Effect of tannins from tropical plants on methane production from ruminants: A systematic review

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ABSTRACT

Methane (CH₄) is a greenhouse gas generated during the feed fermentation processes in the rumen. However, numerous studies have been conducted to determine the capacity of plant secondary metabolites to enhance ruminal fermentation and decrease CH₄ production, especially those plants rich in tannins. This review conducted a descriptive analysis and meta-analysis of the use of tannin-rich plants in tropical regions to mitigate CH₄ production from livestock. The aim of this study was to analyse the effect of tannins supplementation in tropical plants on CH₄ production in ruminants using a meta-analytic approach and the effect on microbial population. Sources of heterogeneity were explored using a meta-regression analysis. Final database was integrated by a total of 14 trials. The ‘meta’ package in R statistical software was used to conduct the meta-analyses. The covariates defined a priori in the current meta-regression were inclusion level, species (sheep, beef cattle, dairy cattle, and cross-bred heifers) and plant. Results showed that supplementation with tropical plants with tannin contents have the greatest effects on CH₄ emission (−0.09), which means that the effect of CH₄ mitigation is increasing as the level of tannin inclusion is higher. Therefore, less CH₄ production will be obtained when supplementing tropical plants in the diet with a high dose of tannins.

1. Introduction

A major problem facing our world today is climate change caused by the emission of greenhouse gases (GHG) of anthropogenic activity (Cardona-Iglesias, Mahecha-Ledesma, and Angulo-Arizala, 2016). Overall, livestock contributes to 14–15% of the anthropogenic GHG emissions, and ruminants are responsible for two-thirds of this production (FAO, 2013; Gerber et al., 2013). These GHG could be methane (CH₄), carbon dioxide (CO₂), and nitrous oxide (NO₂) which are major contributors to global warming (Ugbogu et al., 2019). In particular, ruminants produce around 115 million tons of CH₄ per year, a gas generated from rumen fermentation, carried out by a microbial complex of bacteria, archaea, protozoa and fungi, called “ruminal microbiota” (Sandoval-Pelcastre, Ramírez-Mella, Rodríguez-Avila, & Candelaria-Martínez, 2020).

Because CH₄ has a global warming effect 23 times greater than CO₂ (Ugbogu et al., 2019), the increase in global temperature is having effects on many species of animals and plants. These effects will increase in the coming years, causing crops and fodder to be affected by extreme weather (Olesen and Bindi, 2002). In the search for solutions to reduce
GHG emissions, the use of tropical plants with anti-methanogenic potential has been suggested (Canul-Solis et al., 2020; Jayanegara et al., 2020; Ku-Vera et al., 2020a,b; Rivera et al., 2015). However, due to the great diversity of tropical plant species with an anti-methanogenic effect, more research is needed in order to assess which ones have major impact on CH\textsubscript{4} emissions and the amount whereby the need to be included in the diet (Sandoval-Pelcastre, Ramirez-Mella, Rodríguez-Avila, & Candelaria-Martínez, 2020). Ruminant production systems in the tropics and subtropical areas are characterized by grazing native and introduced grasses varying in quantity and quality throughout the year (Becholje, Tamir, Terrill, Singh, and Kassa, 2005). Tropical trees (TT) as Leucaena leucocephala, Acacia pennatula, Enterolobium cyclocarpum, Gliricidia sepium may contribute to an improvement in ruminants’ feeding due to their high nutritive value (Topps, 1992). Furthermore, TT contain a range of plant secondary metabolites (PSM) (Montoya-Flores et al., 2020; Píneiro-Vázquez et al., 2018), which could alter rumen fermentation and consequently reduce CH\textsubscript{4} emissions (El-Zaiat et al., 2020; Píneiro-Vázquez et al., 2018).

Tannins are contained into the PSM, and reduce methane due to their inhibitory effect on methanogens, protozoa and other hydrogen-producing microbes (Patra and Saxena, 2010; Pavendale et al., 2005). Temperate climate plants, rich in tannins such as Lotus pedunculatus, have been shown to reduce methane production up to 30% (Woodward, Waughorn, and Laboryrie, 2004) and can replace other forages in the diet. Therefore, the objective of this review and meta-analysis is to show the main tropical tannin plants that can be used as natural additives for mitigation of CH\textsubscript{4} emissions in ruminants.

2. Controlling rumen-level methane production

Reducing the output of CH\textsubscript{4} generated by ruminal fermentation is a great challenge for nutritionists. In fact, the digestive system of ruminants has evolved over the years to use cellulose and polysaccharides by means of a pre-gastric fermentation system that produces CH\textsubscript{4}, however, this system represents a disadvantage for the environment in terms of contamination (Gill, Smith, and Wilkinson, 2010). On the other hand, the level of CH\textsubscript{4} yield emitted by ruminant animals related to the amount of feed intake (Monteny, Bannink, and Chadwick, 2006). This means that, although CH\textsubscript{4} yield levels increase directly with feed intake (Benchaar, Pomar, and Chiquette, 2001). It is important to consider, that not all feed ingredients will ferment in the same way in the rumen, as different amounts of CH\textsubscript{4}/unit of fermented carbohydrate are produced. Within concentrate feeding, soluble sugars will produce more CH\textsubscript{4} than starch per MJ of GE intake, so replacing sugars with starch in concentrated feeds will decrease CH\textsubscript{4} by 15%, as well as the emission of other gases in the environment (Mills et al., 2001). Most studies on dairy cows have reported that increasing the proportion of concentrate in the diet increases milk production since feed digestibility is improved; however, these studies were conducted with cows that produce more than 20 kg of milk per day in temperate climates (Olijhoek et al., 2018; Yan et al., 2010). However, Robles-Jiménez et al. (2021) reported that crossbred F1 dual-purpose cows (½ Bos taurus – ½ Bos indicus) grazing in tropical systems and supplemented with 150 – 450 g of concentrate per kg of daily milk production did not improve milk yield but increased CH\textsubscript{4} and N\textsubscript{2}O production per cow as the concentrate increased in the diet, which agrees with Lawrence, O’Donovan, Bolan, Lewis, and Kennedy (2015) and Dale, McGettrick, Gordon, and Ferris (2015).

