducing GHG in Scotland. The assessment aims to update the conclusions from the FAO report and to provide an analysis of the practicability of implementing strategies on-farm. The FAO report highlighted specific issues, relevant to the current exercise:

- There are very few long term studies that have examined the persistency of CH₄ mitigation practices
- The importance of the metric used to quantify CH₄ emissions with EI per unit animal product preferred
- Uncertainty / variation should be considered in quantifying the effect of mitigation practices.
- The challenge of implementing mitigation strategies in extensive livestock systems.

2. Methodologies

The authors searched the published literature for the 10 year period 2007 – 2016 to capture all recent studies. Keywords were set wide (methane and rumen) which resulted in 1913 citations. Irrelevant material and studies which only considered laboratory (in vitro) conditions were rejected and only studies which measured CH₄ by accepted methodologies retained giving 156 relevant studies. In only 60% of studies was some form of animal performance response reported. Therefore, CH₄ emissions expressed per unit feed intake (g/kg DM intake (DMI)), **CH₄ yield**, was the metric adopting for reporting the effectiveness of mitigation strategies; the effects of strategies upon EI were qualitatively assessed by considering the effect of strategies on animal performance.

In assessing whether there was an evidence base for individual strategies, the classification of strategies and conclusions of the FAO were adopted as a starting point. The strategies are classified in Annex 1 together with an updated conclusion upon the efficacy of each strategy. For strategies for which there was sound evidence for efficacy, a more detailed assessment of the practical implementation of each strategy, using published and grey literature was carried out. Detailed information for all strategies is presented in Annex 2. The costs of producing diets which included each strategy were explored using FeedByte to formulate rations for dairy cows and growing and finishing beef cattle (Annex 3). Finally the GHG and wider environmental implications of the individual strategies were estimated (Annex 4).

3. Results

The assessment of mitigation strategies with proven effectiveness and issues relating to practical implementation are summarized in Table 1 and a summary of all other strategies given in Table 2.

The conclusions of the FAO report were generally confirmed as there was good evidence of efficacy for only lipids and nitrates. However, 3NOP, a novel compound, for which there was consistent evidence of reductions in CH₄ emissions, was also considered. Other strategies including essential oils, saponins and tannins reduced CH₄ in laboratory / in vitro studies but were not effective in vivo. Results from in vitro studies cannot frequently be replicated in vivo because of (a) adaptation by the rumen microbial population to the active compounds and (b) the dose rates used in vitro were not practicable in vivo. In addition compounds such as monensin are not permitted for use in the EU. Therefore a detailed summary is given for nitrate, lipids and 3NOP (Table 1).

Nutritional strategies to reduce enteric greenhouse gas emissions

Table 1. Summary of CH₄ mitigation strategies with evidence-base for efficacy.

Strategy	Inclusion method	Inclusion rate (/kg diet DM)	Mitigation potential [†] (%; DMI base)		Grazing [‡]	Feed additive [*]	Diet Cost % Change ^{††}	Emissions intensity of feed production % Change ^{**}	Limitations to use
			Mean	95% confidence					
Lipid	Protected fat	< 70 g total lipid	11	8-14	X	N	-3 to +6	0	Reduction in fibre digestion and feed intake if more than 70 g/kg DM diet with adverse consequences for performance
Lipid	Whole oilseed	< 70 g total lipid	11	8-14	X	N	-1 to +2	+2	
Lipid	By-product	< 70 g total lipid	11	8-14	X	N	-5 to 0	-8	
Nitrate	Nitrate	Up to 20 g	21	18-23	X	Y?	-1 to -4	0	Risk of toxicity if fed inappropriately
3-NOP	3-nitrooxypropanol	Up to 0.2 g	21	13-29	v?	Y	NA	NA	No evidence of toxicity to date

[†]Mean values from studies reviewed irrespective of inclusion rate; inclusion rate dependant; for lipid mean value is that achieved by increasing lipid from a baseline concentration of 30 g /kg DM to 70 g/ kg DM.

[‡]Likelihood of adoption with grazing animals: X, difficult to adopt; v, could be adopted.

^{*}Classified as feed additive under EU legislation

^{††} Change relative to standard diet (range; see Annex 3)

^{**} Emissions associated with production of feed (Change relative to baseline diet; kg CO₂e / kg DM basis); see Annex 4

NA: not applicable

Nutritional strategies to reduce enteric greenhouse gas emissions

Table 2 Summary of CH₄ mitigation strategies with no consistent evidence for efficacy.

Strategy	Material / compound	Hristov et al. (2013) conclusion	Updated	Additional comment
Inhibitor	Bromochloromethane	Might be effective but ozone-depleting compound	Not considered	
Electron receptor	Fumaric acid	May reduce CH ₄ production when applied in large quantities Most results indicate no mitigating effect	Conclusion confirmed	
Electron receptor	Sulphate	May reduce CH ₄ production; may be toxic	No recent studies	
Ionophore	Monensin	May reduce CH ₄ per unit feed intake through increased efficiency. Effect variable	Conclusion confirmed	Banned in EU
Plant bioactive compounds	Tannins	May reduce CH ₄ but no long term studies	Recent long term studies; variable results	Several different tannin sources used: responses may be source specific
Plant bioactive compounds	Saponins	Tea saponins seem to have potential to reduce CH ₄	No response or evidence for long term efficacy	
Plant bioactive compounds	Essential oils	Most essential oils do not reduce CH ₄ production and long-term effects not established	No effect; no long term studies	Several different plant sources used: responses may be source specific.
Exogenous enzymes	Cellulases	Inconsistencies in data; cannot be recommended	Limited evidence; conclusion confirmed	
Direct fed microbials (inoculants)		Insufficient evidence for direct mitigating effect of microbials	Conclusion confirmed; no long term studies	Several different microbial species used: responses may be source specific

Effective strategies – Lipid

Mode of action: Multi-factorial. The fatty acid component of lipids is not fermented in the rumen. Therefore increasing dietary lipid reduces carbohydrate available for fermentation and hence CH₄ production. Lipids also inhibit rumen micro-organisms and coat fibre preventing digestion. Bio-hydrogenation of unsaturated fatty acids diverts hydrogen from CH₄ formation.

FAO recommendation: “Our conclusion is that lipids are effective in reducing enteric CH₄ emissions, but the feasibility of this mitigation practice depends on its cost-effectiveness and potential effects on feed intake (negative), productivity (negative) and milk fat content in lactating animals (positive or negative). High-oil by-product feeds such as distiller’s grains, and meals from the biodiesel industry can serve as cost-effective sources of lipids with potential CH₄ suppressing effect. Their mitigating potential, however, has not been well-established and in some cases CH₄ production may increase due to increased fibre intake.” This was confirmed in recent studies that used feeds relevant to Scottish agriculture

Material: All common feeding stuffs contain lipids and thus typical diets contain 30 – 40 g lipid / kg diet DM. Feeds used to increase dietary lipid content can be grouped as oils (usually from oilseeds), protected fats, whole oilseeds or by-products of either oil extraction or starch extraction and / or fermentation

Practical implementation: Oils extracted from oilseeds are intended to enter the human food chain; these should not be considered as strategies to mitigate CH₄ emissions. Rumen-protected fat products (mainly derived from palm oil) are available and can reduce CH₄ emissions by decreasing carbohydrate available for fermentation; these are established products which increase the energy density of the diet and are targeted mainly at dairy cows. Entire oilseeds can be fed but require treatment to crush/ crack oilseed to release lipid; oil destined for human consumption will be diverted into animal feed. By-products such as distiller’s dark grains are established feeds which contain higher lipid contents. Their usefulness is limited as these feeds contain substantially lower lipid contents than intact oilseeds and there is also a need to avoid excessive protein intakes.

Potential reduction in CH₄ emissions: Mean reduction in percentage terms for the recent studies was 11.2% (95% confidence intervals 8-14). The wide confidence interval reflects the range of materials used and therefore the practically possible inclusion levels (Annex 3). Studies where lipid was increased to greater than 70 g/kg DM were excluded.

Health & welfare implications: Excess dietary lipid has adverse effects upon rumen fibre digestion, feed intake and potentially animal performance. Inclusion of lipid should be limited to a maximum of 70 g/kg DM.

Other climate change impacts: If formulation guidelines are observed to avoid inclusion of excess dietary protein in diets, then important climate change impacts are unlikely.

GHG emissions etc. associated with production of material: Inclusion of lipid sources in diets has little effect on emissions intensity associated with producing diets (EIF) on a DM basis but reduces EIF on a DE basis (see Annex 4).

Impact on feed costs: Source dependant but for indicative diets, costs ranged from 96 to 106% of reference diets (Annex 3).

Likely impact Because of the amounts that need to be fed to achieve mitigation, lipids cannot easily be applied to grazing animals. Overall the mitigating effect will be limited by the necessity to impose an upper limit on inclusion. However, rumen-protected lipid sources and by-products are recognized feeding stuffs.

Effective strategies – Nitrate

Mode of action: Nitrate is an electron acceptor. Nitrate is reduced progressively to nitrite and ammonia by rumen micro-organisms. The reduction of nitrate to ammonia consumes hydrogen which would otherwise be used for CH₄ formation.

FAO recommendation: “Overall, nitrates may be promising enteric CH₄ mitigation agents. When nitrates are used, it is critically important that the animals are properly adapted to avoid nitrite toxicity. More in vivo studies are needed to fully understand the impact of nitrate supplementation on whole-farm GHG emissions (animal, manure storage and manure-amended soil), animal production and animal health”. Recent evidence confirms that nitrate is an effective and consistent means of reducing CH₄ emissions.

Material: Nitrate has been fed in research studies as a calcium ammonium nitrate salt (from fertilizer).

