Review: Strategies for enteric methane mitigation in cattle fed tropical forages

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(Received 11 December 2019; Accepted 20 July 2020; First published online 18 August 2020)

Methane (CH₄) is a greenhouse gas (GHG) produced and released by eructation to the atmosphere in large volumes by ruminants. Enteric CH₄ contributes significantly to global GHG emissions arising from animal agriculture. It has been contended that tropical grasses produce higher emissions of enteric CH₄ than temperate grasses, when they are fed to ruminants. A number of experiments have been performed in respiration chambers and head-boxes to assess the enteric CH₄ mitigation potential of foliage and pods of tropical plants, as well as nitrates (NO₃⁻) and vegetable oils in practical rations for cattle. On the basis of individual determinations of enteric CH₄ carried out in respiration chambers, the average CH₄ yield for cattle fed low-quality tropical grasses (>70% ration DM) was 17.0 g CH₄/kg DM intake. Results showed that when foliage and ground pods of tropical trees and shrubs were incorporated in cattle rations, methane yield (g CH₄/kg DM intake) was decreased by 10% to 25%, depending on plant species and level of intake of the ration. Incorporation of nitrates and vegetable oils in the ration decreased enteric CH₄ yield by ~6% to ~20%, respectively. Condensed tannins, saponins and starch contained in foliages, pods and seeds of tropical trees and shrubs, as well as nitrates and vegetable oils, can be fed to cattle to mitigate enteric CH₄ emissions under smallholder conditions. Strategies for enteric CH₄ mitigation in cattle grazing low-quality tropical forages can effectively increase productivity while decreasing enteric CH₄ emissions in absolute terms and per unit of product (e.g. meat, milk), thus reducing the contribution of ruminants to GHG emissions and therefore to climate change.

Keywords: tropical grasses, legumes, cattle, rumen fermentation, greenhouse gases

Implications

Feeding strategies designed to mitigate enteric CH₄ emissions in cattle have been tested by incorporating a range of tropical plants (foliages, pods and seeds), nitrates and vegetable oils to rations based on low-quality tropical grasses. These strategies have proved to be effective in decreasing enteric methane emissions by cattle. This could lead to an improvement in profitability of production by reducing the incorporation of expensive feedstuffs in rations and thus to sustainable ruminant production systems for smallholders in the tropical regions of the world.

Introduction

Animal agriculture, in developing countries, will face tremendous challenges with respect to mitigation and adaptation to climate change in the following years as an increasing demand for meat and milk by the burgeoning population.
is expected. This increase in demand is projected to result in an increase in livestock numbers in the near future. Considerable research efforts have been invested in accurately predicting enteric methane emissions from cattle (Escobar-Bahamondes et al., 2016; Eugène et al., 2019; van Lingen et al., 2019) as well as in implementing appropriate enteric CH4 mitigation strategies (Beauchemin et al., 2020) in ruminant production systems.

During the dry season, most tropical grasses have a high NDF and low CP contents, resulting in limited fermentation of DM, long retention time of digesta and low absorption of volatile fatty acids (VFA) from the rumen, leading to modest daily weight gains in growing cattle and considerable emissions of enteric methane. More than 200 000 plant secondary compounds such as condensed tannins, saponins, flavonoids, organosulfur compounds and essential oils (EOs) have been identified as having potential to mitigate CH4 emissions in grazing ruminants (Jafari et al., 2019; Molina-Botero et al., 2019a). Secondary compounds in plants should induce a reduction in CH4 production (Figure 1) without negatively affecting rumen fermentation or being toxic to the animal, and should ideally improve productive performance, increase economic gains, be easy to handle and be of low cost to farmers. The effectiveness of these plant secondary compounds depends on their type, concentration and ingested amount. In the medium term, it is important to identify cost-effective secondary compounds which could improve rumen fermentation, VFA production and mitigate CH4 emissions with a lasting effect. One effective methane mitigation strategy is the inclusion of low levels of oils in rations which induce biohydrogenation of unsaturated fatty acids, increase energy density and inhibit protozoa (Knapp et al., 2014). A number of comprehensive reviews on methane mitigation in ruminants have been published recently (Berndt and Tomkins, 2013; Knapp et al., 2014; Beauchemin et al., 2020), but relatively few have emphasized the current situation in tropical cattle production systems in developing countries.

Ruminant production in developing countries (mostly in the tropics) faces enormous challenges to both increase productivity and decrease greenhouse gas (GHG) emissions (Ayantunde et al., 2005). The achievement of CH4 mitigation at the farm level can bring environmental benefits and increase profits for small farmers in tropical regions. Small-scale cattle farmers in the tropical regions of Latin America and the Caribbean, Asia and Africa confront formidable obstacles in achieving sustainable intensification of animal production (Ndung’u et al., 2019). Poverty, poor literacy, scarcity of financial resources (credits), limited access to markets, lack of technical support and paradoxically, the ominous impacts of climate change (droughts, floods and fires) are just but a few of the present constraints.

