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REVIEW ARTICLE

Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity

Potencial de los taninos condensados para reducir las emisiones de metano entérico y sus efectos en producción de rumiantes

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RESUMEN

El metano (CH_4) es un gas subproducto de la fermentación de los carbohidratos en el rumen. La agricultura contribuye con el 47% de las emisiones antropogénicas de CH_4, siendo los rumiantes responsables del 39% de las emisiones de metano entérico. Este gas de efecto invernadero (GEI) tiene un potencial de calentamiento global 25 veces más que el CO_2 y representa una pérdida de hasta el 12% de la energía total consumida por los rumiantes. Este trabajo describe los mecanismos de acción de los taninos condensados contenidos en follajes y frutos y su efecto en los microbios del rumen, así como su potencial en la mitigación de las emisiones de CH_4. Los hallazgos sugieren que los taninos condensados reducen la población de protozoos hasta 79%, reduciendo además los metanogenos del rumen hasta en 33%. Los taninos condensados se unen a proteínas y polisacáridos formando complejos, reduciendo así la digestibilidad de la meteria seca y orgánica y la producción de H_2 metabólico, el que es usado por metanogenos para la reducción del CO_2 a CH_4. Estudios *in vitro* sugieren que los taninos condensados pueden reducir el CH_4 en el rumen hasta 63%, mientras que *in vivo* se han registrado reducciones hasta 58% en la producción de CH_4. Además, la incorporación de taninos condensados en la ración de rumiantes tiene el potencial de aumentar la ganancia de peso en 26%, relacionando esto al aumento en el flujo de proteína de baja degradación ruminal al intestino delgado o la reducción de la energía perdida en forma de CH_4 en el rumen. Se concluye que la incorporación de taninos condensados en la ración de los rumiantes en concentración de 3-6% de materia seca puede reducir las emisiones de CH_4 así como mejorar la ganancia de peso y producción de leche de los animales productivos.

Palabras clave: metano, gas de efecto invernadero, taninos condensados, leguminosas tropicales.

SUMMARY

Methane (CH_4) gas is a by-product of anaerobic fermentation of carbohydrates in the rumen of ruminant species. Agriculture contributes with 47% of anthropogenic emissions of CH_4, being the ruminants responsible for 39% of enteric emissions of CH_4. This greenhouse gas (GHG) has twenty-five times higher global warming potential than CO_2 and represents a loss of up to 12% of the gross energy consumed by ruminants. The aim of this review is to describe the mechanisms of action of condensed tannins contained in foliage of trees and shrubs, and their effect on rumen microbes, as well as their potential for mitigation of CH_4 emissions. The findings suggest that condensed tannins reduce protozoa population up to 79% and along with this, rumen methanogens are decreased by up to 33%. Condensed tannins bounded to proteins and polysaccharides of the ration form complexes which reduce digestibility of dry and organic matter and production of metabolic H_2, used by methanogens to reduce CO_2 to CH_4. *In vitro* studies suggest that condensed tannins may reduce rumen CH_4 up to 63% *in vitro* and up to 58% *in vivo*. Additionally, incorporation of condensed tannins in the ration of ruminants might increase weight gain by 26%, maybe due to the increase in the flow of protein of low rumen degradability to the small intestine or to the reduction of energy losses as CH_4 in the rumen. It is concluded that incorporation of condensed tannins in the ration of ruminants at 3-6% of dry matter concentrations can reduce CH_4 emissions as well as to improve weight gain and milk yield of productive animals.

Key words: methane, greenhouse gases, condensed tannins, tropical legumes.

INTRODUCTION

The growth of the human population, mainly in developing countries, will impact on the increasing demand of livestock products such as meat and milk and in the growth of the inventories of ruminant animals (Eckard *et al* 2010). These factors will exacerbate the emissions of methane (CH_4) to the atmosphere if strategies for mitigation are not devised and applied (Gerber *et al* 2013). Every year, 80 million tons of CH_4 are produced in the world (Eckard *et al* 2010), agriculture contributes with 47% of those emissions being the ruminants responsible for 39% of that (Gerber *et al* 2013).