Practical implementation: The main practical barrier to use of nitrate is the risk of nitrate toxicity. Critically, animals must be adapted to feeding nitrate by gradually increasing the amount of nitrate fed. Rapid consumption of large amounts of nitrate should be avoided. Use of nitrate requires thorough mixing of nitrate with and dilution by other feed constituents. This is probably only achievable by the use of total mixed rations or by inclusion of nitrate in pelleted compound feeds. Nitrate should replace other protein sources in the diet to avoid excess excretion of nitrogen in manure.

Health & welfare implications: The main practical issue is nitrate toxicity, particularly in animals not or incorrectly adapted to nitrate (Lee & Beauchemin, 2014). Acute symptoms of nitrate toxicity are anoxia leading to death. Subacute or chronic effects are reported to include retarded growth, lowered milk production and increased susceptibility to infection. Diagnosis is by measurement of met-haemoglobin (met-Hb) status of blood. Although, most studies have reported no adverse effects of feeding nitrate, individual animals do display elevated (but sub-toxic) concentrations of met-Hb. In some experiments animals have had to be removed from trials because of toxicity. The risks associated with (a) inadvertent inclusion of excess nitrate in feeds and (b) access of unadapted animals to nitrate-containing feed are significant.

Potential reduction in CH₄ emissions: In recent studies a mean reduction of 21% (95% confidence interval, 18% -23%) was achieved in CH₄ yield (g/kg DMI) for a mean dose of 21 g nitrate / kg diet DM.

Impact on feed costs: Source dependant but for indicative diets, costs range from 96 to 99% of reference diets (Annex 3).

Likely impact: At present, nitrate cannot be recommended to reduce CH₄ emissions because of (a) the risks of nitrate toxicity; (b) it is not feasible to administer nitrate to grazing animals and (c) nitrate is likely to be classified as a feed additive under EU Regulations. Development of products which ensure slow release of nitrate in the rumen may reduce the risks of nitrate toxicity (Pegoraro & Araujo 2012).

Effective strategies 3-nitro oxypropanol (3NOP)

Mode of action: 3NOP is a structural analogue of methyl-coenzyme M which specifically inhibits methyl-coenzyme M reductase, the final step of CH₄ synthesis by archaea (Duin et al. 2016).

FAO recommendation: 3NOP is still under development as a product and there were no published reports available when the FAO study was produced. In all reports published since then using 3NOP there have been significant reductions in CH₄ emissions. There have been no reported adverse effects on performance (live-weight gain, feed conversion efficiency, milk yield, milk quality).

Material: The compound is one of a family of synthetic compounds which have been patented (Duval & Kindermann 2012) for their ability to inhibit CH₄ production

Practical implementation: Optimum dose rates are yet to be established but median dose reported was 106 mg/kg diet DM (approximately 1 (beef cattle) to 2 (dairy cows) g/day). The patent states that (a) the product could be supplied as a premix for incorporation into diets on farm or (b) a bolus delivered into the rumen to release 3NOP over an extended time period and therefore compatible with the grazing situation. Recent information from the manufacturers states that optimum dose rates are established as 60 mg / kg feed for dairy cattle and between 100 and 200 mg / kg feed for beef cattle, depending on the diet.

Potential reduction in CH₄ yield: Reduction in CH₄ for a dose of less than or equal to 111 mg 3NOP/ kg diet DM was 21% but with a large degree of uncertainty (95% confidence interval, 13 – 29%) due to the variation in dose and method of administering 3NOP. Recent information from the manufacturers states that the method for administering 3NOP would appear to be critical and they currently recommend addition to a total mixed ration to ensure coupling of feed intake to intake of inhibitor. When this is applied, at the recommended dose of 60 mg/kg for dairy cattle, the potential decrease in CH₄ production due to 3NOP is 30%.

Health & welfare implications: Toxicological studies are being carried out currently to support an application for registration of 3NOP as a feed additive under EU regulations.

Impact on feed costs: Not known – product still under development.

Likely impact: Product still under development and only likely to be available in medium term (approximately 3 years). However 3NOP has potential to be applicable to both grazing and housed livestock.

4. Discussion

This rapid evidence assessment which has built on the FAO report of 2013 indicates that for nutritional methods of reducing enteric CH₄ emissions, the overall picture has little changed. Inclusion of nitrate in the diet or increased dietary lipid were the only strategies for which there was convincing evidence of efficacy. The main difference since 2013 has been the increasing body of evidence for the efficacy of 3NOP.

Key questions are related to the practical feasibility of widespread on-farm use of these options. For use to be truly widespread then this must include the grazing animal. The daily intakes of lipid and nitrate required for effective mitigation are not compatible with the use of slow release intra-ruminal boluses. Equally, since the intake of nitrates and lipids must be controlled to avoid adverse effects, then the use of feed blocks is also not an option as individual animal intakes from blocks can vary widely. Further in the case of nitrate, grazed grass can contain high concentrations of nitrate and the additive effects of nitrate from grazed and administered nitrate are likely to make issues relating to nitrate toxicity worse. 3NOP has the potential to be administered as a bolus because daily intakes are likely to be 1-2 g for cattle. Thus the mitigation strategies which have proven efficacy do not currently provide a route by which CH₄ emissions from grazing can be reduced.

Nitrate was the most consistent and quantitatively effective strategy for reducing CH₄ emissions. However, first, nitrate is not an accepted feed ingredient and so is likely to be classed as a feed additive under EU Regulations and so cannot be legally included in animal feeds at present. Although it is likely that protocols can be established to minimize risks of nitrate toxicity, the adverse consequences of isolated occurrences of misuse of nitrate for animal health are high and thus the risk to acceptance of mitigation strategies by the agricultural industry considerable. For these reasons, currently nitrate should not be recommended as a practical mitigation strategy. Since 3NOP is currently only at the development stage as a product, until its characteristics are better defined and approval for use as a feed additive obtained then of the three nutritional options only increasing the lipid content of the diet is currently viable.

Three types of feed have been used as sources of lipid (Table 3). The maximum inclusion for any in the diet is limited by the need to keep lipid below 70 g/kg DM to avoid adverse effects on digestion. There is also the need to avoid feeding excess protein and potentially exchanging enteric CH₄ emissions for manure GHG emissions. The main source of distiller's dark grains with solubles (DDGS) in the UK is from bio-ethanol plants which use wheat as the main feedstock. Barley DDGS are mainly produced as a by-product of the whisky industry and while small amounts of maize DDGS are produced in Scotland, most is imported. The amounts of DDGS which can be fed are limited by the low lipid to protein ratio in these feeds and the need to avoid over-feeding protein.

Table 3. Characteristics of lipid-containing feeds (g/kg DM)

Feed	CP	Lipid	Lipid to CP ratio
Protected fat	0	850	NA
Crushed rapeseed	215	485	2.2
DDGS			
Maize	310	108	0.35
Barley	265	85	0.32
Wheat	340	70	0.21

The above discussion and indeed the entire document have to date considered each mitigation strategy as independent. In fact, limited evidence from SRUC and other studies (Klop et al. 2016) does support the conclusion that the responses obtained by combining individual mitigation strategies are additive. Therefore, risks associated with individual strategies may be reduced by combining lower inclusion rates of individual strategies.

In general, studies measuring enteric CH₄ emissions have focussed on CH₄ emissions per se and have not identified consequences for (a) animal performance or (b) other sources of GHG, most importantly manure emissions. This explains why CH₄ emissions have been expressed as CH₄ yields (per kg DMI) and not as an EI. However, the effects of mitigation strategies on animal performance were recorded where available. Overall, for the three selected strategies, albeit with limited evidence in some cases, the effects of these strategies on animal performance have been neutral. Thus reductions in CH₄ yield are likely to be a good proxy for EI. Diets in Annex 3 were formulated to be iso-nitrogenous when nitrate and protein-containing lipid sources were included so that any changes in manure nitrogen output were minimized. For nitrate where nitrogen excretion has been measured, there was no evidence for increased excretion. In particular, nitrate excretion was only a small proportion of nitrate intake (Lee et al. 2015) and there were no increases in nitrate in animal tissues including meat.

In addressing the remit of this evidence assessment, it is apparent that only a few strategies have both a sound evidence base and are immediately applicable particularly in the grazing situation. Hristov et al (2013) in compiling the FAO report classified mitigation strategies under three headings: enteric CH₄, manure management and animal husbandry. Animal husbandry included animal genetics, feeding, reproduction and health. The impact of animal disease and manure management on GHG emissions are subjects of other rapid evidence assessments (Skuce et al., 2016). Genetic selection of animals with lower CH₄ emissions is feasible but is a longer term solution. Selection for improved feed efficiency whether the output is milk or meat is possible now and will lead to a reduction in EI. Improved fertility can lead to a reduction in EI, for example, through reductions in the number of replacement animals required to maintain herd/flock size and / or the number of days an individual female is non-productive (neither pregnant or lactating). In both cases, improvements in fertility reduce the number of days when CH₄ emissions are produced from feed used solely to maintain the animal.

Other possible nutritional strategies which may lead to reductions in EI are:

- Increasing the proportion of concentrate in the diet. On Scottish diets, a high-concentrate (90% concentrate) reduces enteric CH₄ yield to 0.67 of that from a diet containing 50% concentrates (Troy et al. 2015). Responses to increased concentrate feeding are variable and care must be taken that more digestible organic matter is not excreted in faeces (starch) thus increasing GHG emissions from manure.
- Increasing forage quality (through increased digestibility) can increase animal performance and thereby reduce EI by reducing for example the days taken to finish cattle and CH₄ emissions assigned to the maintenance function over the entire finishing period. Improving grazing management to increase the nutritive value of grass on offer will have similar effects.
- Increasing the proportion of maize silage in a grass silage based-diet can reduce emissions (Hammond et al. 2015) through a combination of increased performance and reduced CH₄ yield. However, the effect of whole-crop silages in this context is less clear. There is some evidence that replacing grass silage with legume silage may also reduce EI.
- Precision feeding and assessment of livestock can reduce EI by more accurate matching individual animal requirements to feed supply (e.g. by more frequent and accurate analysis of forage quality), by identification of animals with sub-clinical disease states, and identification of animals at the optimum time for slaughter.

