Feed additives in ruminant nutrition

Can feed additives reduce methane and improve performance in ruminants?

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Summary

There is a growing range of feed additives, aimed for use in ruminant diets, that offer potential to improve rumen fermentation efficiency while reducing methane output. This review aims to determine how effectively those additives on the market and under development are meeting these claims.

Methane production is the dominant mechanism for hydrogen disposal in the rumen, a necessary activity for healthy rumen functioning. Potential opportunities to reduce methane production involve: inhibiting the methanogenic archea and/or the accompanying protozoa, supplying an alternative hydrogen disposal mechanism, encouraging other natural hydrogen disposal routes and slowing fermentation to improve fermentation efficiency.

A recent review completed by Herefordshire University for the European Food Safety Authority and a Food and Agriculture Organisation report on technical options for methane mitigation form the basis of this report. From these, the most promising feed additives were identified and effectiveness discussed.

Plant extracts, in various forms, hold significant potential for reducing methane, including: fatty acids, dietary lipids, essential oils and condensed tannins. Polyunsaturated fatty acids and essential oils are thought to have a similar mode of action to monensin (an ionophore currently prohibited for use in the EU); they favour propionate producing bacteria in the rumen, thereby encouraging another hydrogen sink.

Dietary lipids, or their constituent fatty acids alone, reduce methane emissions but at high intakes they can reduce digestibility and dry matter intake.

Plant saponins are thought to reduce rumen protozoa numbers which can affect the methane producing bacteria and slow down protein turnover in the rumen, increasing transport of microbial nitrogen to the duodenum.

Garlic oil is the essential oil with most supporting evidence for methane reduction, but effects on performance are variable.

A promising group of chemical additives are electron receptors. Nitrate is an electron receptor that acts as an alternative hydrogen sink to methane, but if used in high protein diets, excess ammonia production is likely. Nitrate toxicity is a risk to ruminants, but this can be overcome with gradual introduction of nitrate to the diet.
Overall, most additives require further long term studies in the live ruminant to determine how effective they are in commercial systems. To be sustainable and taken up by the industry, the feed additive would need to be effective over long periods of time, non-toxic for animals, the environmental and consumers and cheap enough for standard use in animal feeds.
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Feed additives in ruminant nutrition

1. Introduction
Feed additives are products used in animal nutrition to improve the quality of feed and the quality of food from animal origin, or to improve the animals’ performance and health. They are categorised as follows:

- **Technological additives** (e.g. preservatives, antioxidants, emulsifiers, stabilising agents, acidity regulators, silage additives)
- **Sensory additives** (e.g. flavours, colorants)
- **Nutritional additives** (e.g. vitamins, minerals, amino acids, trace elements)
- **Zootechnical additives** (e.g. digestibility enhancers, gut flora stabilizers)
- **Coccidiostats and histomonostats** (additives used in poultry diets for health reasons)

In recent years, zootechnical additives\(^1\) (the focus of this review from here on) have shown vast growth in the research field and, consequently, in the feed market. Their suggested mode of action varies, but in general, they aim to manipulate the rumen fermentation environment to achieve greater efficiencies.

This range of feed additives has potential to deliver the following improvements in ruminant nutrition:

1. Increase feed conversion efficiency (FCE) and productivity
2. Stabilise rumen pH to reduce acidosis risk
3. Increase dry matter intake (DMI)
4. Reduce methanogenesis
5. Enhance rumen development
6. Reduce pathogen load and shedding
7. Improve meat quality
8. Enhance rumen stability during dietary transitions
9. Buffer against dietary health risks (e.g. mycotoxins)

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\(^1\) For the remainder of this review, zootechnical feed additives will be referred to solely as feed additives
This review aims to:

- Inform producers/advisors of the most promising feed additives for ruminants in terms of methane reduction
- Provide information on the other effects, risks and practicalities of the feed additives
- Where the feed additive is not commercially available, describe those most promising for future development

1.1 The potential of feed additives

Methane (CH$_4$) is a by-product of carbohydrate breakdown in the reticulo-rumen. Methane production is energy inefficient, wasting 2-15% of digested energy (McCrabb and Hunter, 1999, Johnson and Ward, 1996, Blaxter and Clapperton, 1965).

Dietary manipulation could reduce methane production while improving performance. A simplified diagram taken from a Food and Agriculture Organisation report (Alexander N. Hristov et al., 2013) demonstrates carbohydrate breakdown in Figure 1.

**Figure 1**: Carbohydrate metabolism in the rumen, taken from Alexander N. Hristov et al. (2013)

Reaction stoichiometry is the term to describe the relationship between substances as they interact in chemical reactions. It is used to estimate the quantity of products formed during a
reaction. If we can understand how carbohydrates break down (the reaction stoichiometry) and have an understanding of the effect of a specific feed additive on the process, we can predict how that additive will affect the various product quantities.

In general, the reaction stoichiometry for carbohydrate breakdown has been described by Van Soest (1994) as follows:

\[
\text{Glucose + ammonia} \rightarrow \text{microbes + methane + carbon dioxide + volatile fatty acids}\]

*butyrate, formate, lactate, propionate, acetate
Note: methanogenic archea are anaerobic, therefore this only occurs under anaerobic conditions

Therefore, the main products of carbohydrate breakdown in the rumen are volatile fatty acids (VFA), methane and carbon dioxide (CO₂). Alcohols and lactate are also formed.

The basic problem in anaerobic metabolism is the storage of oxygen (i.e. as carbon dioxide) and disposal of hydrogen (i.e. as methane) (Van Soest (1994)). Methane works as a hydrogen sink to remove hydrogen from the rumen for healthy rumen functioning.

Methane is commonly referred to as “2H” sink because pairs of protons and electrons are donated and accepted in metabolic reactions. If we can replace this process with an alternative, there is potential to reduce methane emissions and improve energy-use efficiency, but in-doing so caution is required not to replace the methane with another pollutant. Ideally, the hydrogen will be redirected to a useful form of nutrition for the animal.