The rationale behind this review is to describe and critically discuss the advantages of one of the simplest and most direct options for enteric CH4 mitigation available to small-scale cattle farmers in the tropics, namely the incorporation of foliages, seeds and pods of widely distributed tropical plants, by-products and additives in practical rations.

**Enteric methane production by cattle fed tropical grasses**

There are many factors influencing CH4 yield such as type of grass (C3 vs. C4), chemical composition, forage:concentrate ratio, rate of passage of digesta through the rumen, physiological stage, breed, sex and it is assumed that all those are integrated within the single expression of DM intake (Archimède et al., 2011 and 2018). Dry matter intake is

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**Figure 1** Metabolic and digestive effects of condensed tannins, essential oils and saponins on methane synthesis and animal production. (−): decrease; (+): increase. Modified from Valencia Salazar (2017).
the result of both grass and cattle interactions which affect rumen fermentation and, as such, represents a convenient basis for estimating enteric methane production. More work is required by research groups in tropical regions on the establishment of robust data sets from which accurate CH₄ yields could be derived for methane inventory purposes (Patra, 2017; Castelán Ortega et al., 2019; Goopy et al., 2020) and for evaluation of effective enteric CH₄ mitigation strategies.

A series of experiments involving methane emissions were carried out in Mexico with cattle housed in open-circuit respiration chambers and fed basal rations of low-quality tropical grass (>70% of ration DM) and a supplement (concentrate). Preliminary results showed a methane yield of 17.4 g CH₄/kg DMI in Nellore (Bos indicus) bulls that were fed chopped Pennisetum purpureum grass (Canul-Solís et al., 2017), a value that compares reasonably well with the predicted methane yield of 18.0 g CH₄/kg DM intake (n = 66; R² = 0.73) in cattle fed low quality tropical grass (~70% ration DM) reported by Ku-Vera et al. (2018) and with the methane yield reported by Noguera and Posada-Ochoa (2017) who reported 16.1 g CH₄/kg DMI in lactating cows fed Pennisetum clandestinum in Colombia. The values reported by the cited studies (Noguera and Posada-Ochoa, 2017; Canul-Solís et al., 2017; Ku-Vera et al., 2018) represent an approximation for cattle fed tropical grasses, and they are comparable to some extent to the data from Charmley et al. (2016) who reported a methane yield of 19.3 g CH₄/kg DMI (n = 133) for Zebu (Brahman) cattle fed tropical grasses (>70% of DM) as a basal ration in Australia. Although it has been claimed that tropical (C4) grasses contribute to enteric methane emissions to a greater extent than temperate (C3) grasses (Archimède et al., 2018), the evidence hereby presented does not support that contention (Benaouda, 2018; Goopy et al., 2018; Table 1). The overall enteric methane yield from experiments carried out in Mexico in respiration chambers is 17.0 g CH₄/kg DMI (n = 125) for cattle consuming low-quality tropical grasses. This relatively low yield could be partially explained by the low rate and extent of fermentation of DM (~40%) in the rumen of cattle fed tropical grass during the dry season. Strategic grazing management (with a canopy with light interception of 95%) results in reduction in CH₄ emission intensity and greater milk yield in grazing cows, as well as 18% less CH₄/kg DM intake (Congio et al., 2018). The so-called rotational grazing management (Savian et al., 2018) also results in a decrease in CH₄ emission intensity by carefully controlling grazing behavior of ruminants. Both approaches support the contention that it is through grazing management that CH₄ emission intensity in ruminants can be decreased without any other further intervention. Nonetheless, Berça et al. (2019) found no reductions in enteric methane emissions of crossbred dairy heifers grazing Urochloa brizantha cv. Marandu either fertilized or with the inclusion of the legume Arachis pintoi in Brazil. There are several possible strategies to mitigate methane emissions in ruminants that are fed low-quality tropical grasses. The selection criteria for a particular strategy must be based on sound economic grounds, as smallholders are limited economically in their capacity to buy expensive methane mitigating additives (e.g. monensin, 3-nitrooxypropanol). Leucaena leucocephala, a native legume shrub from Mexico and Central America, ranks first among the options available to develop sustainable beef cattle farming systems in tropical regions (Shelton and Dalzell, 2007; Harrison et al., 2015; Ramírez-Avilés et al., 2019). In Colombia, Molina et al. (2016) fed Lucerna heifers with a Cynodon plectostachys (74% DM) and Leucaena leucocephala (26% DM) ration while cattle were housed in polytunnels for methane measurements and recorded a lower intensity of methane emissions compared to heifers fed only the tropical grass. Also in Colombia, Sierra-Montoya et al. (2017) found a reasonably good milk yield (~10 to 12 l cow/day) in recently