Another aspect to consider is to know that, in diets rich in concentrates, the ruminal pH decreases (this is due to the yield of a large amount of volatile fatty acids, VFA), which facilitates the production of more propionate, acting as a sink for H\textsubscript{2} and, consequently, producing less CH\textsubscript{4}/unit organic matter fermented (OMF) in the rumen (Monteny et al., 2006). A second approach aims to reduce the production of CH\textsubscript{4} by using ingredients or additives specifically intended for that purpose. The function of these ingredients is to directly or indirectly inhibit the process of methanogenesis. Some PSM and plant extracts are included in this category as main secondary compounds that directly inhibit methanogens (García-González, González, and López, 2010).

3. Effect of secondary plant metabolites on CH\textsubscript{4} emission

Due to their availability, TT and fodder is often the main ingredient in the diet of animals in tropical and subtropical regions of the world (Ayasan, Cetinkaya, Aykanat, and Celik, 2020; Canul-Solis et al., 2020; Schultz-Kraft et al., 2018). Feed ingredients (tree foliage, grasses, and legumes) from these regions differs from those from temperate regions, due to their structure, chemical composition and digestibility (Assoumaya, Sauvant, and Archiméde, 2007). Phytochemicals, circumscribed but appropriately chosen (primarily PSM) are attractive because they are naturally produced by plants and can be include it in feed rations. However, forages from tropical regions may contain secondary metabolites that can alter rumen methanogenesis, decreasing the CH\textsubscript{4} yield (Bodas et al., 2012; Canul-Solis et al., 2020; Jouany and Morgavi, 2007; Velez-Terranova, Campos-Guona, and Sanchez-Guerrero, 2014). Ruminants fed tropical forage and pasture have been reported to produce more enteric CH\textsubscript{4} than ruminants fed temperate forage and pastures under different climatic conditions (Ku-Vera et al., 2020b), because in each climatic region the chemical composition and content of PSM will vary. It should be noted that while the IPCC (2006) provides default values for emission calculations (i.e., Ym 6.5%), which are used in most publications, it also specifies that emission factors need to be precise and validated in each country (Van Lingen et al., 2019). In this sense the values of Ym change (Ym 0.54% of energy intake) under tropical conditions as well as the type of animals genetics used in this region (Montoya-Flores et al., 2020).

4. Tannin chemistry

Tannins are natural chemical substances that belong to the group of PSM and are produced by plants in their intermediate metabolism. Plant secondary metabolites play a role of protection from herbivores, pests and pathogens. Secondary metabolites prevent toxicity and act as precursors to physical defence systems (Bennett and and Wallsgrove, 1994). Tannins are polyphenolic compounds of high molecular weight and are able to precipitate protein (Patra and Saxena, 2009). Tannins found in plants are presented as condensed tannins (CT) and hydrolysable tannins (HT) and both vary between fodder (Naumann, Tedeschi, Zeller, and Huntley, 2017).

Due to their lower risk of toxicity for the animal, anti-methanogenic activity has been studied mainly for CT-rich plants or extracts than HT (Beauchemin, Kreuzer, O’Mara, and McAllister, 2008). However, there are few studies related to the addition of tropical plants containing tannins and their antimethanogenic effect. These polyphenolic compounds chemically have variable molecular weights and the ability to bind to natural polymers such as proteins and carbohydrates, and are found in the wood, bark, fruits, flowers, nuts, leaves, and roots of most plant species (Min et al., 2020; Mueller-Harvey, 2006; Ortiz-Dominguez, Posada, & Noguera, 2014). Compared to tropical plants, temperate climate plants such as Lotus pedunculatus, which are rich in tannins, have also been shown to reduce CH\textsubscript{4} excretion by up to 30% (Woodward et al., 2004) and can replace the use of other forages in the diet.

In this way, knowing the plants, tree foliage, legumes and other natural resources with high potential in the mitigation of CH\textsubscript{4} would be beneficial for environment protection. However, what is currently known is that these substances are antimicrobial compounds that have the ability to inhibit abundance of some ruminal microorganisms. This is because they have bactericidal or bacteriostatic activities, which prevent growth or activity of methanogens in the rumen, which is due to the binding of microbial cell proteins and enzymes (Liu, Vaddella, and Zhou, 2011; Pavendale et al., 2005). The challenge in ruminant nutrition is to implement the use of these natural resources with high tannin content in
arid and subtropical areas, since in production systems where it is possible to use these supplements, today is a viable alternative to reduce environmental pollution. Furthermore, most of the research published today on the use of tannins shows positive results (Albores-Moreno et al., 2018; Alves, Dall-Orsosetta, and Ribeiro-Filho, 2017).

5. Effect of tannins on rumen microbial population

The diet has been reported as a predominant factor affecting the microbial community composition in the rumen on the host and the rumen environment (Henderson et al., 2015). Therefore, when PSM are included in the feed, they alter the availability of nutrients and metabolites and/or inhibit ruminal microbial metabolism of bacteria, protozoa, fungi and archaea populations (Bodas et al., 2012; Henderson et al., 2015; Vasta et al., 2019).