Although methane can be produced from VFA and alternative sinks for hydrogen do exist in other environments (acetate, for example), these processes appear to be of little significance in the rumen (Russell and Wallace, 1997). As shown in Figure 1, acetate and butyrate produce four and two hydrogen molecules respectively and propionate is a 2H sink. Therefore propionate is the VFA to encourage if the objective is to reduce methane production as it decreases the overall amount of 2H available to reduce carbon dioxide to methane.

There is potential to manipulate carbohydrate breakdown to reduce methane production and potentially improve performance in the following ways:
- Encourage propionate production, thereby providing an alternative H sink (problem with this approach is that propionate has a lower affinity for hydrogen than methane)
- Adding an alternative hydrogen sink into the diet, e.g. nitrate (this has health risks and may increase ammonia output unless utilised by microbes)
- Encourage the production of acetate and butyrate, as these utilise the hydrogen before it can be directed to produce methane (less potential here as acetate and butyrate still produce hydrogen)
- Inhibit or eliminate the methanogenic microorganisms, thereby forcing the use of other hydrogen sink pathways (problematic if these organisms have other functions in the rumen)
- Slow down fermentation to improve nitrogen and energy-use efficiency

To be sustainable and taken up by the industry, the feed additive would need to be effective over long periods of time, non-toxic for animals, the environment and consumers and cheap enough for standard use in animal feeds.

### 1.2 The relationship between methane and dry matter intake
To determine how effective an additive is at reducing methane, it is important that the studies report the effect using the same units, per land area or per unit of product, for example. In addition, we should also look out for a reduction in methane that is simply due to a reduction in feed intake and/or a reduction in performance.

Dry matter intake is an important determinant of methane output. A meta-analysis showed on average, for every kilogram of dry matter intake approximately 30 litres of methane is produced (Mills et al., 2008). Therefore, when studies report a reduction in methane and a reduction in dry matter intake, the additive may be reducing digestibility or palatability of the feed.
2. Feed additives to reduce methane production in ruminants

2.1 EU regulation
According to EU Regulation 1831/2003, only additives that have been authorised by the European Food Safety Authority (EFSA) can be placed on the market in the EU.

Authorised additives can be put on the market solely for the specific use described for authorisation. Companies or researchers wishing to get an additive authorised must submit an application and a dossier to EFSA which must:

- Enable assessment of additives based on the current state of knowledge
- Demonstrate compliance with the fundamental principles for authorisation
- Have a minimum of three long term *in vivo* studies showing significant effects for the relevant target species/category. These should be carried out at least two different locations, one of which should be in the EU. The studies should include the lowest dose proposed by the applicant to enable recommendations on dose to be made.
- Demonstrate the safety of the product (EFSA, 2012).

Probiotics (fungi, bacteria, enzymes) are the only feed additives categorised under zootechnical additives for beef and sheep production and therefore, technically, should be the only feed additives in that category on the market in the UK. However, some additives, with the claim of offering a dietary nutritional component, may appear on the catalogue of feed materials European Commission (2013).

2.2 European Food Safety Authority report
A recent review completed by Herefordshire University for the European Food Safety Authority (EFSA) conducted a meta-analysis of feed additives aimed at mitigation of environmental impacts of livestock (Lewis *et al.*, 2013).

Many meta-analyses on feed additives report substantial variation in additive effects on animal performance and other measured variables due to differences in the methodology employed across the different experiments (Eugène *et al.*, 2008, Sales, 2011, Desnoyers *et al.*, 2009).

This makes it difficult to determine the efficacy of a substance, as it is hard to disentangle the effect of the additive from any interaction with other factors. Additionally, the variation in
different diets would likely influence the results (Desnoyers et al., 2009). The review covered 246 substances in ten major groups, as illustrated in Table 1.

Table 1: The substance groups covered by the EFSA report (Lewis et al., 2013)

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Number of substances</th>
<th>Number of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids &amp; their salts</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Amino acids</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Plant &amp; animal oils, fats</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Plant extracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Saponins</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>Tannins</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Essential oils &amp; spices</td>
<td>42</td>
<td>48</td>
</tr>
<tr>
<td>Antibiotic drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Ionophores</td>
<td>9</td>
<td>47</td>
</tr>
<tr>
<td>Bacteria, enzymes &amp; yeasts</td>
<td>29</td>
<td>62</td>
</tr>
<tr>
<td>Mineral salts and complexes</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Miscellaneous substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical substances</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>Natural substances</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>246</strong></td>
<td><strong>-</strong></td>
</tr>
</tbody>
</table>

2.1.1. Additive development

Unfortunately, many of the additives, particularly those that had the greatest effect (Table 2) were only tested *in vitro*, and not *in vivo*. *In vitro* research gives insight into the potential of the additive with different substrates, but it does not account for the capacity for rumen microbes to adapt to the additives and degrade them (Benchaar and Greathead, 2011). Additionally, dose rate *in vitro* can be significantly higher than when fed to the ruminant, therefore a response can be induced that may not be practically feasible in a live animal (Benchaar and Greathead, 2011).
Table 2: Summary of the top ten feed additives for methane reduction taken from the EFSA report (Lewis et al., 2013)

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Substance</th>
<th>Methane reduction (%) and range in brackets</th>
<th>No. of studies</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oils</td>
<td><em>Mentha microphylla</em></td>
<td>-96 (-92 to -100)</td>
<td>1</td>
<td>Sheep</td>
</tr>
<tr>
<td>Plant extracts: tannins</td>
<td><em>Rheum officinale</em> (root)</td>
<td>-75 (No range)</td>
<td>1</td>
<td>Sheep</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Linolenic acid</td>
<td>-71 (-46 to -99)</td>
<td>3</td>
<td>Sheep (1) and cattle (2)</td>
</tr>
<tr>
<td>Miscellaneous chemical substances</td>
<td>9, 10-anthraquinone</td>
<td>-70 (-58 to -91)</td>
<td>2</td>
<td>Cattle (1) and goats (1)</td>
</tr>
<tr>
<td>Miscellaneous chemical substances</td>
<td>2-iodopropane</td>
<td>-61 (-25 to -97)</td>
<td>1</td>
<td>Cattle</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Lauric acid</td>
<td>-59 (-11 to -99)</td>
<td>4</td>
<td>Cattle</td>
</tr>
<tr>
<td>Plant and animal oil, fats</td>
<td>Palm kernel oil</td>
<td>-58 (-32 to -85)</td>
<td>2</td>
<td>Cattle</td>
</tr>
<tr>
<td>Miscellaneous chemical substances</td>
<td>Pyromellitic diimide</td>
<td>-57 (-7 to -100)</td>
<td>3</td>
<td>Cattle</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Carvacrol</td>
<td>-56 (-13 to -98)</td>
<td>1</td>
<td>Sheep</td>
</tr>
<tr>
<td>Plant extracts: general</td>
<td>Cashew nut shell liquid</td>
<td>-55 (-36 to -70)</td>
<td>2</td>
<td>Cattle</td>
</tr>
</tbody>
</table>