<table>
<thead>
<tr>
<th>Cattle (breed, sex)</th>
<th>LW (kg)</th>
<th>Ration</th>
<th>DMI (kg/day)</th>
<th>CH₄ (g/day)</th>
<th>CH₄ (g/kg DMI)</th>
<th>Yᵢ (%)</th>
<th>GEI</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holstein cows</td>
<td>541</td>
<td>Pennisetum clandestinum</td>
<td>18.5</td>
<td>287</td>
<td>16.1</td>
<td>3.8</td>
<td>Colombia</td>
<td>Noguera and Posada-Ochoa (2017)</td>
<td></td>
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<tr>
<td>Holstein × Gyr cows</td>
<td>–</td>
<td>TMR (maize silage, Tifton hay, concentrate)</td>
<td>20.2</td>
<td>382.7</td>
<td>18.9</td>
<td></td>
<td>Brazil</td>
<td>Machado et al. (2016)</td>
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<tr>
<td>Crossbred heifers</td>
<td>327</td>
<td>Megathyrsus maximus hay + supplement</td>
<td>8.1</td>
<td>129.4</td>
<td>16.0</td>
<td>5.2</td>
<td>Mexico</td>
<td>Zavala-Escalante, L. unpublished</td>
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<tr>
<td>Boran steers</td>
<td>162.3</td>
<td>Chloris gayana hay</td>
<td>4.6</td>
<td>133.3</td>
<td>29.0</td>
<td>9.14</td>
<td>Kenya</td>
<td>Goopy et al. (2020)</td>
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<tr>
<td>Brahman steers</td>
<td>227</td>
<td>H. contortus C. ciliaris</td>
<td>4.0</td>
<td>99.3</td>
<td>19.6</td>
<td>6.3</td>
<td>Australia</td>
<td>Kennedy and Charmley (2012)</td>
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<tr>
<td>Brahman steers</td>
<td>309</td>
<td>H. contortus hay ad libitum</td>
<td>4.9</td>
<td>94.7</td>
<td>19.3</td>
<td>5.7</td>
<td>Australia</td>
<td>Charmley et al. (2016)</td>
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<tr>
<td>Brahman steers</td>
<td>266.4</td>
<td>Chloris gayana hay ad libitum</td>
<td>4.1</td>
<td>80 to 5</td>
<td>19.5</td>
<td>5.8</td>
<td>Australia</td>
<td>Perry et al. (2017)</td>
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<tr>
<td>Brahman steers</td>
<td>342.7</td>
<td>Digitaria eriantha</td>
<td>3.5</td>
<td>119.3</td>
<td>34.1</td>
<td>11.4</td>
<td>Thailand</td>
<td>Chaokaur et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>Tropical cattle</td>
<td>277</td>
<td>Crop residues and by-products</td>
<td>4.6</td>
<td>123</td>
<td>26.7</td>
<td>8.2</td>
<td>Thailand</td>
<td>Kaewpila and Sommart (2016)</td>
<td></td>
</tr>
</tbody>
</table>

LW = live weight; DMI = DM intake; CH₄ = methane; TMR = total mixed ration; GEI = gross energy intake; Yᵢ = CH₄ loss expressed as percentage of GEI.

https://doi.org/10.1017/S1751731120001780 Published online by Cambridge University Press
calved or early lactating Gyr × Holstein cows grazing a silvopastoral system consisting of *Cynodon plectostachyus* and *Leucaena leucocephala*, obtaining a good balance of metabolizable protein to metabolizable energy.

**Enteric methane mitigation strategies in cattle under small-scale farming conditions**

Before adoption or implementation at farm level, an enteric methane mitigation strategy must induce a significant extent of mitigation as demonstrated in various experiments, published equations applicable to various types of ruminants, feasibility of adoption by farmers, cost-effectiveness analysis and repeatability (Eugène et al., 2019), as well as persistency. This review addresses three different options for mitigation: secondary compounds contained in plant species available in tropical regions that can be used in silvopastoral systems, high-energy compounds such as oils and finally a chemical compound exemplified by the feeding of additives such as nitrates. Nitrates require attention, particularly under the conditions of tropical ruminant production, since they supply N, an element which is usually deficient in C4 grasses during the long dry season.

**Foliage and pods of tropical plants as a methane mitigation strategy**

Foliages, seeds and pods of tropical trees and shrubs have been considered as important components of ruminant rations long ago (Topps, 1992; Shelton and Dalzell, 2007). Secondary compounds contained in such plant species exert different effects on the rumen microbial population (Patra and Saxena, 2009). Piñeiro-Vázquez et al. (2018) reported that foliage of the tropical legume *Leucaena leucocephala* induced a reduction in methane yield when it was included in ration DM in high proportions (40% to 80%) in crossbred heifers (*Bos indicus* × *Bos taurus*) housed in respiration chambers that were fed a basal ration of low-quality tropical grass (Table 2). However, the increase in CP intake from *L. leucocephala* induced higher N excretion (as urea) in the urine, probably leading to higher nitrous oxide emissions (Montoya-Flores et al., 2020). This agrees with data reported by Harrison et al. (2015) who also found a reduction in enteric methane emissions, although nitrous oxide emissions increased in cattle fed *Leucaena leucocephala* in Australia. In a set of experiments involving the incorporation of foliages such as *Leucaena leucocephala* or *Gliricidia sepium* (Molina-Botero et al., 2019a; Montoya-Flores et al., 2020) or ground pods of *Enterolobium cyclocarpum* or *Samanea saman* (Lazos-Balbuena, 2015; Valencia Salazar et al., 2018), a reduction in enteric methane yield of different magnitudes was recorded in cattle housed in respiration chambers (Table 2).