Many of the above strategies which improve animal productivity have the potential for win-win situations, both improving profitability and reducing EI.

5. Policy implications

When considering whether strategies should be implemented and in what order, several factors come in to play: the economic impact of strategies; likelihood of uptake; risks and barriers associated with each strategy and overall effectiveness of each strategy. Of the 12 strategies originally considered, 9 have not been discussed in detail as there was no good evidence for efficacy. Further, some strategies were not considered here as they were either known ozone-depleting agents, not permitted in the EU, or the materials tested were heterogeneous and so there was no evidence for specific materials (e.g. essential oils and garlic).

Considering the three strategies which were examined in detail, there are barriers to uptake for two of them given their likely status under EU Regulations. Both 3NOP and probably nitrate are classified as feed additives and therefore require successful submission of dossiers to gain acceptance as permitted feed additives. In the case of 3NOP, this process is underway but likely to take several years. The status of nitrate is unknown but since it is a generic material, then it is unlikely that a feed manufacturer will take on the financial burden of gaining approval; it is more likely that a differentiated product with specific properties may be submitted for approval.

The likelihood of uptake by farmers must also be considered. If mitigation strategies have no production (economic) benefit and are not mandatory or supported by government then uptake is unlikely. Lipids are the most likely to be favourably viewed as lipid-containing feeds

Nutritional strategies to reduce enteric greenhouse gas emissions

are currently used in practice as long as they can be economically substituted for other feeds (e.g. DDGS for rapeseed meal) or there is a possible production benefit (protected fats in dairy cows).

A further consideration is the risk associated with the strategies. This is a particular concern with nitrate. Although it is likely that the risks associated with nitrate toxicity can be minimized by gradual introduction of nitrate to the diet, there is the risk that an isolated misuse of nitrate and consequent animal deaths will have an adverse effect on acceptance of policy out of proportion to the actual event.

The overall benefits in reducing GHG emissions from use of nutritional strategies need to be considered. The difficulties in using nutritional strategies in the grazing situation have been noted and therefore any reduction in CH₄ emissions achieved by animals receiving the strategy will be diluted by grazing animals not receiving the strategy. The benefit of nutritional strategies must therefore be ranked against other strategies which may have win-win benefits (improvement in profitability and reduction in GHG) which are applicable across the production spectrum.

6. Recommendations

- Discuss implementation of mitigation strategies with industry stakeholders.
- Assess the net benefit of strategies in relation to availability of feed resources and the livestock sectors and systems in which strategies could be implemented.
- Consider in more detail the extent to which emissions swapping may occur with strategies
- Appraise the cost-benefit of nutritional mitigation strategies against other strategies such as improvements in animal health and fertility.

7. References

- Czerkawski JW 1986. An introduction to Rumen Studies. Oxford: Pergamon Press.
- Duin EC, Wagner T, Shima S, Prakash D, Cronin B, Yanez-Ruiz DR, Duval D, Rumbeli R, Stemmler RT, Thauer RK & Kindermann M 2016. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proceedings of the National Academy of Sciences of the United States of America* 113, 6172-6177.
- Duval D & Kindermann M 2012. Use of nitrooxy organic molecules in feed for reducing methane emission in ruminants, and/or to improve ruminant performance. Patent No. WO2012084629 <https://www.google.com/patents/WO2012084629A1> (last accessed 26 SEPTEMBER 2016)
- Hammond KJ, Humphries DJ, Crompton LA, Kirton P & Reynolds CK 2015. Effects of forage source and extruded linseed supplementation on methane emissions from growing dairy cattle of differing body weights. *Journal of Dairy Science* 98, 8066-8077.
- Hristov AN, Oh J, Lee C, Meinen R, Montes F, Ott T, Firkins J, Rotz A, Dell C, Adesogan A, Yang W, Tricarico J, Kebreab E, Waghorn G, Dijkstra J & Oosting S 2013. Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. (Ed Gerber PJ, Henderson B & Makkar HPS) FAO Animal Production and Health Paper No. 177. FAO, Rome, Italy.
- Johnson KA and Johnson DE 1995. Methane emissions from cattle. *Journal of Animal Science* 73, 2483–2492.
- Klop G, Hatew B, Bannink A & Dijkstra J 2016. Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science* 99, 1161-1172
- Lee C & Beauchemin KA 2014. A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance. *Canadian Journal of Animal Science* 94, 557-570
- Lee C, Araujo R, Koenig K & Beauchemin K 2015. Effects of encapsulated nitrate on enteric methane production and nitrogen and energy utilization in beef heifers. *Journal of Animal Science* 93, 2391-2404
- Pegoraro AH & Araujo RC 2012. Use of encapsulated nitrates and sulfates to reduce methane emission derived from ruminal fermentation. Patent No. WO2012159186. <http://www.google.com/patents/WO2012159186A1>. (last accessed 26 SEPTEMBER 2016)
- Scotland Greenhouse Gas Emissions 2014 (available at <http://www.gov.scot/Publications/2016/06/2307/0>) (last accessed 26 SEPTEMBER 2016)
- Skuce PJ, Bartley DJ, Zadoks RN & MacLeod M 2016 Livestock Health and Greenhouse Gas Emissions. ClimateXChange. <http://www.climateexchange.org.uk/reducing-emissions/livestock-health-greenhouse-gas-emissions/> (last accessed 26 SEPTEMBER 2016).
- Troy S, Duthie CA, Hyslop J, Roehe R, Ross D, Wallace R, Waterhouse A & Rooke J 2015. Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets. *Journal of Animal Science* 93, 1815-1823.

Annex 1: Updated nutritional mitigation strategies as classified by FAO (Hristov et al. 2013) updated

Strategy	FAO (2013)	This project	Updated conclusion	Annex 2
1.0 Inhibitors				
1.1 Bromochloromethane	Y	N	Bromochloromethane might be an effective inhibitor but as an ozone-depleting compound, it is not considered here.	N
1.2 Nitro oxypropanol	N	Y	Novel inhibitor of CH ₄ production by archaea with clear evidence of efficacy.	2.1
2.0 Electron receptors				
2.1 Nitrate	Y	Y	Nitrates are promising mitigation agents: confirmed in this project. Critically important animals are properly adapted to avoid nitrate toxicity at high inclusion rates	2.2
2.2 Fumaric acid	Y	Y	Fumaric acid may reduce CH ₄ production when applied in large quantities, but most results indicate no mitigating effect; confirmed in this project.	2.3
2.3 Sulphate	Y	N	May reduce CH ₄ production but no recent studies and evidence for efficacy sparse; may be toxic	N
3.0 Ionophores				
3.1 Monensin	Y	Y	May reduce CH ₄ yield through increased efficiency but effect variable; confirmed in recent studies. Banned in EU	2.4
4.0 Plant bioactive compounds				
4.1 Tannins	Y	Y	Tannins may reduce CH ₄ but no long term studies. Recent studies have included long term studies but variable results and range of different tannins used preclude recommendation	2.5
4.2 Saponins	Y	Y	Tea saponins seem to have potential to reduce CH ₄ . Recent studies indicate no response nor any evidence for long term efficacy	2.6
4.3 Essential oils	Y	y	Most essential oils do not reduce CH ₄ production and long-term effects not established. Recent studies show no effect of essential oils	2.7
5.0 Dietary lipids	Y	Y	Lipids are effective in reducing enteric CH ₄ emission, but the feasibility of use depends on cost-effectiveness and potential effects on feed intake and productivity. Confirmed in this project which focussed on Scotland relevant lipid sources	2.8
6.0 Exogenous enzymes	Y	N	Inconsistencies in data mean that exogenous enzymes cannot be recommended. Limited evidence in this project confirms conclusion	N
7.0 Direct fed microbials (inoculants)	Y	Y	Insufficient evidence for a direct mitigating effect of yeast and other microbials. Recent studies have not increased evidence base and no long term studies	2.9

Annex 2: Strategy specific evidence

For each of the mitigation strategies listed in Annex 1, the expert opinions reported by the FAO (Hristov et al. 2013) were re-examined and updated from published studies from the period 2006 – 2016 to ensure overlap with the 900 references reviewed in the FAO study. The studies identified were subjected to quality control to ensure relevance, adequacy of study design and that an appropriate method of measuring CH₄ was used.

For each strategy listed in Annex 1 and evaluated by FAO, only those where additional information was available are described in more detail in Annex 2. Thus, bromochloromethane, sulphate and exogenous enzymes were excluded. The exception is 3NOP which has been introduced since the FAO report and is included in Annex 2.

Information was compiled for all strategies on:

- Description of material and mode of action.
- Summary of FAO recommendation.
- Brief description of recent evidence
- Conclusion concerning efficacy and likely utility on Scottish farms
- References

For those strategies for which there was good evidence for efficacy more detailed information was included on:

- Any health and welfare implications of employing the strategy
- Practical issues of implementing strategy on Scottish farms
- An estimate of the likely reduction in CH₄ emissions achievable in practice.
- Consequence of employing strategy for other GHG gases and environmental concerns.
- GHG emissions and environmental considerations associated with the production of material.
- Implications for diet costs of implementing strategies on farm. A list of references used as evidence is provided for each strategy.

2.1 3NOP (3-nitro oxypropanol)

Material: The compound is one of a family of synthetic compounds which have been patented for their ability to inhibit CH₄ synthesis.

Mode of action: 3NOP is a structural analogue of methyl-coenzyme M which specifically inhibits methyl-coenzyme M reductase, the final step of methanogenesis (Duin et al. 2016))

FAO recommendation: 3NOP is still under development as a product and there were no published reports available when the FAO study was published.