CH_4 is one of the by-products of anaerobic fermentation of structural carbohydrates in the rumen and along with

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carbon dioxide (CO_2) and nitrous oxide (N_2O), represent the main greenhouse gases (GHG) produced by ruminants (Moss *et al* 2000). CH_4 has a global warming potential twenty-five times greater than that of CO_2.

Within the livestock sector, ruminants are the main source of emission of CH_4 and their production represents a loss of between 2 to 12% of the gross energy consumed, being these losses greater in ruminants fed tropical grasses, because of the low digestibility during some seasons of the year, which induce a lower rate of passage and longer time of retention in the rumen, which lead to an increase in the emissions of methane (Johnson and Johnson 1995; Kennedy and Charmley 2012).

However, in the tropics there is diversity of plants that due to its good nutritive value hold potential for ruminant feeding (Bhatta *et al* 2013[a]), additionally several of those plants possess condensed tannins (CT), which have the ability to modify rumen fermentation due to their different mechanisms of action on rumen microorganisms (Tavendale *et al* 2005) and on the structural components of rations, thus decreasing emissions of enteric methane.

The state of knowledge on mechanisms of action of condensed tannins from foliages of trees and shrubs, and their effects on rumen microbes, animal performance, and their potential to mitigate emissions of methane are dealt with in this review.

RUMINAL METHANOGENESIS

During the process of anaerobic fermentation of soluble and structural carbohydrates of the ration in the rumen, volatile fatty acids (VFA's) and other by-products are formed**,** among them: H_2, CO_2 and CH_4, these last two are known as greenhouse gases (GHG) (Moss *et al* 2000), being CH_4 the most studied due to its greater potential as GHG and to the amount of energy lost (55.2 MJ/kg CH_4) in ruminant species (Brouwer 1965, Johnson and Johnson 1995).

Of the total amount of methane produced daily, 95% is synthesised in the rumen and the remaining 5% in the caecum. Methane production is mainly influenced by the type and quality of the ration, for example rations rich in starch (i.e. concentrates) yield lower CH_4 emissions (Hales *et al* 2014) due to the synthesis of propionic acid and to the improvement in the ratio of methane:organic matter fermented in the rumen, compared to rations based on cellulose and lignin (i.e. forages) (Kennedy and Charmley 2012), which favour the synthesis of acetic acid and the production of H_2 which is used to reduce CO_2 to CH_4.

Metabolic H_2 present in the rumen is used by the methanogenic bacteria, particularly by the *Archaea*, which use H_2 to reduce CO_2 to CH_4 (Czerkawski 1986). Some authors have mentioned that there are two main pathways for the synthesis of CH_4: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ and $CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$ (Czerkawski 1986), those pathways use H_2 as an intermediary in the reduction of CO_2 to CH_4 in anaerobic conditions. These processes are controlled by means of the reduction of cofactors such as nicotinamide adenine dinucleotide reduced (NADH); NAD phosphate reduced (NADPH) and flavin adenine dinucleotide reduced (FADH), which are oxidized to NAD^+, $NADP^+$ and FAD^+ which in turn donate H^+ ions to synthesise H_2 (Martin *et al* 2010; Kittelmann and Janssen 2012).

Rumen protozoa also represent an important factor in methane synthesis, since they live in symbiosis with the *Archaea* with whom there may be an interspecific transfer of H_2 (Johnson and Johnson 1995), rumen defaunation and therefore of the population of methanogens, leads to a reduction of the emission of enteric methane (Puchala *et al* 2012).

METHANE EMISSIONS FROM RUMINANTS

Ruminants are the main source of methane arising from the agricultural sector, in this sense Eckard *et al* (2010) reported that cattle produce around 120 to 450 l/day of methane and sheep may produce between 20 to 55 l/day depending on dry matter intake (DMI).