n.b. these were all *in vitro* studies

Looking only at the substances with five or more studies and some *in vivo* experimentation, we can ascertain which substances have the greatest evidence to reduce methane production (Table 3). Tables 2 and 3 were used to compile a list of the most promising feed additives on which to base the rest of the review (Table 4).
Table 3: Summary of substances with five or more studies including some *in vivo* experiments from the EFSA report (Lewis et al., 2013)

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Substance</th>
<th>Average methane change <em>in vivo</em></th>
<th>Number of <em>in vivo</em> studies</th>
<th>Species</th>
<th>Total studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acids</td>
<td>Myristic acid</td>
<td>-36.3</td>
<td>2</td>
<td>Sheep</td>
<td>5</td>
</tr>
<tr>
<td>Plant &amp; animal oils, fats</td>
<td>Coconut oil</td>
<td>-23.8</td>
<td>5</td>
<td>Cattle (4) and Sheep (1)</td>
<td>10</td>
</tr>
<tr>
<td>Plant &amp; animal oils, fats</td>
<td>Canola oil</td>
<td>-16.0</td>
<td>3</td>
<td>Cattle</td>
<td>6</td>
</tr>
<tr>
<td>Essential oils &amp; Spices</td>
<td><em>Allium arenarium</em> oil</td>
<td>-12.0</td>
<td>1</td>
<td>Sheep</td>
<td>9</td>
</tr>
<tr>
<td>Organic acids and their salts</td>
<td>Fumaric acid</td>
<td>-11.5</td>
<td>2</td>
<td>Cattle and Sheep</td>
<td>5</td>
</tr>
<tr>
<td>Antibacterial drugs: Ionophores</td>
<td>Monensin sodium</td>
<td>-11.3</td>
<td>10</td>
<td>Cattle (9) and Sheep (1)</td>
<td>21</td>
</tr>
<tr>
<td>Plant extracts: Saponins</td>
<td>Tea saponins</td>
<td>-10.1</td>
<td>7</td>
<td>Cattle (3) and Sheep (4)</td>
<td>7</td>
</tr>
<tr>
<td>Plant extracts: Saponins</td>
<td><em>Quillaja saponaria</em> extract</td>
<td>-4.3</td>
<td>4</td>
<td>Cattle (3) and Sheep (1)</td>
<td>9</td>
</tr>
<tr>
<td>Plant extracts: Saponins</td>
<td><em>Yucca Schidigera</em> extract</td>
<td>-2.4</td>
<td>5</td>
<td>Cattle (4) and Sheep (1)</td>
<td>10</td>
</tr>
<tr>
<td>Organic acids and their salts</td>
<td>Fumaric acid, sodium salt</td>
<td>0.0</td>
<td>1</td>
<td>Cattle</td>
<td>12</td>
</tr>
</tbody>
</table>
2.3 Food and Agriculture Organization review

A recent report, *Mitigation of Greenhouse gas emissions in livestock production; a review of technical options for non-CO2 emissions*, of the Food and Agriculture Organization of the United Nations (Alexander N. Hristov *et al.*, 2013) discussed the major feed additive groups with potential to reduce methane as follows:

**Inhibitors (i.e. targeted chemical compounds that inhibit rumen archea, e.g. bromochloromethane)**

Verdict: Bromochloromethane is promising but cannot be used because it is an ozone depleting substance, there was no sufficient data for other substances in this category.

**Electron receptors, e.g. nitrate**

Verdict: Nitrate may be promising in low protein diets that may benefit from non-protein nitrogen supplementation. An adaptation period is crucial and must beware of pollution swapping (i.e. ammonia). Long term effects unknown.

**Ionophores**

Verdict: Likely to provide a moderate CH₄ mitigating effect, prohibited in Europe.

**Plant bioactive compounds (includes tannins, saponins, essential oils)**

Verdict: hydrolysable and condensed tannins show promise but intake and animal production may be compromised. Tea saponins require more long term studies.

Most essential oils or their active ingredients do not reduce methane. Studies that demonstrated a reduction were over short timescales, therefore longer studies are required.

**Dietary lipids (also termed plant and animal oils)**

Verdict: Effective at reducing methane but uptake will depend on cost-effectiveness and potential negative effects on feed intake and productivity. Distillers grains were also categorised here, but results have been variable, some increases in methane observed due to increased fibre intake.
Exogenous enzymes

Verdict: May increase feed efficiency but inconsistent results.

Direct fed microbials

Verdict: Insufficient evidence, although yeast appears to stabilise pH and promote rumen function in high concentrate diets.

Using the results of the two reviews, the most promising additives are summarised in Table 4.