Pods of tropical legumes such as *Enterolobium cyclocarpum* and *Acacia penpellata* contain either condensed tannins or saponins, and when fed ground at 45% of ration DM, weight gains of up to 240 g/lamb per day were obtained in small-scale sheep farms (Briceno-Poot et al., 2012). Pods from those legumes can be hand collected by smallholders, stored in bags, ground and directly fed to cattle and sheep up to 50% of ration DM (Briceno-Poot et al., 2012). Pods are palatable and they are eagerly consumed by cattle and sheep after falling from the trees during the dry season. Rumen DM degradability of ground pods is high: 87% for *Enterolobium cyclocarpum* (Piñeiro-Vázquez et al., 2013) and 80% for *Samanea saman* (Valencia Salazar et al., 2018); they provide (through anaerobic fermentation) the necessary ATP for the maintenance and growth of bacteria in the rumen. The high level of rumen DM degradation of tropical pods suggests that they contain a high concentration of readily available carbohydrates (i.e. pectins, starch), which may change the pattern of rumen fermentation (Valencia Salazar et al., 2018) and decrease methane production per kg of DM consumed. Table 2 shows the magnitude of reduction in enteric methane yield when cattle were fed foliage or pods from tropical trees. Both options are simple and straightforward strategies for reducing methane emissions in farms under smallholder conditions in the tropics.

The proportion of gross energy intake (GEI) lost as methane gas: $Y_m$ (Table 2) ranged from 5.0% to 9.5% throughout the experiments, and it generally decreased as the levels of mitigating compounds (i.e. foliage, pods) were incorporated at increasing levels in the rations. Those authors found that $Y_m$ fluctuated between 4.8% and 13.7% and that IPCC (2006) procedures underestimated $Y_m$ by ~26.1%. Therefore, the derivation of precise $Y_m$ values remains an important challenge for estimating CH$_4$ emissions from *Bos indicus* type of cattle fed low-quality forages in tropical developing countries. In Thailand, Chaokaur et al. (2015) working with Brahman cattle fed Pangola (*Digitaria eriantha*) grass as basal ration found that $Y_m$ ranged between 7.3% and 11.5% (above the IPCC (2006) default value of 6.5%) and that enteric methane emissions were linearly increased from the maintenance level of feeding up to ad libitum intake.

There are several possible explanations for the reduction of enteric methane emissions as a result of feeding foliage and pods of tropical plant species to cattle and sheep (see Figure 1). Condensed tannins contained in such plants may reduce CH$_4$ in two different ways, first, by directly inhibiting methanogenic archaea, and second by decreasing H$_2$ availability in the rumen and the apparent digestibility of DM and protozoal population (Patra et al., 2017; Piñeiro-Vázquez et al., 2018; Aboagye (2018 and 2019) reported a reduction in fiber digestibility, rather than a decrease in the amount of structural carbohydrates. Nonetheless, Canul-Solis et al. (2014) found no effect of saponins (6 g/day) of *Yucca schidigera* on methane emissions of hair sheep fed low-quality tropical grass; this could be associated with the lack of effect on methanogenic archaea or the relatively low levels of incorporation of the mitigating commercial compound.

Saponins in pods such as those in *Samanea saman* may form complexes with sterols in the protozoal membrane.
surface and induce lysis of such membrane (Anantasook et al., 2014), thus reducing the population of methanogenic archaea which are usually associated with protozoa. Incorporation of foliage and pods of plants containing condensed tannins and steroidal saponins in ruminant rations can increase the molar proportion of propionic acid in rumen liquor and decrease the availability of metabolic H which is the specific substrate for carbon dioxide reduction to methane (Valencia-Salazar et al., 2018). Feeding of starch-containing ingredients in the ration such as in the pods of the legume tree *Samanea saman* (rain tree) may also drive a reduction in methane emissions by changing the pattern of rumen fermentation, increasing the molar proportion of propionic acid and lowering that of acetic acid in the rumen (Valencia-Salazar et al., 2018). Anantasook et al. (2014) similarly reported an increase in propionic acid concentration in the rumen and a reduction in methane production, when they fed pods of *Samanea saman* to dairy steers fed urea-treated rice straw as basal ration. Ground pods of *Samanea saman* have been successfully used for feeding dairy cows (Anantasook et al., 2014) in Thailand. Changes in the molar proportions of VFA in the rumen (i.e. increasing