Recent evidence: There have been 7 published reports using 3-NOP in the last 3 years. The product has been fed at different dosages to sheep, dairy and beef cattle in a range of production systems. The studies include extended feeding periods (over 200 days) and production responses have been measured.

Efficacy: There have been significant reductions in CH₄ emissions in every study reported. At higher dose rates there is some evidence of reduced feed intake and diet digestibility in some studies. There have been no reported adverse effects on performance (live-weight gain, feed conversion efficiency, milk yield, milk quality).

Health & welfare implications: Toxicological studies are being carried out currently to support an application for registration of 3NOP as a feed additive under EU regulations.

Practical implementation: The optimum dose rates are yet to be established but the median dose in studies reported was 106 mg/kg diet DM. Therefore, the product could be supplied as a premix for incorporation into diets on farm. The daily dose is small enough that it may be practicable to administer the compound as a bolus into the rumen to release 3NOP over an extended time period and therefore compatible with the grazing situation. Current view of the manufacturers is that optimum dose rates will be 60 mg / kg in feed for dairy cows and 100 – 200 mg / kg diet for beef cattle

Potential reduction in CH₄ emissions: The mean reduction in published studies where 3NOP has been administered at 111 mg / kg diet DM or less was 21% but with a large degree of uncertainty (95% confidence interval, 13 – 29%) due to the variation in dose and method of administering 3NOP. Current view of the manufacturers is that the method for administration would appear to be critical and they currently recommend dosing of a total mixed ration to ensure coupling of feed intake to intake of CH₄ inhibitor. When this is applied, at the recommended dose of 60 mg/kg for dairy cattle, the decrease in CH₄ production due to 3NOP would be 30%. Extreme diets, such as finishing diets for beef cattle (composed mainly of cereals, with little fibre) seem to influence the response to 3NOP supplementation and large variations have been observed for various doses, ranging from 10 to 80% CH₄ reduction.

Other climate change impacts: Not known.

GHG emissions associated with production of material: Not known

Impact on feed costs: Not known – product still under development.

References:

Duin E. C. et al. (2016). Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proceedings of the National Academy of Sciences of the United States of America* 113, 6172-6177.

Haisan, J., et al. (2014). The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. *Journal of Dairy Science* 97:3110-19.

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Hristov, A. N., et al. (2015) An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. *Proceedings of the National Academy of Sciences of the United States of America* 112:10663-68

Lopes, J. C., et al. (2016) Effect of 3-nitrooxypropanol on methane and hydrogen emissions, methane isotopic signature, and ruminal fermentation in dairy cows. *Journal of Dairy Science* 99:5335-44

Martinez-Fernandez, G., et al. (2014) Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. *Journal of Dairy Science* 97:3790-99

Reynolds, C. K., et al. (2014) Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. *Journal of Dairy Science* 97:3777-89

Romero-Perez, A., et al. (2014) The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. *Journal of Animal Science* 92:4682-93

Romero-Perez, A., et al. (2015) Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. *Journal of Animal Science* 93:1780-91

Vyas, D., et al. (2016) Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. *Journal of Animal Science* 94:2024-34

2.2 Nitrate

Material: Nitrates are present as sodium, ammonium or calcium inorganic salts in fertilizers. In assessment of their potential to reduce CH₄ emissions, a fertiliser (Calcinit) in which nitrate is presented as a calcium ammonium nitrate has most commonly used as the nitrate source.

Mode of action: Nitrate is an electron acceptor. Nitrate is reduced progressively to nitrite and ammonia by rumen micro-organisms that possess the necessary enzymes. The reduction of nitrate to ammonia consumes hydrogen and yields more energy than reduction of carbon dioxide to CH₄. Thus hydrogen is utilized for nitrate reduction rather than for CH₄ formation. If nitrate reduction is complete, then for 1 mole nitrate (62 g) fed there is a reduction of 1 mole (16 g) in CH₄ emissions.



FAO recommendation: “Overall, nitrates may be promising enteric CH₄ mitigation agents. When nitrates are used, it is critically important that the animals are properly adapted to avoid nitrite toxicity. More in vivo studies are needed to fully understand the impact of nitrate supplementation on whole-farm GHG emissions (animal, manure storage and manure-amended soil), animal production and animal health”.

Recent evidence: A large number of studies (18) have been reported. The studies were diverse including sheep and dairy and beef cattle fed a range of forages for periods lasting from 28 to more than 90 days. Production responses were reported in 8 (of 18) studies.

Efficacy: In all but two studies there was a significant reduction in CH₄ emissions quantified as g/kg DMI. In only one study (albeit under Scottish conditions) was there an adverse effect on animal performance. Therefore nitrate is confirmed as an effective and consistent means of reducing CH₄ emissions.

Health & welfare implications: The main practical issue is nitrate toxicity, particularly in animals not or incorrectly adapted to nitrate. Nitrite, an intermediate product in the reduction of nitrate to ammonia can accumulate in the rumen because the rate of nitrate reduction is faster than that of nitrite reduction. The nitrite is then absorbed into the bloodstream and converts haemoglobin to met-haemoglobin (Met-Hb). Met-Hb cannot transport oxygen and thus acute symptoms of nitrate toxicity are anoxia leading to death. Subacute or chronic effects are reported to include retarded growth, lowered milk production and increased susceptibility to infection. Most studies have reported no adverse effects of feeding nitrate although individual animals display elevated (but sub-toxic) concentrations of Met-Hb. In experience from SRUC research, these elevated Met-Hb concentrations were animal-specific and persisted for at least 10 weeks. In some experiments animals have had to be removed from trials because of toxicity although this has not been encountered in SRUC trials.

Practical implementation:

There are few studies which have used grass and cereal silages as the main forages. However, the studies which best reflected Scottish conditions were broadly in line with the above conclusion about efficacy and mitigation potential. The main practical barrier to use of nitrate is the risk of nitrate toxicity. Critically, animals must be adapted by gradually increasing the amounts of nitrate fed. Rapid consumption of large amounts of nitrate should be avoided. Importantly, there is no standard protocol for adapting animals to nitrate. Nitrate must be thoroughly mixed and diluted by other feed constituents. This is probably best achieved by the use of total mixed rations or by inclusion of nitrate in pelleted compound feeds. The risks associated with (a) inadvertent inclusion of excess nitrate in feeds and (b) access of unadapted animals to nitrate-containing feed are high. Nitrate has been ineffective when included in intensive finishing beef diets and should not be used this situation.

Potential reduction in CH₄ emissions: Over all in recent studies a mean reduction of 21% (95% confidence interval, 18% -23%) was achieved in CH₄ yield (g/kg DMI) for a mean dose of 21 g nitrate / kg diet DM.

Other climate change impacts: The mean reduction in CH₄ (above, 21%) is equivalent to 75% of the nitrate fed being reduced to ammonia. Concerns are that the 25% of nitrate not accounted for may be excreted in urine and potentially contribute to nitrous oxide production from manure. In studies where nitrogen excretion has been measured there is no evidence for excretion of significant quantities of nitrate or overall increases in nitrogen excretion. However, as the reduction of nitrate to ammonia is a component of both the dissimilatory and assimilatory routes of nitrate metabolism, then nitrogen gas and nitrous oxide are possible products of nitrate reduction in the rumen. Nitrous oxide production has been rarely measured but in one study in which nitrate was fed, nitrous oxide was produced in the rumen and the GHG benefits of nitrate feeding were reduced by 15% when nitrous oxide was accounted for.

GHG emissions etc. associated with production of material: Relative to a baseline diet, emissions associated with diets including nitrate were similar.

Impact on feed costs: Source dependant but for indicative diets, costs ranged from 96 to 99% of reference diets (Annex 3).

References:

de Raphelis-Soissan, V., et al. (2014) Use of nitrate and *Propionibacterium acidipropionici* to reduce methane emissions and increase wool growth of Merino sheep. *Animal Production Science* 54:1860-66.

El-Zaiat, H. M., et al. (2014) Encapsulated nitrate and cashew nut shell liquid on blood and rumen constituents, methane emission, and growth performance of lambs. *Journal of Animal Science* 92:2214-24.

Guyader, J., et al. (2015) Nitrate but not tea saponin feed additives decreased enteric methane emissions in nonlactating cows. *Journal of Animal Science* 93:5367-77.

- Guyader, J., et al. (2015) Additive methane-mitigating effect between linseed oil and nitrate fed to cattle. *Journal of Animal Science* 93:3564-77.
- Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.
- Hulshof, R. B. A., et al. (2012) Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. *Journal of Animal Science* 90:2317-23.
- Klop, G., et al. (2016) Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science* 99:1161-72.
- Lee C & Beauchemin KA (2014) A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance. *Canadian Journal of Animal Science* 94, 557-570
- Lee, C., et al. (2015) Effects of feed consumption rate of beef cattle offered a diet supplemented with nitrate ad libitum or restrictively on potential toxicity of nitrate. *Journal of Animal Science* 93:4956-66.
- Li, L., et al. (2012) An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. *Animal Production Science* 52:653-58.
- Li, L., et al. (2013) Effect of added dietary nitrate and elemental sulfur on wool growth and methane emission of Merino lambs. *Animal Production Science* 53:1195-201.
- Newbold, J. R., et al. (2014) The effect of incremental levels of dietary nitrate on methane emissions in Holstein steers and performance in Nelore bulls. *Journal of Animal Science* 92:5032-40.
- Nguyen, S. H., M. C. Barnett, and R. S. Hegarty. (2016) Use of dietary nitrate to increase productivity and reduce methane production of defaunated and faunated lambs consuming protein-deficient chaff. *Animal Production Science* 56:290-97.
- Nolan, J. V., et al. (2010) Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. *Animal Production Science* 50:801-06.
- Troy, S. M., et al. (2015) Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets. *Journal of Animal Science* 93:1815-23.
- Troy, S. M., et al. (2016). The effects of dietary nitrate addition and increased lipid concentration on methane (CH₄) and hydrogen (H₂) emissions from beef cattle are independent. *Advances in Animal Biosciences*, 7, 50
- van Zijderveld, S. M., et al. (2010) Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. *Journal of Dairy Science* 93:5856-66.
- van Zijderveld, S. M., et al. (2011) Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. *Journal of Dairy Science* 94:4028-38.
- Velazco, J. I., D. J. Cottle, and R. S. Hegarty. (2014) Methane emissions and feeding behaviour of feedlot cattle supplemented with nitrate or urea. *Animal Production Science* 54:1737-40.
- Veneman, J. B., et al. (2015) Does Dietary Mitigation of Enteric Methane Production Affect Rumen Function and Animal Productivity in Dairy Cows? *Plos One* 10

2.3 Fumaric acid

Material: Fumaric acid is an organic acid and is an intermediate product of the Krebs cycle (a biochemical reaction which occurs in aerobic organisms and generates energy from carbohydrates, lipids and proteins).