Some studies point out that ruminants produce around 39% of anthropogenic emissions being cattle responsible for 77% of that amount, buffalos 13% and small ruminants 10% (Gerber *et al* 2013). McMichael *et al* (2007) suggested that ruminants fed in extensive systems (grass) produce higher CH_4 emissions (1.8×10^9 of CO_2-equivalents) compared to cattle fed in intensive (grain) systems (0.4×10^9 of CO_2-equivalents). On the other hand, Gerber *et al* (2013) suggested that beef production contributes with 2.9 and milk production with 1.4 Gigatons of CH_4.

The emissions of CH_4 above described may be augmented due to the increase in human population (9.5 billion for 2050) which will elicit an increase in the demand of milk and meat (73 and 58% respectively), this will in turn determine an increase in livestock inventories at the global level (Robinson *et al* 2011). In this way**,** it has been predicted that emissions of CO_2, CH_4 and N_2O, will follow the same pattern of growth, as it was observed in 1996 when the world emissions of CH_4 reached 65,000 Gg (equivalents to CO_2) while in 2011 the emissions of CH_4 increased to 92,000 Gg[1] (FAOSTAT 2011)**.** Due to this increase in the inventories of CH_4, a series of strategies have been developed to decrease emissions of CH_4, among them: the search for more efficient breeds of livestock (Bezerra *et al* 2013), rumen defaunation with secondary metabolites (tannins and saponins), the use of various additives and inmunizations, the use of halogenated compounds and organic acids (Kumar *et al* 2014). However, the use of secondary metabolites (tannins and saponins) is one

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[1] Food and Agriculture Organization of the United Nations (FAOSTAT); 2011. Disponible en http://faostat.fao.org/site/291/default.aspx. Accesed on April 10, 2013.

of the most appropiate method due to their abundance in tropical plants at a small cost.

PLANTS CONTAINING CONDENSED TANNINS USED IN RUMINANT FEEDING

In different areas of the world there exist a wide diversity of plant species both trees and shrubs**,** which possess components of defense against herbivores, which are known as secondary metabolites, being the condensed tannins (CT) an important example of such metabolites which are found in foliages, fruits and cortex of plants (Perevolotsky 1994).

In addition to their high crude protein content (10-25%) and good digestibility (50-60%) (table 1), plants rich in condensed tannins have the capacity to reduce bacterial and protozoal populations in the rumen and the emissions of CH_4 (Puchala *et al* 2012), furthermore, condensed tannins have the ability to create complexes with the polysaccharides and proteins thus increasing the amount of protein of low rumen degradability which flow to the small intestine, favouring the increase in daily live weight gain and milk production (Min *et al* 2006).

CONDENSED AND HYDROLYZABLE TANNINS

Even when tannins are substances not very well defined from the chemical point of view, they belong to the family of the polyphenols, which are characterised by a large number of phenolic compounds, and their molecular weight ranges from 500 to 20,000 daltons (Karus *et al* 2003).

Condensed tannins (CT) have a strong affinity for other molecules and have the ability to precipitate alkaloids, carbohydrates and proteins. CT are widely distributed in the plant kingdom, and are used as defense against predators, pathogens and competitors and sometimes act in primary metabolism (Harborne 1993).

Conventionally, tannins are classified in two main groups: condensed tannins (CT) and hydrolyzable tannins (HT). Condensed tannins or proanthocyanidins are formed by polymers of chatequins, leucoanthocyanidins and its derivatives, joined by bonds C–C or C–O–C, while hydrolyzable tannins are composed of a nucleus formed with a glucid, and its hydroxilated compounds may be esterified by polymers of galic acid, egalic acid, fecarboxilyc acid or of hexahydrodiphenyl acid, on the other hand, when condensed tannins are consumed by the animals, they are no toxic as compared to hydrolyzable tannins, which are degraded by the microorganisms and absorbed as pyrogallol (Reed 1995).