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Level of incorporation (% DM)</th>
<th>DMI (kg/day)</th>
<th>CH₄ (g/day)</th>
<th>CH₄ (g/kg DMI)</th>
<th>Yᵣ (%)</th>
<th>Design and statistical analysis</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterolobium cyclocarpum</em> (ground dry pods)</td>
<td>Cattle</td>
<td>0</td>
<td>8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108.20</td>
<td>12.86</td>
<td>–</td>
<td>4 × 4 crossover</td>
<td>Lazo-Balbuena (2015) (Thesis)</td>
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<td></td>
<td></td>
<td>12</td>
<td>9.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>97.80</td>
<td>10.02</td>
<td>–</td>
<td>LSD</td>
<td>Tukey’s test</td>
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<td></td>
<td></td>
<td>24</td>
<td>10.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.02</td>
<td>10.00</td>
<td>–</td>
<td>Surface response</td>
<td>Piñeiro-Vázquez et al. (2018)</td>
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<tr>
<td></td>
<td></td>
<td>36</td>
<td>10.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.68</td>
<td>9.89</td>
<td>–</td>
<td>LSD</td>
<td>Analysis</td>
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<tr>
<td></td>
<td>SE</td>
<td>0.26</td>
<td>6.90</td>
<td>1.01</td>
<td>–</td>
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<td></td>
<td>P-value</td>
<td>0.018</td>
<td>ns</td>
<td>ns</td>
<td>–</td>
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<td>–</td>
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<tr>
<td><em>Leucaena leucocephala</em> (fresh forage)</td>
<td>Cattle</td>
<td>0</td>
<td>7.03</td>
<td>104.51</td>
<td>15.30</td>
<td>5.42&lt;sup&gt;–4&lt;/sup&gt;</td>
<td>5 × 5 crossover</td>
<td>Piñeiro-Vázquez et al. (2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>7.15</td>
<td>77.03</td>
<td>11.18</td>
<td>4.00&lt;sup&gt;–24&lt;/sup&gt;</td>
<td>LSD</td>
<td>Analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>7.07</td>
<td>66.52</td>
<td>9.21</td>
<td>3.44&lt;sup&gt;–60&lt;/sup&gt;</td>
<td>Surface response</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>60</td>
<td>7.00</td>
<td>57.01</td>
<td>7.99</td>
<td>2.95&lt;sup&gt;–80&lt;/sup&gt;</td>
<td>–</td>
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<td></td>
<td></td>
<td>80</td>
<td>7.00</td>
<td>40.72</td>
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<td>SE</td>
<td>0.60</td>
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<td>1.59</td>
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<tr>
<td></td>
<td>Linear contrast</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td><em>Samanea saman</em> (ground dry pods)</td>
<td>Cattle</td>
<td>0</td>
<td>6.26</td>
<td>87.14</td>
<td>13.73</td>
<td>4.70&lt;sup&gt;–20&lt;/sup&gt;</td>
<td>4 × 4 crossover</td>
<td>Valencia-Salazar et al. (2018)</td>
</tr>
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<td></td>
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<td>10</td>
<td>6.44</td>
<td>64.64</td>
<td>10.06</td>
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<td>Analysis</td>
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<tr>
<td></td>
<td></td>
<td>20</td>
<td>6.16</td>
<td>51.94</td>
<td>9.12</td>
<td>2.82&lt;sup&gt;–60&lt;/sup&gt;</td>
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<td>6.49</td>
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<td>2.15&lt;sup&gt;–80&lt;/sup&gt;</td>
<td>–</td>
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<tr>
<td></td>
<td>SE</td>
<td>0.28</td>
<td>6.77</td>
<td>0.78</td>
<td>0.31</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Linear contrast</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Leucaena leucocephala</em> (dry leaves)</td>
<td>Cattle</td>
<td>0</td>
<td>8.36</td>
<td>174.2</td>
<td>20.8</td>
<td>6.5&lt;sup&gt;–12&lt;/sup&gt;</td>
<td>4 × 4 crossover</td>
<td>Montoya-Flores et al. (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>8.32</td>
<td>162.9</td>
<td>19.7</td>
<td>6.07&lt;sup&gt;–24&lt;/sup&gt;</td>
<td>LSD</td>
<td>Analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>8.63</td>
<td>154.8</td>
<td>18.0</td>
<td>5.54&lt;sup&gt;–36&lt;/sup&gt;</td>
<td>Surface response</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
<td>8.54</td>
<td>140.0</td>
<td>16.5</td>
<td>5.05&lt;sup&gt;–54&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.13</td>
<td>3.03</td>
<td>0.41</td>
<td>0.13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Linear contrast</td>
<td>0.09</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>E. cyclocarpum</em> (ground dry pods) + <em>Glicidícea sepium</em> (dry foliage) 50%</td>
<td>Cattle</td>
<td>0</td>
<td>5.18</td>
<td>144.8</td>
<td>28.50</td>
<td>9.57&lt;sup&gt;–15&lt;/sup&gt;</td>
<td>4 × 4 crossover</td>
<td>Molina-Botero et al. (2015a)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.23</td>
<td>140.1</td>
<td>26.80</td>
<td>9.09&lt;sup&gt;–45&lt;/sup&gt;</td>
<td>LSD</td>
<td>Analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.57</td>
<td>141.3</td>
<td>25.56</td>
<td>8.57&lt;sup&gt;–75&lt;/sup&gt;</td>
<td>Surface response</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>5.57</td>
<td>143.3</td>
<td>25.89</td>
<td>8.80&lt;sup&gt;–100&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td></td>
<td>SE</td>
<td>0.33</td>
<td>4.88</td>
<td>1.50</td>
<td>0.51</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Linear contrast</td>
<td>0.989</td>
<td>0.788</td>
<td>0.050</td>
<td>0.051</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Quadratic contrast</td>
<td>0.258</td>
<td>0.218</td>
<td>0.299</td>
<td>0.281</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Cubic contrast</td>
<td>0.500</td>
<td>0.655</td>
<td>0.689</td>
<td>0.683</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>E. cyclocarpum</em> (ground dry pods) + <em>G. sepium</em> (dry foliage) 50%</td>
<td>Cattle</td>
<td>E + G : 0</td>
<td>6.11</td>
<td>145.99</td>
<td>23.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Repeated measures</td>
<td>Molina-Botero et al. (2019b)</td>
</tr>
<tr>
<td></td>
<td>E + G : 15</td>
<td>6.62</td>
<td>147.78</td>
<td>23.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>Tukey’s test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.20</td>
<td>3.02</td>
<td>0.33</td>
<td>0.11</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>P-value diet</td>
<td>0.598</td>
<td>0.631</td>
<td>0.016</td>
<td>0.016</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>P-value time</td>
<td>0.018</td>
<td>0.262</td>
<td>0.885</td>
<td>0.886</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>P-value (diet × time)</td>
<td>0.383</td>
<td>0.440</td>
<td>0.682</td>
<td>0.682</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