Mode of action: Fumarate is an electron acceptor. Fumarate is reduced by hydrogen in the rumen to produce succinate; succinate is then converted to propionate which is a good energy source for the animal. Thus hydrogen is utilized for fumaric acid reduction rather than for CH₄ formation. If fumarate reduction is complete, then for one mole fumaric acid (116 g) fed there is a reduction of one mole (16 g) in CH₄ emissions.

FAO recommendation: “Fumaric (and malic acids) may reduce CH₄ production when applied in large quantities, but most results indicate no mitigating effect. The long-term effects of these compounds have not been established and cost is likely to prohibit their applicability.”

Recent evidence: Five recent studies have been reported. The studies included sheep, beef cattle and goats fed on a variety of forages for periods lasting from 21 to 56 days. Production responses were reported in two of these five studies.

Efficacy and likely utility on Scottish farms: There was a significant reduction in CH₄ emissions in only two of the five recent studies, one of which used large quantities of fumaric acid. Overall, the FAO recommendation is supported and at levels of inclusion where effective, the cost of fumaric acid as a feed additive precludes use.

References:

Beauchemin, K. A. and S. M. MCGINN. (2006) Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. *Journal of Animal Science* 84:1489-96

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Molano, G., T. W. Knight, and H. Clark. (2008) Fumaric acid supplements have no effect on methane emissions per unit of feed intake in wether lambs. *Australian Journal of Experimental Agriculture* 48:165-68

van Zijderveld, S. M., et al. (2011) Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. *Journal of Dairy Science* 94:3094-104.

Wood, T. A., et al. (2009) Encapsulated fumaric acid as a feed ingredient to decrease ruminal methane emissions. *Animal Feed Science and Technology* 152:62-71.

Yang, C. J., et al. (2012) Effect of disodium fumarate on microbial abundance, ruminal fermentation and methane emission in goats under different forage: concentrate ratios. *Animal* 6:1788-94.

2.4 Monensin

Material: Monensin is an ionophore antibiotic. These are naturally occurring, lipid soluble substances which are products of microbial fermentation.

Mode of action: Monensin acts to change the end products of fermentation of certain ruminal microbes. It does this by favouring the production of propionate, a preferential energy source, over acetate. While hydrogen is produced in the formation of acetate, it is consumed in the formation of propionate and so a reduction in the formation of acetate could lead to a reduction in CH₄ emissions.

FAO recommendation: “Our conclusion is that ionophores, through their effect on feed efficiency and reduction in CH₄ per unit of feed, would likely have a moderate CH₄ mitigating effect in ruminants fed high grain or mixed grain-forage diets. The effect is dose-, feed intake-, and diet composition dependent. The effect is less consistent in ruminants that are mainly fed pasture.”

Recent evidence: There have been 8 recent studies reported. These studies were all conducted on cattle fed a variety of forages for periods lasting from 26 to 200 days. Production response was measured in 6 of these studies.

Efficacy and likely utility on Scottish farms: In 2 of these studies there was a significant reduction in CH₄ yield (g/kg DMI). The result was dose dependant. A further study had a significant absolute reduction but DMI was not measured. These recent studies do not disagree with the FAO recommendation. Monensin is currently banned for use in the EU.

Potential reduction in CH₄ emissions: Over all in recent studies a mean reduction of 7% (95% confidence interval, 0.8 – 14.4%) was achieved in CH₄ yield (g/kg DMI) for a mean dose of 0.03 g monensin /kg diet DM.

References:

Benchaar, C. (2016) Diet supplementation with cinnamon oil, cinnamaldehyde, or monensin does not reduce enteric methane production of dairy cows. *Animal* 10:418-25.

da Fonseca, M. P., et al. (2016) Intake, apparent digestibility, and methane emission in bulls receiving a feed supplement of monensin, virginiamycin, or a combination. *Animal Production Science* 56:1041-45.

Grainger, C., et al. (2008) Use of monensin controlled-release capsules to reduce methane emissions and improve milk production of dairy cows offered pasture supplemented with grain. *Journal of Dairy Science* 91:1159-65.

Grainger, C., et al. (2010) A high dose of monensin does not reduce methane emissions of dairy cows offered pasture supplemented with grain. *Journal of Dairy Science* 93:5300-08.

Guan, H., et al. (2006) Efficacy of ionophores in cattle diets for mitigation of enteric methane. *Journal of Animal Science* 84:1896-906.

Nutritional strategies to reduce enteric greenhouse gas emissions

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. Animal Production and Health Paper No. 177. FAO, Rome, Italy.

Odongo, N. E., et al. (2007) Long-term effects of feeding monensin on methane production in lactating dairy cows. *Journal of Dairy Science* 90:1781-88.

Tomkins, N. W., et al. (2015) Manipulating rumen fermentation and methanogenesis using an essential oil and monensin in beef cattle fed a tropical grass hay. *Animal Feed Science and Technology* 200:25-34.

Waghorn, G. C., et al. (2008) Monensin controlled-release capsules for methane mitigation in pasture-fed dairy cows. *Australian Journal of Experimental Agriculture* 48:65-68.

2.5 Tannins

Material: Tannins are natural phenolic compounds found in plants. Plants which contain higher tannin contents have lower nutritional value. Tannins can be added to the diet by inclusion of the plant as a forage or as a plant extract.

Mode of action: Tannins bind with proteins in the rumen thus reducing the utilisation of protein by microbes. Hydrolysable tannins tend to act by directly inhibiting rumen methanogens, while the effect of condensed tannins on rumen CH₄ production is more through inhibition of fibre digestion.

FAO recommendation: “In conclusion, hydrolysable and condensed tannins are plant bioactive components that may offer an opportunity to reduce enteric CH₄ production, although intake and animal production may be compromised. The agronomic characteristics of tanniferous forages must be considered when they are discussed as a GHG mitigation option”

Recent evidence: There have been five recent studies where sheep and goats have been supplemented with tannins. These included feeding tannin rich forages or tannin extracts for periods lasting from 19 to 190 days. Production response was only measured in two of these studies.

Efficacy and likely utility on Scottish farms: A significant reduction in CH₄ yield (g/kg DMI) was observed in only one study where the tannin source was a tropical legume. Therefore, there is no recent evidence to support the use of tannins especially as a variety of tannin sources were tested. Mean reduction in CH₄ (g/kg DMI) was 8% but very variable (95% confidence interval, 0.2% to 25%). In addition, supplementation with tannins may impair protein utilization, reducing animal performance.

References:

Animut, G., et al. (2008) Methane emission by goats consuming different sources of condensed tannins. *Animal Feed Science and Technology* 144:228-41.

Guyader, J., et al. (2015) Nitrate but not tea saponin feed additives decreased enteric methane emissions in nonlactating cows. *Journal of Animal Science* 93:5367-77.

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Liu, H., V. Vaddella, and D. Zhou. (2011) Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. *Journal of Dairy Science* 94:6069-77.

Moreira, G. D., et al. (2013) Tropical tanniferous legumes used as an option to mitigate sheep enteric methane emission. *Tropical Animal Health and Production* 45:879-82.

Ramirez-Restrepo, C. A., et al. (2016) Methane production, fermentation characteristics, and microbial profiles in the rumen of tropical cattle fed tea seed saponin supplementation. *Animal Feed Science and Technology* 216:58-67.

Nutritional strategies to reduce enteric greenhouse gas emissions

Soltan, Y. A., et al. (2013) Contribution of condensed tannins and mimosine to the methane mitigation caused by feeding *Leucaena leucocephala*. *Archives of Animal Nutrition* 67:169-84.

Wischer, G., et al. (2014) Effects of long-term supplementation of chestnut and valonea extracts on methane release, digestibility and nitrogen excretion in sheep. *Animal* 8:938-48.

2.6 Saponins

Material: Saponins are natural compounds found in plants. Saponins have a detergent like quality.

Mode of action: Saponins selectively bind with the cell membranes of ruminal protozoa causing cell death. Methanogenic archaea have a close relationship with ruminal protozoa and so suppression of protozoa by saponins may lead to a reduction in CH₄ production.

FAO recommendation: “Tea saponins seem to have potential, but more and long-term studies are required before they could be recommended for use..”

Recent evidence: There have been only two recent studies using cattle supplemented with tea saponin and fed hay-based diets for periods lasting from 10 to 35 days. Production responses were not measured in either of these studies.

Efficacy and likely utility on Scottish farms: No significant reductions in CH₄ emissions were observed in either of the recent studies. The FAO recommendation remains valid especially as tea saponins would be imported.

References:

Guyader, J., et al. (2015) Nitrate but not tea saponin feed additives decreased enteric methane emissions in nonlactating cows. *Journal of Animal Science* 93:5367-77.