Table 4: Most promising feed additives of the EFSA and FAO reports, as categorised by the reports

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acids</td>
<td>Myristic acid Linolenic acid, Lauric acid (table 2)</td>
</tr>
<tr>
<td>Plant &amp; animal oils, fats/dietary lipids</td>
<td>Coconut oil, Canola oil, Palm Kernel oil</td>
</tr>
<tr>
<td>Essential oils and spices</td>
<td><em>Allium arenarium</em> oil, <em>Mentha microphylla</em>, Carvacrol</td>
</tr>
<tr>
<td>Plant extracts: Saponins</td>
<td><em>Tea saponins, Quillaja saponaria</em> extract, <em>Yucca Schidigera</em> extract</td>
</tr>
<tr>
<td>Electron receptors</td>
<td>Nitrate, sulphate</td>
</tr>
</tbody>
</table>

Taking into account the findings of the EFSA and FAO reports, the next section will provide further details of the mode of action of these additives, describe the practicality of feeding them, health or environmental risks and effects on other pollutants.
2.3 The most promising feed additives for reducing methane

2.3.1 Fatty acids and plant oils

**Table 5:** Characteristics of the most promising fatty acids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Characteristics</th>
<th>Main sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>Saturated, Medium chain</td>
<td>Coconut oil, Palm kernel oil</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>Saturated, Medium chain</td>
<td>Coconut oil</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>Polyunsaturated, Long chain</td>
<td>Linseed</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>Unsaturated, long chain, omega 6</td>
<td>Soybean, sunflower</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>Monounsaturated, long chain</td>
<td>Rapeseed meal, Canola oil</td>
</tr>
</tbody>
</table>

Medium-chain saturated fatty acids have strong antimicrobial properties (Hristov et al., 2004), particularly affecting methanogenic archea (Machmüller et al., 2003). This inhibition should reduce methane production. Conversely, it has been suggested that long chain fatty acids do not have this mode of action (Mosoni et al., 2008).

Polyunsaturated fatty acids have toxic effects on cellulolytic bacteria and protozoa (Doreau and Ferlay, 1995). They act against lactate producers (Kubo et al., 1993, Nagaraja et al., 1997) thereby favouring propionate producers.

Coconut oil contains lauric and myristic acid. Both fatty acids have substantial potential to reduce methane production (Tables 2 and 3) but there is a large range in effects observed and lauric acid is only explored *in vitro* in the EFSA report. There is some indication that the effect of the two fatty acids on methane production is additive (Machmüller and Kreuzer, 1999, Soliva et al., 2004).

Linolenic acid and linoleic acid also reduce methane by an average of 71% and 51%, respectively, *in vitro* (Lewis et al., 2013) but further work is required *in vivo*. 
Diet effect

Diet composition will affect the success of the additive, but results are not definitive.

Machmuller et al. (2003) investigated the interaction of myristic acid supplementation with diet type (concentrate: forage ratios: 1:1.5 and 1:0.5) and dietary calcium (Ca) content (4.2 and 9g/kg DM) in sheep. Myristic acid suppressed methane in both diets, although more in the concentrate diet than the forage diet and the highest dose of Ca reduced this effect.

When averaged across both diets, myristic acid increased DMI, as a result, methane emissions reported in kg/megajoule (MJ) of gross energy intake and g/kg organic matter digested were reduced for both diet types.

Lovett et al. (2003) showed no interaction between diet (forage to concentrate ratios: 65:35, 40:60 and 10:90) and coconut oil supplementation in finishing beef heifers. Although supplementing with coconut oil reduced dry matter intake, this was outweighed with greater reductions in methane, therefore methane produced per kg DMI, liveweight gain, carcase gain and as percentage of gross energy intake was reduced.

Martin et al. (2009) explored linseed supplementation in dairy cows on a hay- and maize silage-based diet. In the four treatments (0, 5, 10 and 15% linseed in ration), the cows on the maize silage diet produced less methane than those on the hay-based diet. The methane reduction effect from linseed supplementation was greater on the hay-based diet than the maize diet, perhaps this is due to a greater potential to improve rumen fermentation efficiency in the hay based diet. 15% extruded linseed with a maize diet had the lowest total methane emissions (l/d). The authors did not present results on dry matter intake, however, they found that supplementation did not alter milk yield, suggesting extruded linseed (providing an expected oil supplementation of 6% of the diet) is a viable methane mitigation option.

Feeding

The best way to feed dietary lipids of interest is unclear. Wholeseeds tend to be cheaper than the refined oils (Martin et al., 2010), but sunflower seeds have been reported to reduce NDF digestibility in heifers (Beauchemin et al., 2007) and average daily gain may be enhanced with the oil form compared to whole seed/bean form (refined soy oil, crossbred beef bulls (Jordan et al., 2006a)).
In terms of methane reduction, the oil form was better at reducing methane when feeding soy (young bulls, Jordan et al. (2006a)) and linseed (lactating dairy cows, Martin et al. (2008)) than the whole form, but sunflower seed has been reported to reduce methane more than sunflower oil (Aberdeen angus heifers, Beauchemin et al. (2007)). For many seeds, the hard seed coating is difficult to digest by enzymes, therefore crushing, bruising, extrusion or expansion is required to release the oil (Raes et al., 2004). These papers suggest the oil form would be the most attractive option in terms of performance and methane reduction as long as the cost of the ingredient is outweighed by improved performance.

**Unintended consequences**

Only five studies additionally looked at ammonia emissions in the EFSA report (three *in vivo* and two *in vitro*), illustrating that lauric acid and myristic acid have potential reduce ammonia emissions too (Lewis et al., 2013).

The fatty acids above are described as slight to strong skin and eye irritants and lauric acid is toxic to aquatic life (Lewis et al., 2013).

**Performance effects**

Many studies report a reduction in methane with various types of fat supplementation to ruminant diets but this is often accompanied by a reduction in dry matter intake and digestibility (Table 6). When this is reported on a fat-corrected milk basis, increased feed efficiency is observed in dairy production (Eugene *et al.*, 2008, Rabiee *et al.*, 2012).