CH₄ = methane; DMI = DM intake; Yᵣ = CH₄ loss expressed as percentage of gross energy intake (GEI); LSD = Latin square design; ns = non-significant (P > 0.05).

<sup>a,b</sup>Means in the same column with different superscript are statistically different according to Tukey’s test (P > 0.05) (for experiments that used Tukey’s test).

**P < 0.01.

https://doi.org/10.1017/S1751731120001780 Published online by Cambridge University Press
propionic acid relative to acetic acid) may improve the efficiency of metabolizable energy utilization by decreasing heat increment and increasing energy retention as protein and fat synthesis (weight gain) in the body (NASEM, 2016) or in milk as fat and lactose. Thus, this kind of mitigation strategy may be more acceptable to smallholders, since they would see their effort compensated in both productivity and probably in financial revenues.

An increase in animal productivity has been observed under production conditions in cattle and sheep in small farms where both leucaena and pod legume incorporation in practical rations were implemented. Growing crossbred bulls showed a daily weight gain of 770 g, while grazing in a silvopastoral system with *Leucaena leucocephala* (Mayo-Eusebio, 2013) and confined hair sheep gained 240 g/day when pods of *Enterolobium cyclocarpum* were incorporated at 50% (DM basis) of their ration (Briceno-Poot et al., 2012) under smallholder farming conditions. These results agree with those of Harrison et al. (2015) with grazing cows on leucaena pastures in Australia and Moscoso et al. (1995) with confined sheep fed ground pods of *Enterolobium cyclocarpum* (36% of ration DM) in Honduras (Central America). We do not fully understand the lack of changes in terms of the rumen microbial population when using tropical feedstuffs containing secondary compounds. So far, the work carried out on DNA (qPCR) techniques for counting the rumen microbial population has been inconclusive with regard to changes in the composition of the microbial population. No changes have been observed so far in the structure of the rumen population when cattle were fed either *Leucaena leucocephala* or *Enterolobium cyclocarpum*. Nonetheless, the length of time of feeding apparently does not lead to an adaptation of the rumen microbial population to secondary compounds (such as the saponins contained in pods of *Enterolobium cyclocarpum*) (Molina-Botero et al., 2019b). Disposal of H₂ in the rumen through methanogenesis is aimed at maintaining the partial rumen pressure of H₂ low so that fermentation can proceed efficiently. So far, there is still not a plausible explanation for the fate of the excess metabolic H as a result of methane mitigation in ruminants fed tropical C₄ grasses (4 mol H₂ remains unused per mol CH₄ decreased; Hegarty and Gerdes, 1999). No alternative H₂ sink has been proposed as yet, although it is recognized that there may be an excess H₂ in the rumen when metabolizing compounds are fed (up to 35.9 g/day in heifers; Romero-Perez et al. (2015)). Some of the excess H₂ in the treatments involving incorporation of mitigating compounds may have been incorporated into rumen microbes (Kennedy and Charmley, 2012). Nevertheless, more work has to be done on this subject. Rumen pH of cattle fed foliage and pods of tropical plants where substantial reductions in methane emissions have been recorded showed no apparent changes 4 h after feeding (Valencia-Salazar et al., 2018; Montoya-Flores et al., 2020). Since rumination is normally carried out in such conditions, rumen pH generally remains within the physiological range (6 to 7), as well as the osmotic pressure (i.e. 250 to 300 mosmol/kg).