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Ramirez-Restrepo, C. A., et al. (2016) Methane production, fermentation characteristics, and microbial profiles in the rumen of tropical cattle fed tea seed saponin supplementation. *Animal Feed Science and Technology* 216:58-67.

2.7 Essential Oils

Material: Essential oils are natural compounds found in plants.

Mode of action: Essential oils possess anti-microbial properties. The exact mode of action varies between different essential oils but includes disruption of cell membranes and inactivation of microbial enzymes. The modes of action described reduce either the total microbial population in the rumen or the activity of the microbial population thus reducing the production of CH₄.

FAO recommendation: “Most essential oils or their active ingredients do not reduce CH₄ production and, when CH₄ production was reduced *in vivo*, their long-term effects were not established.”

Recent evidence: There have been 6 recent studies on cattle and sheep fed a variety of different forages supplemented with a variety of essential oils. These studies lasted only from 21 to 28 days. Production response was measured in three of these studies.

Efficacy and likely utility on Scottish farms: No significant reductions in CH₄ emissions were observed in any of the recent studies and therefore the above conclusion is supported especially as essential oil sources used were derived from a variety of plant sources.

References:

Benchaar, C. (2016) Diet supplementation with cinnamon oil, cinnamaldehyde, or monensin does not reduce enteric methane production of dairy cows. *Animal* 10:418-25.

Benchaar, C., F. Hassanat, and H. V. Petit. (2015) Dose-response to eugenol supplementation to dairy cow diets: Methane production, N excretion, ruminal fermentation, nutrient digestibility, milk production, and milk fatty acid profile. *Animal Feed Science and Technology* 209:51-59.

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Klevenhusen, F., et al. (2011) Diallyl disulphide and lovastatin: effects on energy and protein utilisation in, as well as methane emission from, sheep. *Archives of Animal Nutrition* 65:255-66.

Klevenhusen, F., et al. (2011) Garlic oil and its principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. *Animal Feed Science and Technology* 166:356-63.

Meale, S. J., et al. (2014) Including essential oils in lactating dairy cow diets: effects on methane emissions. *Animal Production Science* 54:1215-18.

Tomkins, N. W., et al. (2015) Manipulating rumen fermentation and methanogenesis using an essential oil and monensin in beef cattle fed a tropical grass hay. *Animal Feed Science and Technology* 200:25-34.

2.8 Dietary lipid

Material: All commonly used feeding-stuffs contain lipid and diets typically contain 30 – 40 g lipid /kg diet DM. Feeds commonly used to increase dietary lipid content can be grouped as extracted oils (usually from oilseeds), oilseeds (with or without some processing) or by-products of either oil extraction (which contain residual oil) or starch extraction and / or fermentation (in which endogenous lipid is concentrated).

Mode of action: Lipids reduce CH₄ emissions in a multi-factorial manner. The fatty acid components of lipids are not fermented in the rumen and therefore increasing dietary fat content reduces carbohydrates available for fermentation and hence CH₄ production. Lipids also have a direct inhibitory effect on rumen micro-organisms and an indirect effect as lipids coat fibre and physically preventing digestion. Finally bio-hydrogenation of unsaturated fatty acids consumes hydrogen and diverts hydrogen from CH₄ formation. The above mechanisms are listed in descending order of importance.

FAO recommendation: “Our conclusion is that lipids are effective in reducing enteric CH₄ emission, but the feasibility of this mitigation practice depends on its cost-effectiveness and potential effects on feed intake (negative), productivity (negative) and milk fat content in lactating animals (positive or negative). High-oil by-product feeds such as distiller’s grains, and meals from the biodiesel industry can serve as cost-effective sources of lipids with potential CH₄ suppressing effect. Their mitigating potential, however, has not been well-established and in some cases CH₄ production may increase due to increased fibre intake.”

Recent evidence: One third (53 of 156) of recent studies tested lipid as a mitigating strategy. Of these, 23 focussed on feeds that were relevant to Scotland and the UK. These feeds were derived from linseed or rapeseed or by-products of the distilling or bio-ethanol industries. The increases in lipid concentration of the diet achieved were greatest for oils and protected fat products, followed by oilseeds and least for by-products, recognizing the lipid concentration in each of these classes. There was more evidence for linseed- than for rapeseed-based materials and for maize DDGS than wheat DDGS. Approximately half the studies reported some aspect of animal production.

Efficacy: The efficacy of lipid in reducing CH₄ yield using feed sources relevant to Scottish agriculture is confirmed although reductions were not always statistically significant. In specific cases where high concentrate diets were fed, increases in emissions were observed.

Health & welfare implications: If excess lipid is included in the diet, it has long been recognized that adverse effects upon rumen fibre digestion and feed intake and thus on performance are likely. Thus in the recent studies, the inclusion of lipid was limited to a maximum of 70 g/kg DM. Reductions in intake and performance were observed in studies where lipid intake was greater than 70 g/kg DM. It should be noted that as the lipid content of the diet increases, so does the energy concentration of the diet, there are examples where DMI is reduced but animal performance is maintained or even increased.

Practical implementation: This is dependant on feeds used as sources of lipid.

Nutritional strategies to reduce enteric greenhouse gas emissions

1. As oils extracted from oilseeds such as rapeseed are intended to enter the human food chain then these oils should not be considered as suitable feeds to mitigate CH₄ emissions.
2. There are specific products in which dietary fat is protected from rumen metabolism for example by treatment with calcium. Feeding these products can reduce CH₄ emissions by decreasing the amount of carbohydrate available for fermentation in the rumen but do so without directly inhibiting the rumen microflora. The primary purpose of protected-fat products has been to increase the energy density of the diet thereby increasing energy intake and consequently milk yield in dairy cows. Products are based on imported palm oil.
3. Rapeseed treated to disrupt the seed coat and release oil in the rumen: crushed or cracked rapeseed. All mechanisms of CH₄ reduction noted above are likely with crushed / cracked rapeseed. However, practical difficulties are availability of equipment to process seed on farm and the risk of rancidity in the crushed / cracked product. Use of intact oilseeds diverts oil away from the human food chain.
4. By-products. Extraction of oil by mechanically treating oilseeds yields a by-product with greater oil concentration (130 g/kg DM) than conventional solvent extracted oilseed meals. Until recently, quantities produced have been small in relation to conventional solvent extracted oilseed meals; however larger quantities are likely to become available. DDGS are a long standing by product of the whisky distillation industry. More recently, the establishment of bio-ethanol plants has made available large quantities of wheat DDGS. However, the lipid content of DDGS is determined by base cereal, ranking (from high to low), maize, barley and wheat. Maize DDGs has an oil content of >100 g/kg DM and is most practicable to reduce CH₄ emissions but most is imported into the UK. In general, DDGS have been shown to be effective in reducing CH₄ emissions but this is dependant on the basal diet fed. Where DDGS replaces starch (cereals) in the diet, increased fibre intake from DDGS can lead to increased emissions.

Potential reduction in CH₄ emissions: Previous meta-analyses of the literature have produced relationships relating increases in oil content to reduction in CH₄. The simplest relationship states that for every 10 g /kg DM increase in dietary lipid, CH₄ yield is decreased by 1 g/kg DMI. More complex is the relationship: CH₄ yield (g/kg DM intake) = Exp (3.15 – 0.0035 x dietary lipid (g/kg DM)). In recent studies increasing the dietary lipid content from 30 to 70 g/kg DM would reduce CH₄ emissions by 4 and 2.8 g / kg DM intake if calculated by the above two methods. This compares with the mean observed value of 2.8 g / kg DM observed (95% confidence interval 1.9 – 3.7). Therefore the simple rule of 1 g reduction in CH₄ / kg DMI for every 10 g increment in dietary lipid may be preferred. The mean reduction in percentage terms for the recent studies was 11.2 (95% confidence intervals 8-14) when total dietary lipid was less than 70 g/kg DM. The wide confidence interval reflects the range of materials used and therefore the practically possible inclusion levels.

Other climate change impacts: If formulation guidelines are observed to avoid inclusion of excess dietary protein in diets, then there are unlikely to be important climate change impacts.

GHG emissions associated with production of material: Relative to a baseline diet, emissions associated with diets including protected fat were on a DM basis (kg CO₂e/kg DM)

were no different (protected fat); increased by 2% (crushed rapeseed) or decreased by 8% (maize DDGS).

Impact on feed costs: Source dependant but for indicative diets, costs ranged from 96 to 106% of reference diets (Annex 3).

References:

Alstrup, L., et al. (2015) Effect of fat supplementation and stage of lactation on methane production in dairy cows. *Animal Feed Science and Technology* 207:10-19.

Beauchemin, K. A., et al. (2009) Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. *Journal of Dairy Science* 92:2118-27.

Benchaar, C., et al. (2013) Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. *Journal of Dairy Science* 96:2413-27.

Benchaar, C., et al. (2015) Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. *Journal of Dairy Science* 98:7993-8008.

Brask, M., et al. (2013) Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation. *Animal Feed Science and Technology* 184:67-79.

Brask, M., et al. (2013) Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. *Journal of Dairy Science* 96:2356-65.

Chung, Y. H., et al. (2011) Linseed suppresses enteric methane emissions from cattle fed barley silage, but not from those fed grass hay. *Animal Feed Science and Technology* 166:321-29.

Duthie, C. A., et al. (2015) Methane emissions from two breeds of beef cows offered diets containing barley straw with either grass silage or brewers' grains. *Animal* 9:1680-87.

Duthie, C. A., et al. (2016) Impact of adding nitrate or increasing the lipid content of two contrasting diets on blood methaemoglobin and performance of two breeds of finishing beef steers. *Animal* 10:786-95.

Fiorentini, G., et al. (2014) Effect of lipid sources with different fatty acid profiles on the intake, performance, and methane emissions of feedlot Nellore steers. *Journal of Animal Science* 92:1613-20.