Table 6 is adapted from a recent review of the benefits of supplementary fats in ruminant rations (Rasmussed and Harrison, 2011). From this table, we can see that, apart from one study, all the plant oils in the studies had potential to decrease methane. However, digestibility and intake were reduced in some studies, particularly with high dose rates therefore the negative effect on production is likely to increase methane output per unit of liveweight gain.
**Table 6: In vivo studies analysing the effects of plant oils on methane and other parameters (adapted from (Rasmussen and Harrison, 2011))**

<table>
<thead>
<tr>
<th>Animal</th>
<th>n</th>
<th>Duration</th>
<th>Ration (forage/concentrate dry matter basis)</th>
<th>Fat source (% of DM)</th>
<th>Methane (%)</th>
<th>NH₃</th>
<th>DMI</th>
<th>Digestibility</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charolais/Limousin heifers</td>
<td>4</td>
<td>93d</td>
<td>50/50</td>
<td>Coconut oil (10%) 250g/day</td>
<td>-18</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>(Jordan et al., 2006c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copra meal* (10%) 250g/day</td>
<td>-14.9</td>
<td>NS</td>
<td>decrease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charolais/Limousin heifers</td>
<td>6</td>
<td>35d</td>
<td>50/50</td>
<td>Coconut oil (14%) 250g/day</td>
<td>-20.5</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>(Jordan et al., 2006b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coconut oil (42%) 375g/day</td>
<td>-39</td>
<td>decrease</td>
<td>decrease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charolais/Limousin bulls</td>
<td>3</td>
<td>103 d</td>
<td>10/90</td>
<td>Soya oil (10%)</td>
<td>-40</td>
<td>NS</td>
<td>decrease</td>
<td></td>
<td>(Jordan et al., 2006a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Soybean (12%)</td>
<td>-25.3</td>
<td>decrease</td>
<td>decrease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating cows</td>
<td>5</td>
<td>12 wk</td>
<td>70/30</td>
<td>Cotton seed (48%)</td>
<td>-23</td>
<td>NS</td>
<td>decrease</td>
<td></td>
<td>(Grainger et al., 2010)</td>
</tr>
<tr>
<td>Lactating cows</td>
<td>1</td>
<td>4 X 28d</td>
<td>45/55 TMR</td>
<td>Sunflower seed (3.3%)</td>
<td>-10</td>
<td>increase</td>
<td>decrease</td>
<td>decrease</td>
<td>(Beauchemin et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Linseed oil (3.3%)</td>
<td>-18</td>
<td>NS</td>
<td>decrease</td>
<td>decrease</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rape seed (3.3%)</td>
<td>-16</td>
<td>NS</td>
<td>decrease</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Huzhou sheep</td>
<td>3</td>
<td>60 d</td>
<td>60/40</td>
<td>Soya oil (3%)</td>
<td>-14</td>
<td>decrease</td>
<td>Decrease</td>
<td></td>
<td>(Mao et al., 2010)</td>
</tr>
<tr>
<td>Swiss White Hill sheep</td>
<td>1</td>
<td>3 x 21d</td>
<td>60/40</td>
<td>Coconut oil (6%)</td>
<td>-26</td>
<td>NS</td>
<td>Decrease</td>
<td></td>
<td>(Machmüller et al., 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sunflower seed (6%)</td>
<td>-27</td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Linseed oil (6%)</td>
<td>-10</td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal</td>
<td>n</td>
<td>Duration</td>
<td>Ration (forage/concentrate)</td>
<td>Fat source (% of DM)</td>
<td>Methane (%)</td>
<td>NH3</td>
<td>DMI</td>
<td>Digestibility</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
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<td>------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Holstein bulls</td>
<td>1</td>
<td>21 d</td>
<td>75/25</td>
<td>Sunflower oil (5%)</td>
<td>-22</td>
<td></td>
<td></td>
<td>decrease</td>
<td>(McGinn et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>400g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating Holstein cows</td>
<td>3</td>
<td>319d</td>
<td>50/50 TMR</td>
<td>Cotton seed (4%)</td>
<td>NS</td>
<td></td>
<td></td>
<td>increase</td>
<td>(Johnson et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td>Cotton seed (5.6%)</td>
<td>NS</td>
<td></td>
<td></td>
<td>increase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rape seed (4%)</td>
<td>NS</td>
<td></td>
<td></td>
<td>increase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rape seed (5.6%)</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating Holstein cows</td>
<td>1</td>
<td>18 d</td>
<td>60/40 TMR</td>
<td>Myristic acid (5%)</td>
<td>-36</td>
<td>NS</td>
<td></td>
<td>decrease</td>
<td>(Odongo et al., 2007b)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating Holstein cows</td>
<td>8</td>
<td>4 wk</td>
<td>65/35</td>
<td>Linseed oil (5.7%)</td>
<td>-64</td>
<td></td>
<td></td>
<td>decrease</td>
<td>(Martin et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extruded linseed (5.7%)</td>
<td>-38</td>
<td></td>
<td></td>
<td>decrease</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Crude linseed (5.7%)</td>
<td>-12</td>
<td></td>
<td></td>
<td>NS decrease</td>
<td></td>
</tr>
<tr>
<td>Huzhou Sheep</td>
<td>8</td>
<td>21 d</td>
<td>60/40</td>
<td>Coconut oil (7%)</td>
<td>-38</td>
<td></td>
<td></td>
<td></td>
<td>(Yuan et al., 2007)</td>
</tr>
</tbody>
</table>

*copra meal based concentrate with 250g of Coconut/day from copra meal

DMI = dry matter intake

TMR = total mixed ration

NS = no statistically significant effect
2.3.4 Essential oils

Essential oils cover a wide variety of products extracted from plants using steam distillation with water or aqueous alcohol.

Mode of action

Similar to ionophores and polyunsaturated fatty acids, essential oils are thought to select against gram positive bacteria, favouring propionate producing bacteria, thereby encouraging the alternative hydrogen sink to methane – propionate.

Response

Within the category 'Essential oils', 42 substances were covered by the EFSA review and 31 were found to offer potential for reducing ruminal methane and ammonia, in many cases reduction in these pollutants occurred simultaneously (Lewis et al., 2013). The most promising essential oil with over 5 studies, that included in vivo work, was Allium arenarium oil (garlic oil) which reduced methane by 12 % in vivo and an average of 36% reduction was reported in vitro (Lewis et al., 2013).

Garlic oil is unique in that some active compounds present in the oil are not present in the whole plant; they are formed during the steam treatment process (Pentz and Siegers, 1996). It is active against a wide range of gram positive and gram negative bacteria, fungi, parasites and viruses – this is likely due to interaction with the sulfhydryl groups (-SH) of other active compounds (Reuter et al., 1996). The activity of the oil is more powerful than the individual compounds alone – suggesting synergism between constituent compounds (Busquet et al., 2005b).