5.1. Effect of tannins on rumen bacteria and methanogens

The high molecular weight and polyphenolic nature of tannins result in the formation of complexes with microbial enzymes or cell walls. Thus, the exerted activity may cause the inhibition of cellulyotic or proteolytic bacteria or methanogens (Mannelli et al., 2019; McSweeney, Palmer, Bunch, and Krause, 2001). The mode of action of tannins is strictly dependent on their chemical structure as well as the bacteria species (Vasta et al., 2019). Condensed tannins (CT) were recognized to have a stronger binding with nutrients than hydrolysed tannins (HT), mainly due to the fact they have a higher grade of polymerization, which makes their degradation in the rumen environment more difficult (Jayanegara, Goel, Makkar, and Becker, 2015). On the contrary, HT have been reported to have a greater protein precipitation capacity that (Jayanegara, Goel, Makkar, and Becker, 2015). On the contrary, HT mainly due to the fact they have a higher grade of polymerization, which makes their mitigation capacity in comparison to CT. Additionally, the HT activity makes their degradation in the rumen environment more difficult strictly dependant on their chemical structure as well as the bacteria (Palmer, Bunch, and Krause, 2001). The mode of action of tannins is strictly dependant on their chemical structure as well as the bacteria species (Vasta et al., 2019). Condensed tannins (CT) were recognized to have a stronger binding with nutrients than hydrolysed tannins (HT), mainly due to the fact they have a higher grade of polymerization, which makes their degradation in the rumen environment more difficult (Jayanegara, Goel, Makkar, and Becker, 2015). On the contrary, HT have been reported to have a greater protein precipitation capacity that has been related to higher biological activity and a higher methane mitigation capacity in comparison to CT. Additionally, the HT activity may be enhanced by the direct toxic methanogenic activity exerted by HT fractions, produced as a consequence of HT degradation by rumen microorganism enzymes, i.e. tannase (Bhat, Singh, and Sharma, 1998; Jayanegara et al., 2015).

The CT have been proposed to directly inhibit some ruminal gram-positive specialized fibrolytic bacteria (Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens, Butyrivirio proteoclasticus) in an in vivo study with fistulated ewes (Costa et al., 2018). In another study, Fibrobacter succinogenes and total methanogens population inhibition (up to 36%), have been reported in vitro, either supplementing CT or HT (Jayanegara et al., 2015).

Salami et al. (2018), included 4% of either CT (Mimosa pudica, Uncaria gambir) or HT (Castanea sp., Caesalpinia spinosa) in lambs’ diet, and did not observe a difference in absolute abundance of bacteria and fungi, while methanogens (~12%) abundance decreased similarly with both types of tannins. In a recent in vitro study, the same concentration of chestnut tannins (HT) was fermented, and methane produced was reduced by 12.5% compared to control, while acetate production increased (Cappucci et al., 2021). Goel and Makkar (2012) suggested that HT directly inhibit methanogenic activity, and, therefore, they might affect less fibre digestibility, which can be compromised by the inclusion of CT in the diet. Tavendale et al. (2005) evaluated in broth culture the growth and methane production of Methanobrevibacter ruminantium testing either polymeric or oligomeric CT fractions from Lotus pedunculatus. The polymeric CT fractions were the only effective in inhibiting the growth, thus demonstrating the importance of PSM chemical structure and synergistic effect of all components to directly inhibiting methanogens along with other rumen microorganism activities (Mannelli et al., 2019). The reduction of fibre digestibility, when CT sources were included in the diet, was thereby supported by the reduction of total VFA production mainly explained by the reduction of acetate production, as evaluated in sheep fed with an inclusion of 16 g/Kg dry matter (DM) intake of quebracho extract (Buccioni et al., 2015). However, total VFA production was not impaired with a level of tannins inclusion less of than 2 g/Kg DM (Table 1). This low dosage might be not always sufficient to achieve a methane mitigating effect. Hence, a dosage above 20 g/Kg of tannins has been proposed by Jayanegara, Leiber, and Kreuzer (2012). In accordance with Salami et al. (2018) both HT and CT extracts could impact the ruminal microbiome when supplemented at moderate levels (<50 g/Kg DM, Mueller-Harvey, 2006), but their detrimental effect on fibrolytic bacteria should be considered when animals are fed with high-fibre diets. The contrasting results concerning rumen fermentation traits, microbial population, and methane production can be at least partially explained by the heterogeneity of tannin chemical structures from plants, the various dosages intake and the feeding regimen (Patra and Saxena, 2011; Vasta et al., 2019). Moreover, microbial adaptation to tannins might occur through mechanisms of some bacteria such as the formation of protective exopolysaccharide layer around the cells, degradation of tannins, and modification of cell membrane (Patra and Saxena, 2011).

5.2. Effect of tannins on rumen protozoa

The antiprotozoal activity of some PSM might be relevant since methanogens colonizing ciliate protozoa were suggested to be responsible for 9 – 25% of methanogenesis in ruminant fluid (Henderson et al., 2015; Newbold, Lassalas, and Jouany, 1995). The antiprotozoal activity of tannins is contrasting, and Patra and Saxena (2009) suggested that the effect is plant dependant, having the tannin structure-activity relationship a major role in the mechanism of action (Mueller-Harvey, 2006). HT have been proposed to permeate through protozoa membranes, thus compromising methanogens associations (Patra and Saxena, 2011). In the study by Malik et al. (2017), male sheep diets were supplemented with tanniferous tropical tree leaves (Ficus benghalensis, Artocarpus heterophyllus and Asadrachta indica) containing 7.1–10.8 g/Kg DM of CT. The digestibility was not compromised, whereas methane production was reduced (up to 26%). The authors suggested that methane reduction can be explained by the decrease of protozoa number (~23%). Moreover, CT appeared to affect Entodinimorphs protozoa more than Holotrichs protozoa (Malik et al., 2017). A similar reduction of protozoa number (~21%) was reported by Salami et al. (2018), including 4% of both CT (mimosa, gambier) or HT (chestnut, tara) in lamb’s diet. However, other studies conducted in vivo and reported in Table 2 showed that methane reduction was not always related to a decrease of protozoa number.