Essential oils are secondary compounds (alcohol, ester or aldehyde derivatives of phenylpropanoids and terpenoids) present in some tropical plants. They are volatile, aromatic substances. Essential oils display antimicrobial actions related to processes in the bacterial cell membrane involving electron transport, ion gradients, phosphorylation and protein translocation (Benchaar et al., 2008). Essential oils exert a high affinity for lipids in cell membranes of bacteria which results from their hydrophobic nature and their antibacterial actions are related to their lipophilic character; thus, they can disrupt the cytoplasmic membrane either directly or by damaging proteins in the membrane, resulting in increased permeability of the membrane and then leakage of cytoplasmic constituents thus affecting the proton motive force (Hart et al., 2008). Scientific evidence on the potential of EOs to reduce enteric methane in ruminants *in vivo* in tropical regions is still scarce (Wang et al., 2018). Effective doses, mode of action, voluntary intake and the cost-benefit ratio must be assessed. Most of the studies involving EOs have been conducted under *in vitro* conditions (Ye et al., 2018) and those results must be validated *in vivo* at the farm level under smallholder conditions. Recent work, carried out at the University of Yucatan in Mexico, with crossbred heifers held in respiration chambers (Jimenez-Ocampo, R. *unpublished* results) has shown that EO from *Citrus sinensis* (0.5% DM) reduced methane yield in heifers fed low-quality tropical grasses by up to ~18% (compared to a control ration without oil), which agrees with experiments carried out in Hu sheep supplemented with EO from grapefruit peels (Wu et al., 2018). Figure 1 shows the mechanisms of action of some secondary compounds for mitigation of enteric methane in ruminants. The mechanisms are rather different: condensed tannins may both affect feed digestibility and inhibit archaea, saponins may disrupt protozoa membranes and reduce the population of methanogenic archaea, while EOs may affect protozoa and methanogens, depending on the type and concentration. In the tropics, there is a wide range of plants which may induce such mechanisms in the rumen of cattle under practical feeding conditions.

*Nitrate supplementation as a methane mitigation strategy* Nitrate (NO₃⁻) has been proposed as a replacement for urea (non-protein N) as a source of rumen degradable N for the rumen microbes (Lee and Beauchemin, 2014). Therefore, nitrate could work well under dry season cattle farming conditions in the tropics when grasses are characterized by deficient N concentration for optimal microbial growth supplying N essential for adequate bacterial protein synthesis (Leng, 1990). Nitrate is an alternative H sink which competes with methane synthesis; the stoichiometric potential of 1 mol of nitrate can reduce methane synthesis by 1 mol (Leng, 2008) and nitrate can decrease the number of rumen protozoa resulting in CH₄ reduction. Nitrate reduction in the rumen to nitrite and then to NH₃ is energetically more favorable than carbon dioxide reduction for methane synthesis, which implies that 4 mol H₂ is redirected toward nitrate reduction. Therefore, methane production can be mitigated by 1 mol per
each mole of nitrate reduced, which is equivalent to a CH₄ emission reduction of 25.8/100 g nitrate fed. However, high levels of nitrate in the rumen may induce toxicity, as nitrate is transformed into nitrite and an excess may induce an increase in methemoglobin (MetHb) in the bloodstream, leading to hypoxia, dyspnea and even death if the animal is not treated immediately (Callaghan et al., 2014). Nitrate toxicity and its potentially hazardous consequences can be avoided by supplying less than 9 g NO₃/kg DM intake in cattle, but the concentrations of basal NO₃ in the consumed grass must also be accounted for.

Concentrations of NO₃ are related to plant species, phenological stage and other plant stressors (e.g. drought, fertilization). According to Callaghan et al. (2014), in Northern Australia they recommended ~15 g/day of NO₃ in breeding beef cows grazing dry season pastures. Nonetheless, there is a risk in the control of the intake of the supplement and the heterogeneous structure of herds of grazing cattle with different feed intakes. An experiment was carried out to evaluate the effect of dietary nitrate on enteric methane emissions in dairy cows grazing pastures during the summer and it was found that 8 g NO₃/kg DM in grazing dairy cows led to a decrease in CH₄ emissions (van Wyngaard et al., 2018). Hulshof et al. (2012) using sugarcane-based rations reported that 22 g NO₃/kg DM reduced enteric methane emission by 32%.

In the dry season, tropical grasses usually contain low concentrations of CP and nitrates could supply the N required for improving rumen fermentation of low-quality basal rations. A gradual adaptation period of 15 days and the use of slow-release NO₃ in the ration are alternative ways of reducing the risk of toxicity (Alemu et al., 2019). El-Zaiat et al. (2014) showed that encapsulated nitrate was not toxic to lambs (4.51% encapsulated nitrate product (60.83% nitrate in the product DM)). At the same time, those authors identified higher risks of toxicity in rations with low levels of readily fermented carbohydrates. For that reason, a practice that reduces feeding problems is to supply non-structural carbohydrates in the ration, as grains (i.e. starch) non-structural carbohydrates in the ration, as grains (i.e. starch) reason, a practice that reduces feeding problems is to supply low levels of readily fermented carbohydrates. For that authors identified higher risks of toxicity in rations with increasing levels of NO₃ (0.1%, 0.2% and 0.3% DM) to a basal ration of low-quality chopped tropical grass (Pennisetum purpureum) resulted in a substantial reduction in enteric CH₄ emissions from 135.5 g/day (0% NO₃) to 93.3 g/day (with 0.3% NO₃ DM), although a reduction in DM intake was also noticed. Nitrate seems to be a viable methane mitigating additive which simultaneously supplies N to the rumen microorganisms during the dry season when N concentration in tropical grasses is deficient for optimal microbial protein synthesis in the rumen.