Performance and practicality

Garlic and other essential oils could affect the palatability of the feed. Garlic oil and diallyl disulphide (garlic oil constituent) additions to mature ewe diets did increase feed refusal initially (Klevenhusen et al., 2011). With time, the sheep adjusted to diallyl disulphide additions and feed intake resumed to the level of the control (no additive). This adjustment did not occur with the garlic oil treatment.
Robertson *et al.* (2006) looked at improving the palatability of straw by adding different food flavourings and found that applying a low rate of garlic flavouring (0.05 g/kg (0.005%)) to straw improved acceptability to sheep. However, higher rates of garlic inclusion (5, 10, 15, 20, and 25% DM) reduced diet palatability (Nolte and Provenza, 1992a, Nolte and Provenza, 1992b).

Performance effects of added garlic to ruminant diets are variable, as illustrated in Table 7.

**Diet effect**

There are few studies investigating the interaction between diet and garlic supplementation. One *in vitro* study suggests garlic oil additives are more effective with moderate concentrate diets (50 : 50 alfalfa hay : concentrate) than high concentrates (15 : 85 barley straw : concentrate) at reducing the acetate:propionate ratios (Mateos *et al.*, 2013). In both diets the garlic oil reduced methane production significantly.

**Risks**

High rates of any plant extracts may be toxic, therefore dose will need careful consideration. No adverse effects on the target species or human health (as typical usage doses) have been identified for these substances.

The main barrier to the use of garlic in dairy diets it the risk of milk taint (Babcock, 1938), but there is no evidence to suggest it will impact meat quality. In fact, Strickland *et al.* (2011) found that lamb reared on diets containing garlic additives was accepted by taste panellists more positively than those reared on garlic-free diets.
### Table 6: Studies reporting the effect of garlic supplementation in ruminants

<table>
<thead>
<tr>
<th>Form</th>
<th>Dose</th>
<th>Diet</th>
<th>Animals</th>
<th>ADG</th>
<th>Dry matter intake</th>
<th>FCE (DM intake:kg growth)</th>
<th>Methane</th>
<th>Other</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic oil</td>
<td>200mg/kg dietary DM</td>
<td>Barley based</td>
<td>10 ewe lambs/treatment</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NA</td>
<td></td>
<td>(Chaves et al., 2008)</td>
</tr>
<tr>
<td>Garlic extract*</td>
<td>250 mg/kg BW/day/calf</td>
<td>Milk, forage and concentrate (~1:6 conc:forage ratio)</td>
<td>12 pre-ruminant Holstein cross calves, weaned just after birth, 5 days old</td>
<td>+18</td>
<td>+8%</td>
<td>NS</td>
<td>NA</td>
<td></td>
<td>(Ghosh et al., 2011)</td>
</tr>
<tr>
<td>Garlic extract*</td>
<td>250 mg/kg BW/day/calf</td>
<td>Milk, forage and concentrate (~1:6 conc:forage ratio)</td>
<td>38 pre-ruminant Holstein cross calves, weaned just after birth, 5 days old</td>
<td>+44%</td>
<td>+12%</td>
<td>-44%</td>
<td>NA</td>
<td></td>
<td>(Ghosh et al., 2010)</td>
</tr>
</tbody>
</table>
Table 6 continued: Studies reporting the effect of garlic supplementation in ruminants

<table>
<thead>
<tr>
<th>Form</th>
<th>Dose</th>
<th>Diet</th>
<th>Animals</th>
<th>ADG</th>
<th>Dry matter intake</th>
<th>FCE (DM intake:kg growth)</th>
<th>Methane</th>
<th>Other</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Garlic **</td>
<td>53 g/kg dietary DM (1.5% DMI)</td>
<td>Concentrate based</td>
<td>Brown swiss x Limousin crossbred bulls</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>(Staerfl et al., 2012)</td>
</tr>
<tr>
<td>Raw Garlic</td>
<td>Diet inclusion: 0.9%</td>
<td>Pelleted ration</td>
<td>40 Merino wether lambs aged 6 months</td>
<td>-18%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NA</td>
<td>(Strickland et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>1.8%</td>
<td></td>
<td></td>
<td>-22%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.6%</td>
<td></td>
<td></td>
<td>-41%</td>
<td>NS</td>
<td>+62%</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic oil***</td>
<td>4g/day</td>
<td>Lucerne hay and concentrate at 45:55 ratio</td>
<td>Four ruminally fistulated Baloochi lambs</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>(Vakili et al., 2011)</td>
</tr>
</tbody>
</table>

*prepared using electric mixer and filtered through muslin cloth, **acacia tannin, maca and lupines also compared, ***Tumeric powder and monensin also compared

ADG = average daily gain, BW = body weight, DM = dry matter, DMI = dry matter intake, FCE = feed conversion efficiency, NA = not applicable to study, NS = no statistically significant effect, VFA = volatile fatty acids.
2.3.5 Plant extracts: Saponins

Although there has been a lot of research interest in tannins and other plant extracts, saponins were the only plant extract-based additive that had sufficient evidence described in the EFSA review to reduce methane emissions.

Saponins are sugar and non-carbohydrate complexes that characteristically foam when shaken in water. They are steroid or triterpene glycoside compounds found in a variety of plants.

Commercial additives are derived from *Yucca shidigera* or *Quillaya saponaria* and *Sapindus sp* (temperate and tropical plant species).

As Table 3 shows, *in vivo* effects of saponin addition in the studies for the EFSA report show mean methane reductions of 2.4%, 4.3% and 10.1% for *Yucca Schidigera* extract, *Quillaja saponaria* and *Tea saponins*, respectively.

**Mode of action**

*In vitro* and *in vivo* experiments show how saponins can be used to remove or reduce protozoa numbers in the rumen (described in a review by Wina *et al.* (2005)). Protozoa cause protein turnover by predating on bacteria. Theoretically their removal should lead to an increase in the number of bacteria in the rumen, this slows the protein turnover leading to an increase in bacterial N flow to the duodenum. Therefore, the removal of protozoa may increase nitrogen utilisation leading to improvements in performance output and reductions in ammonia produced. This theory has been supported by *in vitro* work of Makkar and Becker (1996), whereby quillaja saponins (0.4-1.2 mg/mL) on a hay substrate increased efficiency of microbial protein synthesis.