6. Effect of tannins on CH4 emission

6.1. In vitro studies

The inclusion of tannins directly from plants or as plant extracts, in ruminant diets, has been showed to decrease CH4 above 20 g/kg (Jayanegara et al., 2011). In this sense, Goel and Makkar (2012) reported that CH4 synthesis from ruminal fermentation has been reduced by by 50% in response to tannin or plant extracts containing these polyphenolic compounds (Patra and Saxena, 2010). Authors who conducted experiments on plants with high tannin content (Molina-Botero et al., 2019; Morgavi, Martin, Jouany, and Ranilla, 2012; Patra and Saxena, 2011; Tavendale et al., 2005) agreed that tannin plants reduce CH4 production due to their antimicrobial properties, for example, Jayanegara et al. (2015) found that all tannins decreased CH4 concentration in a linear or quadratic manner, and they also reported that the magnitude of the decrease was greater for plants containing hydrolysable tannins than for those plants rich in condensed tannins. The mode of action and the effects that tannins have on the animal will continue to be the subject of research. Reduction of nematode egg excretion and worm burden have been also reported in small ruminants fed with tanniferous plants (Birhan, Gesses, Kenumih, Dejene, and Yayeh, 2020; Marley, Cook, Keatinge, Barrett, and Lampkin, 2003; Mengistu et al., 2017; Minho, Filippsen, Amarte, & Abdalla, 2010;
The inclusion of tropical tanniferous plants in vitro studies has been reported (Table 3). For example, Albores-Moreno et al. (2018) reported that supplementation with *Leucaena leucocephala* at a concentration of 950 g/kg DM in an in vitro study on diets for cattle based on *Pennisetum purpureum* grass, is a feeding alternative that can promote greater efficiency and synthesis of microbial biomass, increase the proportions of propionic and butyric acid, and decrease the output of enteric CH₄ up to 15.6 to 31.6%. Rodriguez, Britos, Rodriguez-Romero, and Fondevila (2011) studied the effect of inclusion of plant tanniferous extracts equivalent to 240 mg of *Acacia cornigera* or *Albizia lebbekoides* added to 800 mg *Pennisetum purpureum*, *A. cornigera*, and *A. lebbekoides* and reported that CH₄ concentration (ml/ml gas) was lower (14 and 7%, respectively) than *Pennisetum Purpureum* as a control after 24 h of incubation. Tan et al. (2011) evaluated the effects of CT from *Leucaena leucocephala* at 15 mg of CT/500 mg DM reducing CH₄ excretion by ~47%, while Carlulli, Kreuzer, Machmüller, and Hess (2005) reported that supplementation of 25 g/kg of CT (12.5 mg CT/500 mg DM) from *Acacia mearnsii* in sheep fed ryegrass with a reduction of CH₄ emissions by ~12%. In an in vitro study, Petlum, Paengkoum, Liang, Vasupen, Kreuzer, Machmüller, and Hess (2005) reported that supplementation of 25 g/kg of CT (12.5 mg CT/500 mg DM) from *Acacia mearnsii* in sheep fed ryegrass with a reduction of CH₄ emissions by ~12%. In an in vitro study, Petlum, Paengkoum, Liang, Vasupen, & Paengkoum, 2019 evaluated the inclusion of CTs of a higher molecular weight as *Asadrichuta indica*, showing stronger effect than those of a lower molecular weight as *Leucaena leucocephala* on CH₄ excretion. The inclusion of Siamese neem suppressed CH₄ output at inclusion levels of 2, 4 or 6 mg/100 g DM, with the long period.

### Table 1

| Plant | Dosage (g/kg DM) | Trial type | Unit | Methane reduction potential (% of control) | Other major effects reported | References |
|-------|-----------------|------------|------|------------------------------------------|-----------------------------|------------|
| Acacia tannins | 50 | In vitro | mL/24h | 15% | –11% of total VFA | Staatl, Kreuzer, and Soliva, 2010 |
| Chestnut and sumarch (HT) and mimosa and quebracho (CT) | 1 g/L | In vitro | mL/L | 3% CT | –14% and 5.8% HT of total VFA | Jazayegh et al., 2015 |
| Chestnut leaves | –24 mg/g DM of HT tannin | In vitro | mL/24h | 28% | –13% total VFA | Terranova, Kreuzer, Braun, and Schwarm, 2018 |
| CT from leaves of Gliciridia sepium, Leucaena leucocephala, and Manihot esculenta. | 0, 0.25, 0.5, 0.75, and 1.0 g CT/Kg, respectively | In vitro and in vivo (rumen-cannulated sheep) | mL/24h | Up to 22% (in vitro) of total VFA | No effect on Methanogens population (in vivo) | Rza et al., 2015 |
| Vaccinium vitis idaea | 140 g of extract containing 2 g of tannins/kg DM | In vivo (Polish Holstein-Friesian dairy) | mM | 8% | –46% rumen NH₃ | Cieslak, Zmora, Pers-Kamczyc, and Szumacher-Strabel, 2012 |
| Acacia mearnsii tannin extract | 7 g/Kg DM | In vivo (dairy cows) | g/day | 32% | No effect on milk production | Alves et al., 2017 |
| Chestnut or Chestnut-Quebracho tannin extract | 1.5 g/Kg | In vivo (crossbred steers) | g/day | No effect | No effect on Protozoa population | Abajo et al., 2018 |

CT, Condensed tannins; HT, Hydrolysable tannins; VFA, Volatile Fatty Acids.