Hales, K. E., N. A. Cole, and J. C. MacDonald. (2013) Effects of increasing concentrations of wet distillers grains with solubles in steam-flaked, corn-based diets on energy metabolism, carbon-nitrogen balance, and methane emissions of cattle. *Journal of Animal Science* 91:819-28.

Hammond, K. J., et al. (2015) Effects of forage source and extruded linseed supplementation on methane emissions from growing dairy cattle of differing body weights. *Journal of Dairy Science* 98:8066-77.

Nutritional strategies to reduce enteric greenhouse gas emissions

Hellwing, A. L. F., et al. (2012) Can rapeseed lower methane emission from heifers? *Acta Agriculturae Scandinavica Section A-Animal Science* 62:259-62.

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Hunerberg, M., et al. (2013) Effect of dried distillers grains plus solubles on enteric methane emissions and nitrogen excretion from growing beef cattle. *Journal of Animal Science* 91:2846-57.

Hunerberg, M., et al. (2013) Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle. *Canadian Journal of Animal Science* 93:373-85.

Livingstone, K. M., et al. (2015) Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows. *Journal of Dairy Science* 98:4000-11.

Martin, C., et al. (2016) Increasing linseed supply in dairy cow diets based on hay or corn silage: Effect on enteric methane emission, rumen microbial fermentation, and digestion. *Journal of Dairy Science* 99:3445-56.

Martin, C., et al. (2008) Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. *Journal of Animal Science* 86:2642-50.

McGinn, S. M., et al. (2009) Use of corn distillers' dried grains to reduce enteric methane loss from beef cattle. *Canadian Journal of Animal Science* 89:409-13.

Pinares-Patino, C. S., et al. (2016) Feed intake and methane emissions from cattle grazing pasture sprayed with canola oil. *Livestock Science* 184:7-12.

Troy, S. M., et al. (2015) Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets. *Journal of Animal Science* 93:1815-23.

2.9 Direct-fed microbials

Material: Direct-fed microbials are cultured yeast colonies or strains of bacteria commonly found in the rumen which divert resources away from the production of CH₄, or increase competition for hydrogen in the rumen.

Mode of action: Direct-fed yeast products mop up oxygen in the rumen creating a more anaerobic environment. Anaerobic conditions are more favourable for the rumen microbial population and so their increased populations due to the introduction of direct-fed yeast should lead to increased digestive efficiency and therefore decreased CH₄ emissions. A further mode of action involves the introduction of *Propionibacterium* species. *Propionibacterium* favour the production of propionate (a favoured energy source), which competes for hydrogen against CH₄ formation.

FAO recommendation: “there is insufficient evidence of the direct enteric CH₄ mitigating effect of yeast and other direct-fed microbials. However, yeasts appear to stabilize pH and promote rumen function, especially in dairy cattle, resulting in small but relatively consistent responses in animal productivity and feed efficiency, which might moderately decrease CH₄ emission intensity.”

Recent evidence: There have been 7 recent studies where sheep and cattle have been supplemented with a variety of different microbial products for periods lasting from 21 to 56 days. Forages were either silage (grass or barley) or grass hay. Production response was measured in three of these studies.

Efficacy and likely utility on Scottish farms: Significant reductions in CH₄ emissions (quantified as g/kg DMI) were observed in only 2 of 7 studies. There were no adverse effects on animal performance. Overall in recent studies a mean reduction of 7.1% (95% confidence interval, 6.5% to 11.7%) was achieved in CH₄ yield (g/kg DMI). However, as microbial preparations were added directly to the rumen, there is still insufficient evidence for direct fed microbials in practical conditions.

References:

Bayat, A. R., et al. (2015) Effect of camelina oil or live yeasts (*Saccharomyces cerevisiae*) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. *Journal of Dairy Science* 98:3166-81.

Chung, Y. H., et al. (2011) Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. *Journal of Dairy Science* 94:2431-39.

de Raphelis-Soissan, V., et al. (2014) Use of nitrate and *Propionibacterium acidipropionici* to reduce methane emissions and increase wool growth of Merino sheep. *Animal Production Science* 54:1860-66.

Hristov, A.N., et al (2013) Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO₂ emissions. Eds Gerber, P.J., Henderson, B. and Makkar, H.P.S. *Animal Production and Health Paper No. 177*. FAO, Rome, Italy.

Nutritional strategies to reduce enteric greenhouse gas emissions

Jeyanathan, J., C. Martin, and D. P. Morgavi. (2016) Screening of bacterial direct-fed microbials for their antimethanogenic potential in vitro and assessment of their effect on ruminal fermentation and microbial profiles in sheep. *Journal of Animal Science* 94:739-50.

Vyas, D., et al. (2016) Enteric methane emissions in response to ruminal inoculation of *Propionibacterium* strains in beef cattle fed a mixed diet. *Animal Production Science* 56:1035-40.

Vyas, D., et al. (2014) Effect of *Propionibacterium* spp. on ruminal fermentation, nutrient digestibility, and methane emissions in beef heifers fed a high-forage diet. *Journal of Animal Science* 92:2192-201.

Vyas, D., et al. (2014) Effects of *Propionibacterium* strains on ruminal fermentation, nutrient digestibility and methane emissions in beef cattle fed a corn grain finishing diet. *Animal* 8:1807-15.

Annex 3: Costs associated with use of nitrate and lipids

Using the SRUC nutritional software, FeedByte, diets were formulated for dairy cows and growing and finishing beef cattle. Baseline diets were formulated with no mitigation strategies and daily cost of the diet calculated. It must be emphasised that the costs shown below are only indicative as changes in costs on the commodities market, actual performance targets for livestock and quality of forage available on individual farms will heavily influence actual costs on any individual farm unit.

Nitrate

Livestock class	Inclusion	Level (g/kg DM)	Cost (£/day)	Change (%)
Dairy cow	-		3.44	
	+	20	3.31	-3.8
Growing cattle	-		0.93	
	+	20	0.92	-1.1
Finishing cattle	-		1.08	
	+	20	1.07	-1.0

Because nitrate (as Calcinit) replaced protein (soya-bean meal for dairy and rapeseed meal for beef cattle), there was a small decrease (2.0%) in diet costs.

Protected fat

Livestock class	Inclusion	Level (g/kg DM)	Cost (£/day)	Change (%)
Dairy cow	-	32	3.44	
	+	52	3.33	-3.2
Growing cattle	-	39	0.93	
	+	69	0.97	+4.0
Finishing cattle	-	39	1.06	
	+	74	1.12	+5.6

While the cost of the diet reduced when protected fat (as Megalac) was added to dairy cows, it increased with growing cattle. This reflects the greater inclusion rate in beef cattle and in relation to other diet constituents, that lipid sources were relatively a more expensive ingredient for beef cattle diets. However, the energy content of the diet (MJ Metabolisable Energy / kg DM) was increased by 0.1 (dairy) and 0.3 (beef) when protected fat was included which partially offsets increased diet costs.

Crushed rapeseed

Livestock class	Inclusion	Level (g/kg DM)	Cost (£/day)	Change (%)
Dairy cow	-	32	3.44	
	+	52	3.41	-0.9
Growing cattle	-	37	0.93	
	+	72	0.95	+2.1
Finishing cattle	-	39	1.06	
	+	70	1.07	+0.1

Including crushed rapeseed had a neutral effect overall on costs. The inclusion rate for dairy cows was deemed the upper practical limit for this material. As for protected fat, any increases in costs will be offset by increases in the Metabolisable Energy content of the diet.

Maize DDGS

Livestock class	Inclusion	Lipid (g/kg DM)	Cost (£/day)	Change (%)
Dairy cow	-	32	3.44	
	+	36	3.22	-5.4
Growing cattle	-	39	0.93	
	+	39	0.89	-3.3
Finishing cattle	-	39	1.06	
	+	42	1.06	0.0

For maize DDGS, the exercise emphasises the modest effect that including DDGS in the diet has on lipid content at the performance levels chosen (35 kg milk /day or 1.0 kg live-weight gain /day). The reduction in feed costs reflects the cost of maize DDGS compared to rapeseed meal. Increasing the performance to 1.5 kg /day, does increase inclusion of dark grains to give 55 g lipid /kg DM but at the expense of increasing dietary protein from 120 to 200 g protein / kg DM which is in excess. In an experiment carried out at SRUC in which rapeseed meal was replaced with maize DDGS (forage was a mixture of grass and whole crop barley silage) an increase in lipid content from 26 to 37 g/kg DM was achieved with an associated increase in feed costs of 2.9% for maize DDGS at feed prices current when the trial was carried out.

Annex 4: Environmental impacts of amending rations

Emissions intensity (EIF) of feed materials and their effect on ration EIF

Method

In order to predict the effect of changes in the diet to the EIF of the ration, the inclusion rates in Table 1 were assumed using the reference dairy cow diet (Annex 3) as a baseline.

Table 1. Dairy cow diet: feed materials g/kg of diet DM

<i>Introduced material</i>	<i>Baseline diet</i>	<i>Adjusted diet</i>	<i>Feed Displaced</i>
Nitrate†	0	20	Rapeseed meal
Protected fat†	0	30	Rapeseed meal
Oilseeds	0	70	Rapeseed meal
Maize DDGS	0	200	Barley

†Calcinit used as nitrate source and Megalac as source of protected fat

A short review was undertaken to determine the EIF of the introduced feed materials (see Table 2).