EFFECTS OF CONDENSED TANNINS IN RUMINANTS

Condensed tannins have been related to several positive effects in animal production (Min *et al* 2006); however, when they are consumed by ruminants they could produce a variety different effects, one the main being the formation of complexes with proteins and polysaccharides, which are formed in the mouth at pH 7, and those complexes are kept as such while in the rumen until they are dissociated in the abomasum at pH between 3-3.5 (Kumar 1984).

The factors which promote the formation of complexes include: their high molecular weight and the structural flexibility of CT; the bond between the condensed tannin and proteins reduce rumen degradability and increase therefore the flow of protein to the small intestine (Soltan *et al* 2012).

In spite of their beneficial effects linked to the increase in the flow of protein of low rumen degradability to the small intestine, CT may reduce voluntary intake (VI) and rumen metababolism, and these responses will obey to the doses of condensed tannins, for example when intake of condensed tannins is above 7% of the DM of the ration, VI is reduced and deleterious effects may occur in animals. However, moderate intakes of condensed tannins (3-6% of DM on the ration) may lead to positive responses, such as the formation of the complex between tannin-protein and

Table 1. Chemical composition of plants foliages containing condensed tannins.
Composición química de follaje de plantas que contienen taninos.

Species	CP	NDF	ADF	CT	TP	Reference
	Composition in %					
Lespedeza cuneata	10.3	40.1	39.9	20.0	–	Puchala *et al* 2005
Guazuma ulmifolia	10.4	42.5	29.5	4.7	2.8	Gómez-Castro *et al* 2006
Acacia farneziana	24.0	42.1	26.7	4.5	10.0	Gómez-Castro *et al* 2006
Swietenia mahagoni	11.2	28.1	22.2	8.6	20.7	Jayanegara *et al* 2011
Myristica fragans	10.1	38.0	36.1	7.2	18.1	Jayanegara *et al* 2011
Prosopis juliflora	17.9	49.4	38.4	0.04	2.9	Soltan *et al* 2012
Acacia saligna	13.8	46.5	42.8	6.3	9.1	Soltan *et al* 2012
Leucaena leucocephala	23.6	78.9	51.1	2.3	5.17	Soltan *et al* 2013

CP= Crude protein; NDF= Neutral detergent fibre; ADF= Acid detergent fibre; CT=Condensed tannins; TP= Total phenols.

tannin-carbohydrate, in addition to their anti-parasitic effect (Barry and Duncan 1984) and reduction of CH_4 emissions.

EFFECTS OF CONDENSED TANNINS IN THE RUMEN SYNTHESIS OF VOLATILE FATTY ACIDS (VFA'S)

Condensed tannins have the ability to reduce the fermentation and digestibility of organic matter in the rumen, they also alter the proportions of VFA's, particularly the ratio acetate:propionate; and the presence of CT may reduce the NH_3 production in the rumen (Jayanegara *et al* 2012, Hassanat and Benchaar 2013).

Under *in vitro* conditions, Hassanat and Benchaar (2013) demonstrated that CT of *Acacia mearnsii* and *Schinopsis balansae* increase the molar proportion of propionate above 100 g/kg dry matter, while the proportion of acetic acid goes unaffected. Gurbuz (2009) evaluated the effect of legumes and observed that the total concentration of VFA's was numerically decreased in relation to the concentration of CT of the forage legumes, but the molar proportion of acetate and propionate remained unaffected.

The consistency in the reduction of VFA's production and the relatively constant ratio acetate:propionate seem to depend on the type of vegetation used. Tiemann *et al* (2008) found that the concentration of the three main VFA's decreased as the concentration of condensed tannins was increased, however, the reduction in VFA's was affected by the source of CT. Concentration of acetate was less affected by the CT of *Flemingia macrophylla* compared to those of other legumes; however, concentration of propionate was higher with CT from *F. macrophylla* and *Leucaena leucocephala* than with those of *Calliandra calothyrsus*.