In addition, some methanogens are closely associated with protozoa, therefore the reduction of protozoa may also reduce methanogen activity.

**Diet effect**

A review of studies by Wina *et al.* (2005) indicated that improvements in liveweight gain are most likely to be seen with saponin addition to high roughage diets, although this was not clear cut, some moderate to high roughage diets still showed no growth enhancing effect (Hussain and Cheeke, 1995). Also, sex can interplay with the response: a higher growth
response was reported in male lambs compared to females lambs with 40 mg quillaja saponins/kg in low roughage diets (Hussain and Cheeke, 1995).

**Performance and practicality**

The main issue with feeding saponins to ruminants is evidence that the rumen microbe adapt and protozoa return to pre-supplementation levels (Newbold et al., 1997), intermittent additions may mitigate this effect (Thalib et al., 1996). There is also evidence that they can be degraded in the rumen (Wang et al., 2000). Further long term experimentation will need to fully explore this effect and develop ways to overcome it.

Saponins have been described to reduce fibre digestibility but not total tract digestibility, however acid detergent fibre (ADF) digestibility of a legume-grass diet was reduced and neutral detergent fibre digestibility of a grass diet were reduced with addition of *S. Saponaria* fruit (Abreu et al., 2004), further emphasising the important diet-additive interactions.

**Risks**

Some saponin containing plants are toxic which can lead to photosensitisation and subsequent liver and kidney degeneration, and gut problems. However it is unlikely these would be used in commercial additives.

**2.3.6 Electron receptors**

Electron receptors such as nitrate and sulphate were not considered in the EFSA review, yet are prominent in other recent reviews on methane mitigation (Alexander N. Hristov et al., 2013, Hristov et al., 2013). They offer an alternative a hydrogen sink to carbon dioxide, thereby reducing methane emissions and potentially improving digestion efficiency (Ungerfeld and Kohn, 2006). Nitrate (NO₃⁻) likely holds the greatest potential for this because it has a greater affinity for hydrogen (H₂) than carbon dioxide therefore – instead of methane – nitrite and ammonia are produced. Ammonia generated would supply fermentable nitrogen for animals receiving protein deficient diets where ammonia is limiting protein synthesis for microbial growth (Dijkstra et al., 1998). Additionally, the reaction to produce nitrite is more energetically efficient than methanogenesis (Ungerfeld and Kohn, 2006).
Risks

Sudden introduction of nitrate into the diet may induce methaemoglobinemia, a state of general anoxia; in mild cases this will depress animal behaviour but in severe cases can be fatal (Ozmen et al., 2005). Methaemoglobinemia is caused by nitrite in the blood. The accumulation of nitrite in the rumen is readily absorbed across the rumen wall and converts blood haemoglobin (Hb) from the ferrous (Fe$^{2+}$) to the ferric (Fe$^{3+}$) form - methaemoglobin (MetHb) - which is incapable of transporting oxygen (Morris et al., 1958). Gradual introduction of nitrate into the diet can allow the rumen microbes to adapt and increase their ability to reduce nitrite (Alaboudi and Jones, 1985). In animals unadapted to nitrate in their diet, the capacity of the microbes to reduce nitrate to nitrite, exceeds the capacity for nitrite reduction (Lewis, 1951). MetHb formation coincides with reduced oxygen intake, carbon dioxide production and metabolic rate (Takahashi et al., 1998).

A comprehensive review suggests that sheep can tolerate up to 4% nitrate in high quality concentrate or forage based diets without showing clinical signs of methamoglobinemia (Leng, 2008).
Table 7: Studies reporting nitrate supplementation in ruminant diets

<table>
<thead>
<tr>
<th>Paper</th>
<th>Additive(s)</th>
<th>Form</th>
<th>Administration</th>
<th>Dose</th>
<th>Animals</th>
<th>Diet</th>
<th>Adaptation period (days)</th>
<th>Methanoglobin &gt;2% of haemoglobin?</th>
<th>Methane change (% difference in output (L/day))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Li et al., 2012)</td>
<td>Nitrate</td>
<td>Calcium nitrate (1.9% nitrate)</td>
<td>Dietary pellet</td>
<td>3% of DM</td>
<td>10 weaned ewe lambs (Poll dorset sire x Dohne ewe)</td>
<td>Limited info.</td>
<td>7</td>
<td>No</td>
<td>-34 (P=0.06)</td>
</tr>
<tr>
<td>(van Zijderveld et al., 2011)</td>
<td>Nitrate</td>
<td>Calcinit: 5Ca(NO₃)₂NO₃·10H₂O; 75% NO₃ in DM</td>
<td>Added to concentrates</td>
<td>8.8% of DM</td>
<td>20 lactating holstein-fresian cows 33.2 ±6.0kg milk/d 104±58 d in milk at start</td>
<td>TMR-Corn silage based ration (66:34, forage:concentrate) Isoenergetic and isonitrogenous</td>
<td>28</td>
<td>Yes, max. observed: 4.2% of Hb</td>
<td>-16</td>
</tr>
<tr>
<td>(van Zijderveld et al., 2010)</td>
<td>Nitrate and Sulphate</td>
<td>5Ca(NO₃)₂·NH₄NO₃·10H₂O; 75% NO₃ in DM (highly soluble and available nitrate) MgSO₄</td>
<td>Added to concentrates</td>
<td>2.6% of DM</td>
<td>20 male cross bred lambs lambs</td>
<td>Maize silage basal ration (90%) plus concentrates based on soybean mean and additives (10%). Urea substitutes nitrate in sulphate experiment</td>
<td>28</td>
<td>Yes-max. observed: 7% of Hb for high nitrate inclusion diet</td>
<td>Nitrate - 32% Sulphate - 16%, Both -47%</td>
</tr>
<tr>
<td>Paper</td>
<td>Additive(s)</td>
<td>Form</td>
<td>Administration</td>
<td>Dose</td>
<td>Animals</td>
<td>Diet</td>
<td>Adaptation period (days)</td>
<td>Methanoglobin &gt;2% of haemoglobin?</td>
<td>Methane change (% difference in output (L/day))</td>
</tr>
<tr>
<td>------------------------</td>
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<td>-------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>(Nolan et al., 2010)</td>
<td>Nitrate</td>
<td>Potassium nitrate (KNO₃)</td>
<td>Sprinkled on hay</td>
<td>4% of DM</td>
<td>8 merino wethers rumen-cannulated sheep</td>
<td>Chaffed oaten hay 1kg/day</td>
<td>18</td>
<td>No</td>
<td>-23</td>
</tr>
<tr>
<td>(Guo et al., 2009)</td>
<td>i) Nitrate-N</td>
<td>Sodium nitrate (NaNO₃)</td>
<td>in vitro</td>
<td>12.6% DM</td>
<td>3 cannulated Simmental x Luxi steers</td>
<td>60% roughage (corn stover cubes and alfalfa hay pellets) and 40% mixed concentrate (ground corn, soybean mean and soy hulls)</td>
<td>NA</td>
<td>Not measured</td>
<td>-74</td>
</tr>
</tbody>
</table>
Table 7 continued: Studies reporting nitrate supplementation in ruminant diets