Points to note:

- The EIF of the feed materials and rations were calculated using GLEAM, and include the following emission categories: direct and indirect N₂O arising during crop production; CO₂ arising from the use of fossil fuels during field work (e.g. tillage) and crop transport and processing; CO₂ from fossil fuel use during the production of non-crop feed materials; CO₂ from fossil fuel use during the production of synthetic N fertiliser; CO₂ from soil carbon loss arising from land use change induced by soya cultivation in Latin America and oil palm cultivation in SE Asia; CO₂ from fossil fuel use during the blending and transportation of compound feed.
- The EIF of a feed material can vary significantly depending on how it is produced and how the EIF is calculated (e.g. which emissions categories are included, method by which emissions are allocated to co-products etc.). In particular, the EIF of protected fat (with Megalac used here as an example) should be treated with caution as the EIF of palm oil (the main ingredient) can vary by more than an order of magnitude depending on the types of land on which it is cultivated.
- The EIF also depends on the functional unit, i.e. whether the emissions are measured per unit DM, CP, lipid or energy etc. For example, feeds with a high fat content (such as rapeseed and Megalac) have high EIF per kg of DM but moderate EIF per MJ of DE.

Results

The effects of introducing the feed materials on diet EIF were calculated assuming the inclusion rates in Table 1, and the results are presented in Table 3. When measured in terms of DM, only the replacement of barley with maize DDGS leads to a marked reduction (8%) in EIF (see Table 4). Maize DDGS and Megalac both lower the EIF per unit of DE.

Nutritional strategies to reduce enteric greenhouse gas emissions

Table 2 EIF of introduced and substituted feed materials

		Barley	Rapeseed meal1	Rapeseed meal2	Maize DDGS 1	Maize DDGS 2	Rapeseed 1	Rapeseed 2	Nitrate	Megalac	Palm oil
Digestible energy	MJ/kg DM	14.8	14.7	14.7		15.1		25.0	0.0	34.8	
Digestible energy	%	90.0	74.8	75.7	78.0	70.9	81.4	86.8	0.0	96.0	
CP content	g/kg DM	116.9	393.8	378.0	237.5	203.0	206.3	209.0	968.8	0.0	
Lipid content	g/kg DM	20.0	27.0	27.0		85.0		460.0	0.0	840.0	
Emissions intensity	kgCO ₂ e/kg DM	0.49	1.04	0.49	0.34	0.30	1.33	1.05	1.24	1.03	1.2 to 5.3
Emissions intensity	kgCO ₂ e/MJ DE	0.03	0.07	0.03	0.02	0.02	0.05	0.04	na	0.03	
Emissions intensity	kgCO ₂ e/kg fat	24.5	38.4	18.1	4.0	3.5	2.9	2.3	na	1.2	
Emissions intensity	kgCO ₂ e/kg CP	4.2	2.6	1.3	1.4	1.5	6.4	5.0	1.3	na	
		<i>Sources</i>									
Digestible energy		2	2	2		2		2	10	6	
Digestible energy		1	1	2	1		1	2		6	
CP content		1	1	4	1	2	1	2	10	10	
Lipid content		2	2	2		2		2		6	
Emissions intensity		1	1	4	1	4	1	4	7	8	9
Emissions intensity		3	3	3	3	3	3	3	3	3	
Emissions intensity		3	3	3	3	3	3	3	3	3	
Emissions intensity		3	3	3	3	3	3	3	3	3	

Sources: 1. GLEAM; 2. Feedipedia; 3. Calculated; 4. Feedprint; 5. Yara (2016); 6. Volac; 7. Vellinga et al. (2013, p67); 8. O'Brien et al. (2014); 9. Based on Carlton (2011, p23); 10. FeedByte

Table 3 EIF of diets for dairy cow, including grazing

Diet		Baseline diet	Rapeseed	Maize DDGS	Megalac	Nitrate
Inclusion (g/kg DM)			70	200	30	20
Gross energy	MJ/kg DM	18.5	19.2	18.9	19.1	18.2
Digestible energy	MJ/kg DM	14.9	15.5	15.0	15.5	14.6
Digestibility	%	80%	81%	79%	81%	79%
Emissions intensity	kgCO ₂ e/kg DM	1.10	1.12	1.01	1.10	1.10
Emissions intensity	kgCO ₂ e/MJ DE	0.073	0.072	0.068	0.071	0.075

Table 4 Change relative to baseline diet

			Rapeseed	Maize DDGS	Megalac	Nitrate
Inclusion (g/kg DM)			70	200	30	20
Gross energy	MJ/kg DM		3%	2%	3%	-2%
Digestible energy	MJ/kg DM		4%	0%	4%	-2%
Digestibility	%		1%	-2%	1%	-2%
Emissions intensity	kgCO ₂ e/kg DM		2%	-8%	0%	0%
Emissions intensity	kgCO ₂ e/MJ DE		-2%	-8%	-4%	2%

Potential non-GHG environmental impacts**Table 5. Summary of impacts of changing diets**

<i>Feed material</i>	<i>Potential environmental impacts</i>
Megalac	Induced land use change in SE Asia, with consequent impacts on biodiversity
Rapeseed	No major effects anticipated.
Maize dark grains	Increase in energy use and potentially non-renewable energy depletion
Nitrate	Increased impacts associated with nitrate manufacture, i.e. non-renewable energy depletion.

Protected fat (Megalac)

Megalac is a rumen protected fat, consisting primarily of palm oil, with the addition of a calcium salt to protect the fat from digestion in the rumen. Depending on how it is produced, palm oil can be a high EIF feed material. Emissions arising from the establishment of oil palm plantations can vary greatly depending on the particular LUC, ranging from extremely high where they are established on cleared peatlands to moderate net sequestration when established on Imperata grassland (Table 6).

Table 6. EIF of palm kernel expeller and palm oil. The palm oil EIF is calculated using the EFA and MFA values in Carlton (2011, p23)

	EIF of Palm Kernel Expeller (mass allocation kgCO ₂ e/ kg DM)	EI of palm oils (kgCO ₂ e / kg DM)	% of palm by plantation type (Carlton 2011, p8)
Peatland forest high	18.22	20.1	15%
Peatland forest low	9.21	10.2	15%
Mineral soil high	1.97	2.2	13%
Mineral soil low	1.13	1.2	13%
Oil palm plantation	0.79	0.9	43%
Imperata grassland	-0.79	-0.9	1%
Weighted average	4.8	5.3	

Using the oil palm production data in the Feedprint database, and the EFA and MFA values in Carlton (2011, p23), gives an EIF for palm oil (including transport and processing) of 1.22 kgCO₂e / kg DM. This is closer to the EI for Megalac cited in O'Brien et al (2014), which give a value 1.03 kg CO₂e / kg DM (based on the Ecoinvent and Feedprint databases (Vellinga et al. 2013).

A proportion of these emissions would be offset by the reduction in enteric CH₄ and the increases in milk yield and cow fertility arising from the inclusion of Megalac in the cow diet. Quantifying this is beyond the current study, as it requires better data on the impacts of Megalac on milk yield and fertility.

Palm oil production can induce LUC, with consequent impacts on biodiversity (Vijay 2016). However the scale of these impacts and the extent to which they are mitigated by sustainable sourcing and initiatives such as the Round Table on Sustainable Palm Oil (RSPO) requires further investigation. Note that Megalac is manufactured by Volac, and “Volac is a member of the Roundtable on Sustainable Palm Oil (RSPO) and our policy is to source palm oil-based ingredients from RSPO members only.”

Rapeseed

HGCA (2005, p4) observed that for soil erosion “risk is generally low for winter-sown cereals and oilseeds compared with spring-sown and row crops, e.g. maize, potatoes and sugar beet”. They also noted that switching from cereal to oilseeds may impact on water quality as “oilseed rape presents a higher risk (of nitrate leaching) due to higher levels of residual fertiliser nitrogen left after harvest”.

The environmental impact of switching from cereals to oilseeds will depend on the specific agronomic and biophysical conditions, for example timing or sowing, mode of tillage, soil type etc. If oilseeds are integrated into crop rotations and good agronomic practice is followed, there should be little net increase in environmental impacts

Maize DDGS

Maize DDGS (like many by-products) can be a low EIF feed. If the emissions arising during crop growing are allocated to the co-products of brewing/distillation on the basis of economic value, then most of the emissions will be allocated to ethanol, rather than the wet grains or stillage. Most of the EIF arises from the use of energy to dry the grains and stillage. Replacing whole grains with maize DDGS therefor reduces the EIF of the diet, and displaces the emissions from soil N₂O to CO₂ from fossil fuel combustion.

Calcium Ammonium Nitrate (CAN)

Increased impacts associated with CAN manufacture, i.e. non-renewable energy depletion.

References

Carlton R (2011) The carbon cost of palm kernel expeller and its contribution to the dairy carbon footprint in New Zealand Auckland: Greenpeace New Zealand

HGCA (2005) Environmental impact of cereals and oilseed rape for food and biofuels in the UK London: HGCA

O’Brien D, Capper JL, Garnsworthy PC, Grainger C & Shalloo L (2014) A case study of the carbon footprint of milk from high-performing confinement and grass-based dairy farms *Journal of Dairy Science* 97, 1835–1851.

Olijhoek DW, Hellwing ALF, Brask M, Weisbjerg MR, Højberg O, Larsen MK, Dijkstra J, Erlandsen EJ & Lund P (2016) Effect of dietary nitrate level on enteric methane production, hydrogen emission, rumen fermentation, and nutrient digestibility in dairy cows *Journal of Dairy Science* 99, 6191-6205.

Vellinga TV, Blonk H, Marinussen M, van Zeist WJ, de Boer LJM & Starmans D (2013) Methodology used in FeedPrint: a tool quantifying greenhouse gas emissions of feed production and utilization Report 674 Lelystad: WUR

Vijay V, Pimm SL, Jenkins CN & Smith SJ (2016) The Impacts of Oil Palm on Recent Deforestation and Biodiversity Loss. *PLoS ONE* 11 (7) e0159668.

Volac (2016) MEGALAC product description, <http://www.volac.com/agriculture/product-by-species/dairy/product1/megalac> (LAST ACCESSED 21 SEPTEMBER 2016)