### Table 2

| Plant | CT (g/kg DM) | Dosages (g/kg DM) | Species | CH₄ Production | % CH₄ reduction | Effect on microbial population | References |
|-------|--------------|-------------------|---------|----------------|----------------|-----------------------------|------------|
| *Leucaena leucocephala* | 2.70, 8.20 and 12.90 | 120, 240 and 360 | Crossbred heifers | 162.9, 154.8 and 140.0 g/d⁻¹ | 6.49, 11.14 and 19.64 | No changes in Protozoa, Bacteria and Methanogens counts | Montoya-Flores et al. (2020) |
| *Samanea saman* + *Pennisetum purpureum* | 1.20, 2.40 and 3.60 | 900, 935 and 965 | Crossbred heifers | 89.63, 72.03 and 59.30 L/d⁻¹ | 25.83, 40.40 and 50.93 | No changes in Protozoa count | (Valencia-Salazar et al., 2017) |
| *Leucaena leucocephala* | 21.00 in all doses | 200, 400 and 800 | Crossbred heifers | 101.20, 87.40, 74.90 and 53.50 L/d⁻¹ | 26.30, 36.25, 45.45 and 61.03 | No changes in Protozoa count | Piteiro-Vázque et al. (2018) |
| Lolium perenne | - | 185 | Dairy cattle | 260.00 g/d⁻¹ | 10.34 | - | Woodward et al. (2002) |
| Hedyaspernum coronarium | 2.72 | 130 | Dairy cattle | 253.90 g/d⁻¹ | 15.37 | - | Woodward et al. (2002) |
| Lolium perenne | - | 161 | Dairy cattle | 360.63 g/d⁻¹ | 10.00 | - | Woodward et al. (2004) |
| Lotus corniculatus | - | 121 | Dairy cattle | 343.24 g/d⁻¹ | 14.19 | - | Woodward et al. (2004) |
| *Sorrel* | 153.00 | 881 | Goat | 6.30 g/d⁻¹ | 12.00 | Protozoa count increased in the long period | Puchala et al. (2012) |
| *Leucaena leucocephala* | 40.00 | 820 | Sheep | 7.80 g/d⁻¹ | 25.71 | - | Dias-Moreira et al. (2013) |
| Stylolobium aterrimum | 40.00 | 690 | Sheep | 10.40 g/d⁻¹ | 0.95 | - | Dias-Moreira et al. (2013) |

¹ CT, Condensed tannins (g/kg DM).
² % CH₄ reduction compared with the control diet, CT, Condensed tannins (g/kg DM).
Table 3
Effect of dietary tropical tanniferous plants on methane production in vitro studies.

| Plant                             | CT (g kg\(^{-1}\) of DM) | Doses (g kg\(^{-1}\) of DM) | CH\(_4\) Production | % CH\(_4\) reduction | References                      |
|----------------------------------|--------------------------|-----------------------------|----------------------|-----------------------|--------------------------------|
| **Pennisetum purpureum + NeomilEspargiha emargiata; P. purpureum + Tabernemontana argyralatifolia; P. purpureum + Fissidixia piscipula; P. purpureum + Leucaena leucocephala; P. + Havardia abicans** | 950                       | 25.80 – 33.00 L/kg of digested DM | 10.31                | 12.47 – 31.57               | Albores-Moreno et al. (2018) |
| **Pennisetum purpureum + Acacia cornigera; P. purpureum + Alliana lebbekoides; P. purpureum + Leucaena leucocephala** | 53                        | 5.50 mL/g DM                | 0.22 mL              | 4.35                  | Rodríguez et al. (2011)       |
| **Leucaena leucocephala + Panicum maximum** | 200                       | 4.46 – 4.77 mL/L            | 11.44, 105.4        | 9.07, 16.22, and 25.20 | Supapong et al. (2017)        |
| **Acacia mearnsii, Schinopsis balansae, Castanea sativa, Quercus acelops** | 97.9                      | 3.3, 1.7 and 0.01 mL/g DM   | 67.00, 83.00, and 99.90 |                       | Perfil, Paengkoum et al. (2019) |
| **Arachis pintoi, Cratylia argentea, Leucaena leucocephala** | 1000                      | 29.6 – 33.6 mL/g DM         | 11.79 – 21.77       |                       | Vandermeulen et al. (2018)    |
| **Leucaena leucocephala, Acacia saligna, Atriplex halimus** | 500                       | 9.5 – 9.7 mL/g DM           | 22.40 – 24.00       |                       | El-Zait et al. (2020)         |
| **Calliandra calothyrsus, Acacia nilotica, Gliciridia sepium, Leucaena leucocephala, Manihot esculenta, Musa spp** | 100                       | 1.41 mL/d                  | 64.04                |                       | Rira et al. (2019)           |
| **Acacia nilotica leafe, Acacia nilotica leaves** | 25, 50, 100               | 1.41 mL/d                  | 64.04                |                       | Rira et al., 2019            |
| **Castanea sativa, Schinopsis lorentzii** | 53.80                     | 15 and 30                  | 54.70 mL/g DM       | 44.40                 | Menci et al., 2021           |
| **Brachiaria humiliscola, Vigna unguiscilata, Calliandra calothyrsus, Flemingia macrophylla** | 29.00                     | 0.76 g/d                   | 50.31                |                       | Hess et al., 2013            |