**Lipid supplementation as a methane mitigation strategy**

Lipid sources as a methane mitigation strategy under tropical conditions (ratios with high NDF and low digestibility and intake) can play an important role in the improvement of cattle performance and therefore in farm productivity. In their comprehensive review, Grainger and Beauchemin (2011) suggested that the mitigation potential of fats is 1 g CH₄/kg DM intake for each 10 g fat/kg DM in the ration and this has shown persistent results, although no effect of the chain length of the fatty acids on the magnitude of mitigation has been detected. Feeding sunflower oil can effectively reduce enteric methane emissions of dairy cows (Bayat et al., 2017). Similar effects of vegetable oils on reducing methane emissions have been reported under in vitro conditions (Vargas et al., 2020). Recent work carried out at the University of Yucatan, Mexico (Flores-Santiago, E. unpublished results), has shown that by feeding palm oil (6% of ration DM) to hair Pelibuey sheep housed in respiration chambers, which were fed a low-quality basal ration of tropical C4 grass (Brachiaria brizantha) hay, methane yield was reduced by ~14%, compared to a control treatment without palm oil. Palm oil contains saturated fatty acids (50%; palmitic acid: 44% and stearic acid: 5%) and unsaturated fatty acids (oleic acid: 40% and polyunsaturated linoleic and α-linoleic acid: 10%). Palm oil is available for ruminant feeding in several Latin American and other tropical countries.

A number of experiments have been carried out in Brazil with grazing cattle supplemented with diverse sources of lipids. Mata e Silva et al. (2017) found that sunflower oil (383 g/day) given to lactating dairy cows grazing a tropical pasture (Urochloa brizantha) decreased enteric CH₄ by ~23%. Neto et al. (2015) reported that grazing Nellore bulls supplemented with a concentrate containing oil was an effective means for reducing enteric methane emissions as a percentage of GEI by 12.8%. Similarly, Carvalho et al. (2016) supplemented Nellore steers grazing Brachiaria brizantha with linseed oil and found a reduction in enteric methane emissions. In Nellore bulls grazing Brachiaria brizantha supplemented with starch, the use of oil (~198 g soybean grain/kg DM) reduced enteric CH₄ emissions (Neto et al., 2019).

**Practical implications of methane mitigation in tropical cattle systems**

There is a shortage of information on enteric methane emissions from dairy and beef cattle production systems in the tropics (Bartl et al., 2011; Niu et al., 2018). Enteric CH₄ mitigation in small-scale cattle farms may be a rather
Conclusions

Foliages, pods and seeds of tropical trees and shrubs containing either condensed tannins, EOs, saponins or starch, as well as other compounds such as nitrates and by-products such as vegetable oils can be fed to cattle to mitigate enteric methane emissions under smallholder conditions. Methane yields can be reduced by approximately 10% to 25% by adopting those practices in ruminant production systems. Silvopastoral systems with high-quality legumes (i.e. leucaena, desmanthus), intensification of grazing management (i.e. rotatinous) and supplementation with nutritional blocks containing CH₄ depressors (pods of Enterolobium cyclocarpum or Samanea saman or seeds of Brosimum alicastrum) are practical options available to smallholders for mitigating enteric methane emissions in cattle raised under tropical conditions.

Acknowledgements

A preliminary version of this paper was published in an abstract form at the 7th Greenhouse Gas and Animal
Agriculture Conference 2019, held at Iguassu Falls, Brazil. We are indebted to CONACYT-Mexico for granting fellowships to students to carry out MSc and PhD studies, as well as postdoctoral research at the University of Yucatan, Mexico. We thank the State Government of Yucatan (SIIDETEY)-Mexico for financial support to keep the respiration chambers running. We are grateful to CONACYT-Mexico for support (CB: A1-S-23910; Convocatoria 2018-1) to carry out experiments on energy metabolism of cattle. This work was implemented as part of the CGIAR Research Programs on Livestock and Climate Change, Agriculture and Food Security (CCAFS), which are carried out with support from CGIAR Fund Donors and through bilateral funding agreements. For details please visit https://ccafs.cgiar.org/donors. The views expressed in this document cannot be taken to reflect the official opinions of these organizations. We thank all donors that globally support our work through their contributions to the CGIAR system. We are indebted to the anonymous referees of this paper, for their kind suggestions towards its improvement.

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Declaration of interest
The authors declare no conflict of interest.

Ethics statement
The authors did not use any live animal to conduct this review.

Software and data repository resources
None of the data were deposited in an official repository.

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Methane mitigation in cattle in tropical regions


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