<table>
<thead>
<tr>
<th>Paper</th>
<th>Additive(s)</th>
<th>Form</th>
<th>Administration</th>
<th>Dose</th>
<th>Animals</th>
<th>Diet</th>
<th>Adaptation period (days)</th>
<th>Methanoglobin &gt;2% of haemoglobin?</th>
<th>Methane change (% difference in output (L/day))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Sar et al., 2004)</td>
<td>Nitrate with or without: Beta 1-4 galacto-oligosaccharides or Nisin</td>
<td>30%(w/v) aqueous solution of NaNO3/kg(^{0.75}) GOS-oligomate 55 Nisin-Sigma</td>
<td>Via fistula 30 mins after morning meal</td>
<td>1.3g NaNO3/kg(^{0.75}) of body wgt GOS 20g/day Nisin 3mg/kg(^{0.75}) of BW</td>
<td>4 ruminally-fistulated wethers</td>
<td>Chopped timothy hay, alfalfa hay cube, and concentrate (40:40:20 on DM basis) Fed at maintenance energy level</td>
<td>7</td>
<td>Yes - peaked at 18.4% in nitrate-only treatment</td>
<td>-50 No diff. with GIS or nisin</td>
</tr>
<tr>
<td>(Alaboudi and Jones, 1985)</td>
<td>Nitrate</td>
<td>Initially low dose of nitrate (NO(_3)-) alone (0.05% of BW) then potassium nitrate (KNO(_3))</td>
<td>Added to ration</td>
<td>2.5g KNO(_3)/kg body wgt/day reached in 0.5g increments</td>
<td>4 Dorset-Columbia crossbred ewes</td>
<td>44% cereal grain, 50% hay</td>
<td>63</td>
<td>No</td>
<td>NA</td>
</tr>
</tbody>
</table>
**Unintended consequences**

Nitrate may divert hydrogen away from other uses such as propionogenesis- therefore the VFA profile often shifts away from propionate and butyrate in favour of acetate (Farra and Satter, 1971, Allison and Reddy, 1984, Alaboudi and Jones, 1985). This could potentially reduce the efficiency of the rumen as acetate production is less energy efficient than propionate and butyrate production.

Additionally, it can lead to an increase in ammonia production, which can be a good source of nitrogen for microbes but in high protein diets it is likely to be excreted, thereby producing another pollutant.

**Effect longevity**

Although overall, potassium nitrate addition did reduce methane emissions in the study by Nolan *et al.* (2010) (further details in table 10) after the immediate reduction observed post feeding, there was a gradual rise in methane over the two hours between feeding periods. The authors suggest a ‘slow release’ form of nitrate might enhance methane mitigation and, in addition, reduce nitrate and nitrite absorption from the rumen and thereby reduce the potential for 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. The long-term effects of these compounds have not been established.
3. Persistence

Of the studies considered in the EFSA review, very few studied the long term effects of feeding additives. Where the mode of action depends on shifting the microbial population of the rumen there is always potential for adaption of the microbes to take place over time and the response to diminish, this issue was highlighted in a review by McAllister and Newbold (2008). Where the mode of action is related to providing exogenous alternative hydrogen sinks then there may be less potential for adaptation over time to occur.

Sauer et al. (1998) reported that ruminal microflora adapted over time to the use of monensin, a view supported by Guan et al. (2006) who found that monensin reduced methane production by around a third but this benefit was short-lived and methane production gradually returned to its pre-supplementation level over a 16 week period. However, Odongo et al. (2007a) studied the effects of monensin on methane production in steers and identified a modest yet long term (6-months) reduction in methane of around 7%.

In a study with dairy cows on pasture, Woodward (2006) examined the effect of vegetable and fish oils on milk production and CH$_4$ emission in short- (14 days) and long-term (12 weeks) experiments. Lipids significantly decreased CH$_4$ production in the short-term study, but this effect was not observed after 11 weeks of feeding lipids in the long-term study.

Promising results in vitro demonstrating inhibition of methane production is not always seen in vivo. Several processes in the live animal, such as: adaptation of the microbes to the additive or degradation of the additive, toxicity for the host animal, negative effects on overall digestion and productive performance can temper the results achieved in vivo (VanNevel and Demeyer, 1996).
4. Conclusion

Several strategies have been explored to mitigate methane production using feed additives in ruminants. Recent reviews suggest that plant extracts (dietary lipids, fatty acids, essential oils and saponins) and some chemical substances (electron receptors) hold the most potential.

Condensed tannins and electron receptors (e.g., nitrate) have known toxicity risks to animals, therefore dose and adaptation periods are necessary. All substances require more in vivo studies over long time periods to determine whether the effect will be sustained.

Their successful implementation will depend on the cost-effectiveness and practicality in commercial farm systems. Solutions must be evaluated in combination with other aspects of livestock production.
5. References


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