1 % CH\(_4\) reduction compared with the control diet.

while supplementation of *Leucaena* leaves showed reductions on CH\(_4\) production at 6 mg/100 mg DM of supplementation, Huang et al. (2010, 2011) suggested that chemical structure and molecular weight of the CTs influenced their efficacy to manipulate rumen fermentation, with specific effect on CH\(_4\) mitigation output. Hassan and Benchaar (2012) added Valonea (*Quercus acelops*; Nutri爱国-Adisseo®) extracts as sources of HT, showing that CH\(_4\) excretion reduced up to 11% at 50 g/kg DM. On the other hand, Vandermeulen et al. (2018) evaluated the effect of *Desmanthus spp.* which emitted less CH\(_4\) (mL/g DM incubated) than the reference grass hay at 72 h (C.gyana) up to 23%. In vitro studies vary in their response to CH\(_4\) production and that seems to depend on various management and environmental factors such as nutrient soil composition, light intensity, and temperature (Albores-Moreno et al., 2019; Frutos, Hervas, Giraldes, and Mantecon, 2004; Yang et al., 2018). Thus, we can notice that the differences in the concentrations of CT amongst studies vary with plant species, and geographical locations of plants. It is difficult to extrapolate in vitro to in vivo results, due to the variation between results and doses. Therefore, it is highly recommended to evaluate the effect of the supplementation of tanniferous plants on CH\(_4\) mitigation in vivo studies.

7. Meta-analysis: methodology

To quantify the overall effect of the inclusion of tannins on CH\(_4\) emissions in ruminants (*In vivo* studies), a meta-analysis process was carried out. A compressed and structured search of articles was carried out using the search engines Google Scholar, PubMed. Different sets of the following keywords were provided to field experts to integrate the study database: ‘ruminants’, ‘tropical plants’, ‘secondary metabolites’, ‘tannins’, ‘methane emission’, ‘treatment’ (control vs tropical plant), ‘in vitro’, and ‘in vivo’.

Only articles peer reviewed, written in English containing an experimental set up were included in the current literature review. To be considered, the studies must meet the following inclusion and exclusion criteria according with Lean, Thompson, and Dunshea (2014): a) studies published in an international peer-reviewed scientific journal, b) specific procedures for random assignment of animals to each treatment (experimental design), c) report minimum means squares and a measure of variability, and c) report the sample size of each group (Fig. 1).

The final database included the publications from 2002 to 2021 and comprised the following information of least squares means, variability measures [standard error of mean (SEM), standard error of differences (SE) or standard deviation (SD)] and number of experimental units for both groups compared to each output variables, animal species as sheep (*Ovis aries*), goat (*Capra hircus*), cattle (*Bos Taurus* and *Bos indicus*), beef and dairy cattle and crossbred heifers, plant, dose, CH\(_4\) emission from the control and tannin groups, as well as the number of repetitions. The CH\(_4\) values from *in vitro* studies were homogenized to mL/g DM, g/d, or mL/d. With regard to *in vivo* studies all were adjusted and expressed in g CH\(_4\)/d. Current analysis, random effects models were fitted to estimate the effect size (ES), the 95% confidence interval and the statistical significance of ES for each outcome variable, using the ‘meta’ package version 4.6–0 (Schwarzer, Carpenter, & Rücker, 2015) in the R statistical software version 3.3.1 (R Core Team, 2016). The ES was calculated as standardized mean difference (SMD) using the methods described by Hedges (1981) for the fixed effects and by DerSimonian and Laird, (2015) for random effects models. The studies that reported outcome variables in the same unit of measure aid to calculate the raw mean difference (RMD), which permits ES interpretation under original measures units (Appuhamy et al., 2013). The current systematic review analyses studies performed in different places with different methods and under different animal management; hence, the heterogeneity was needed (Higgins, 2008). Heterogeneity of results amongst trials was reported using the I\(^2\) statistic (Higgins & Green, 2011). The I\(^2\) represents the approximate proportion of total variability and indicate estimates that can be attributed to heterogeneity, which was calculates as:
Fig. 1. PRISMA flow diagram of the systematic review from initial search and screening of publications included in the meta-analysis.

Fig. 2. Forest plot of methane production, expressed as Dry Matter Intake (DMI, g tannins/d) from studies focused on tannins supplementation in ruminants.
Where $Q$ is the $X^2$ heterogeneity statistic and $K$ is the number of trials. $I^2$ values of 25%, 50% and 75% represented small, moderate, and high levels of heterogeneity, respectively. For output variables that showed substantial heterogeneity ($I^2$ >50%), mixed effects regression models (meta-regression analysis) were constructed to explore sources of heterogeneity using the ‘metaphor’ package (Viechtbauer, 2010). The covariates defined a priori in the current meta-regression were inclusion level, species (sheep, beef cattle, dairy cattle, and cross-bred heifers) and plant.

8. Results from meta-analysis

A total of 14 articles were analysed to assess the effect of tannins supplementation on CH$_4$ emissions of ruminants (Fig. 2). According to the obtained database, two meta-analysis were carried out in the current work. The first meta-analyses assessed the effect of tannins supplementation on CH$_4$ enteric emission in ruminant using in vivo studies (n = 19 trials). In vivo techniques database allowed to estimate the raw mean difference (RMD) and standardized mean difference (SMD) because all studies reported the CH$_4$ emission in the same unit (g/d). The second meta-analysis evaluated the effect of tannins supplementation on CH$_4$ emissions of overall studies (in vivo = 19; vitro = 45 trials). However, because those studies reported CH$_4$ emission in different units of measurement, only the SMD was estimated (Table 4). In both meta-analyses the values of heterogeneity ($I^2$) were greater than 25%, therefore the sources of heterogeneity were explored.

8.1. In vivo studies meta-analysis

The in vivo studies showed a positive response in mitigating CH$_4$ emission due to the inclusion of tannins in the diets of ruminants through the feeding of tropical plants (SMD = −0.86; $P = 0.005$) (Fig. 2). The response to tannin content has a moderate heterogeneity ($I^2 = 50.5\%$) that can be explained by the type of plant offered, level of inclusion and animal species. With regard to the type of animal that was fed Leucaena leucocephala and the combination of Samanea saman + Pennisetum purpureum, showed the greatest mitigation effects of CH$_4$ according to the meta-regression analysis (Fig. 3, Table 4). The effect of tannins was most evident in heifers with an effect size of −1.3 compared to dairy cows ($ES = −0.06$), beef cattle ($ES = 0.02$) and sheep ($ES = −0.32$) (Fig. 2). Finally, a negative relationship was observed between the level of inclusion of tannins and CH$_4$ emission (−0.09), by increasing the dose of tannins, the difference between control and treatment increases, although in a negative direction (Fig. 3). This means that, the higher the dose of tannins, the treatment group will emit less CH$_4$ compared with control, showing differences between the type of plant used, with a rather interesting effect on Leucaena leucocephala and Samanea saman, being mostly condensed tannins in ruminant animal production.

8.2. Overall meta-analysis

The global response of tannins supplementation in ruminants (in vivo and in vitro studies) when all available studies where analysed depicts a SMD of −0.60 to the random effect model. The heterogeneity was considerably lower than overall meta-analysis ($I^2 = 27\%$) in comparison with the meta-analysis of in vivo studies ($I^2 = 50\%$). Sub-group analysis revealed differences of tannins supplementation response according with the measure technique of CH$_4$ emission. The effect size of in vitro studies was lower (−0.51; 95% CI −0.76 −0.26) compared with in vivo studies (−0.86; 95% CI −1.35 −0.38) (Fig. 4). With regard to sources of tannins (Table 4), the highest mitigation response was observed in Flemingia macrophylla (−2.21; 95% CI −3.53 −0.89) followed by Samanea saman (−2.02; 95% CI −3.17 −0.86) and Leucaena leucocephala (−1.46; 95% CI −1.95 −0.97). The studies that supplemented Schinopsis lorentzii showed a higher effect size (−3.31; 95% CI −6.88 −0.26), however the confidence intervals were wide and included zero value.

9. Discussion from meta-analysis

The combination of Samanea saman and Pennisetum purpureum (Valencia-Salazar et al., 2017) in cattle diets have been shown to contribute to the reduction of CH$_4$ up to 50.9% (Table 2), showing the greatest mitigation effects of CH$_4$ according to the results of the meta-regression analysis. On the other hand, in an in vivo study with lambs, Dias-Moreira et al. (2013) evaluated the effect of three forages, Leucaena leucocephala, Stylosanthes achras and Mimosas caesalpiniaefolia, reporting that with the use of Leucaena leucocephala there is a greater reduction in CH$_4$ emissions (−25%). El-Zaait et al. (2020) carried out in vivo and in vitro studies on sheep (Table 3), confirming that in the in vitro study, the supplementation of Leucaena leucocephala, Atriplex halimus or Acacia saligna to the diet (50/50) reduced CH$_4$ output to almost 23% compared with the control group, and in the in vivo study in sheep diets, showed reductions of 11.45% in the CH$_4$ production. Tiemann et al. (2008) in an in vitro study found an 80% reduction of CH$_4$ by including Flemingia macrophylla, followed by Leucaena leucocephala with variations in CH$_4$ reduction (30-60%) in vitro studies (Table 3), which may be due to the different levels of inclusion, which coincided with our results (Table 4).

Ku-Vera et al. (2020a) confirmed that the use of Leucaena leucocephala in beef cattle has a mitigating effect on CH$_4$ when fed at levels of up to 30–35% DM. Furthermore, Ku-Vera et al. (2020a) mentioned that the legume Samanea saman which contains saponins, has demonstrated to have a mitigating effect on enteric CH$_4$ in cattle and sheep housed in respiration chambers, since saponins break the membrane of the rumen protozoa thus decreasing the number of methanogenic protozoa and archaea. This result of the use of tropical plants was confirmed by Ku-Vera et al. (2020b) who, by incorporating ground foliage and pods.
from tropical trees and shrubs into beef cattle rations, obtained a decrease of between 10% and 25% in CH\textsubscript{4} (g CH\textsubscript{4}/kg DM intake), and those responses depended on the species of plant and the level of intake of the ration.

Piñeiro-Vázques et al. (2018) evaluated the use of Leucaena leucocephala in crossed heifers and reported that, the higher the dose concentration, the lower the CH\textsubscript{4} emission, indeed they obtained a 61% decrease in CH\textsubscript{4} at a dose of 800 g/kg DM of Leucaena leucocephala. This result agrees with Montoya-Flores et al. (2020) and Valencia-Salazar et al. (2017) in another study with crossed heifers, reporting that the use of Samanea saman + Pennisetum purpureum pod meal decreases CH\textsubscript{4} emissions as its inclusion increases, since, from the inclusion of 0, 10, 20 and 30%, the latter decreased 50.9% of CH\textsubscript{4} in L/d.

Rumen CH\textsubscript{4} yield represents an energy loss of up to 0.12 of the total feed intakes (Olijhoek et al., 2018). In this sense, if the inclusion of tannins reduce CH\textsubscript{4} output (Ku-Vera et al., 2020a,b), plants containing these compounds should have a positive impact on energy utilization, as well as a reduction of the environmental impact of livestock production (Vázquez-Carrillo, Montelongo-Pérez, González-Ronquillo, Castillo-Gallegos, & Castelán-Ortega, 2020). However, a selective effect of tannins on fibrolytic bacteria occurred, with Ruminococcus albus being most affected, in agreement with the negative effects of saponins on this species (Galindo et al., 2016). These different bacterial responses to tannins might be due to the specific attachment mechanisms to the substrate and the fermentation pattern (Koike and Kobayashi, 2009), as well as by the different modes of action of tannins depending on their source (Tiemann et al., 2008). In the present study, the inclusion of tropical forage rich in tannins seems to reduce CH\textsubscript{4} emission in vivo trials, but responses vary amongst plant sources, doses and animal species (Figs. 2, 3).

Since the concentration of tannins varies depending on the plant, (Fig. 3, Table 4) it is observed that Leucaena leucocephala shows a better effect in terms of CH\textsubscript{4} reduction compared with Styzolobium têrimum at the same concentration. Likewise, it was observed that Leucaena leucocephala and Samanea saman showed a greater effect in the decrease of CH\textsubscript{4}, compared to other plants such as Brachiaria brizantha, Gliricidia sepium, Enterolobium cyclocarpum, this effect was found in cross breed cattle. Puchala et al. (2012) found a 12% decrease in CH\textsubscript{4} in goats when adding Sericea lespedeza (Table 2). Likewise, Dias-Moreira et al. (2013) obtained a reduction of CH\textsubscript{4} in sheep up to 25% when supplementing Leucaena leucocephala, being lower when supplementing Styzolobium têrimum (0.99%), although both plants contained the same concentration of tannins (40 g CT/kg of DM), this effect may be due to the fact that 19% more L. Leucocephala was administered compared to Styzo - lobium têrimum. On the contrary, when supplementing Mimosa caesalpiniaefolia there was no effect on the reduction of CH\textsubscript{4}, possibly due to the amount administered in the diet (530 g/kg DM), being 34% less TC compared with L. leucocephala. Beauchemin, McGinn, Martinez, and

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**Fig. 3.** Relationship between the level of inclusion of tannins (g/kg dry matter intake) from tropical plants and methane production in ruminants.

**Fig. 4.** Forest plot of methane production (SMD) according with measurement technique from studies of ruminants supplemented with tannins. SMD is the standardized mean difference estimated of the random model.
McAllister (2007) when supplementing Schinopsis balansae in beef cattle, found a CH4 reduction of 0.96%, being very similar to that found by Puchala et al. (2012) when supplementing Mimosa caesalpinioidea. When supplementing Hedysarum coronarium, Woodward, Wagahn, Lassey, and Laboyrie (2002) found CH4 reductions of 14%, with a concentration of condensed tannins of 2.72 g/100 g DM in Hedysarum coronarium. However, in general a negative relationship was observed between the level of tannin inclusion and CH4 emissions. The reduction in CH4 production observed with the use of tannins could be attributed to the fact that they inhibit the activity of microbial enzymes, decrease the populations of protozoa and cellulyotic bacteria and form links with forage proteins, reducing the degradation of ruminal protein (Jakh-mola, Taruna, and Raghuvans, 2010; Moscoso et al., 2017). However, an important factor to consider is that the concentration of plant tannins (HT and CT) are known to have both adverse and beneficial effects depending on their concentration and nature, besides other factors such as season, geographical region, animal species and genetics, animal physiological stage and dietary composition (Goel and Makkar, 2012; Piluzza, Sulas, and Bullitta, 2014) and derived from it, the effect on the decrease of CH4 excretion in ruminants (Fig. 2). Therefore, supplementing tropical and subtropical plants in the diet with a high dose of tannins will result in less CH4 production.

10. Final remarks

The efficacy of CTs from plant materials to reduce CH4 emission depends on the plant species and possibly to the environment in which they are grown. Supplementation of tannin-rich plants such as Leucaena leucocephala, Flemingia macrophylla and Samanea saman in vitro and in vivo studies, have a positive effect on the reduction of CH4 in ruminants. Other tropical tannin-rich plants such as Shinopsis lorentzii, Musa spp., Acacia spp., and Albizia spp. can reduce CH4, but further in vivo studies are suggested to determine rumen microbiome and rumen metabolites.

Funding

This project was financed by UAEMex 4335/2017

Compliance with ethical standards

Due to the nature of the work (systematic review), the authors have nothing to declare.

Ethical statements

The authors state that no animals were used in this study, as it is a review of previous work in the tannin supplementation in ruminants and the effect on CH4

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Dr. Lizbeth Robles was supported by a grant of the CONACyT during her studies of Doctorate, in the Programa de Doctorado en Ciencias Agropecuarias y Recursos Naturales, Universidad Autonoma del Estado de Mexico. During the study, Dr. Einar Vargas-Bello-Pérez was a visiting scholar, also supported by project number 4974/2020CIB, and Dr Angeles-Hernandez was granted by Secretaria de Educacion Publica Mexico Grant numbers: UAEH-PTC-823

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