Soltan *et al* (2012) evaluated four plants rich in CT and determined that the molar proportion of acetate was not significantly different among plants, while the concentration of propionate was higher with a reduction in the ratio acetate:propionate with *Prosopis juliflora* and *L. leucocephala*, This suggests that the effect of the CT in *L. leucocephala* on the molar proportions of VFA's, will depend on the concentration used.

EFFECTS OF CONDENSED TANNINS ON RUMEN MICROORGANISMS AND THE REDUCTION OF ENTERIC METHANE EMISSIONS

Even when the specific effect of CT on the reduction in emissions of CH_4 is unknown, some authors have pointed out that they form complexes with dietary proteins and carbohydrates in the rumen (pH = 7.0) thus reducing digestibility of DM and organic matter (OM) and the release of H_2 (indirect effect) (Jayanegara *et al* 2011, Patra 2014). Furthermore, CT have the ability to reduce the activity of the protozoa population (Galindo *et al* 2008) and the bacteria (*Archaea*) in the rumen, particularly of the population of *Methanobrevibacter ruminantium* (Tavendale *et al* 2005) and *Methanosphaera spp* (Min *et al* 2014).

Some authors have pointed out that there are three mechanisms of action of CT on rumen microorganisms (inhibition of enzymatic activity, lack of substrate and direct action on the cell membrane and lack of metallic ions) (McSweeney *et al* 2001), these effects constraint growth and activity of the rumen population (Goel *et al* 2005); however, even when partially unknown, some reports indicate that there are bacterial populations with the ability to degrade tannins and to develop enzymatic mechanisms to avoid the microbial effect of tannins (Goel *et al* 2007). Due to the antimicrobial mechanisms it is possible that the protozoa, bacterial and fungi populations may adapt to the presence of tannins in the ration and the effects of these on methane reduction may be reduced or may only show their effect during a short period of time before the microorganism become adapted to the mechanisms of tannin action (Wischer *et al* 2014).

Nonetheless, Galindo *et al* (2008) found that when *L. leucocephala* was included at the level of 30% under *in vitro* conditions, there was a reduction in the protozoa and bacterial populations by 39.42 and 43.8% respectively; similar results were reported by Bhatta *et al* (2013[a]) who found a reduction of up to 53.5% in the population of rumen protozoa.

Under *in vitro* conditions Goel *et al* (2008) registered a reduction of 44 and 47% in the rumen population when they added 66 mg of *Sesbania sesban* and *Trigonella foenun-graecum L.* to the basal susbstrate. Cieslak *et al* (2012) reduced by 23% rumen protozoal population when they included 2 g of CT/kg DM in cows fed forage and concentrate.

In addtion to the reduction in the protozoal population, CT exert important effects on the population of methanogenic bacteria, on this respect it has been found that their incorporation results in a reduction in *Methanobrevibacter ruminantium* (Tavendale *et al* 2005). Min *et al* (2014) observed that when they fed goats with a ration with 30% of DM as pine cortex (10.3% CT), there was a reduction of 33 and 37% in bacteria of the genus *Methanosphaera spp* and *Methanobacteriaceae spp* respectively.

Under *in vitro* conditions, Min *et al* (2006) found that the incorporation of 2% of CT in the DM from extract of quebracho (with 75% of CT kg/DM), reduced CH_4 production by 31%. Also, Goel *et al* (2008) reported a reduction of 19.9% in CH_4 production when they added 150 mg of *Carduus pynochepalus* to the substrate.

Tan *et al* (2011) found a linear reduction in gas production and when they included condensed tannins from *L. leucocephala*, gas production was reduced up to 43%, in a similar way CH_4 production was decreased by 63%. Jayanegara *et al* (2011) evaluated gas production with plants containing condensed tannins and found that *Swietenia mahagoni, L. leucocephala* and *Acacia magnium* had the ability to reduce CH_4 production by 78.3, 43.4 and 65%

respectively. Similar results were found by Delgado *et al* (2012) when evaluating twelve plants rich in condensed tannins, who observed a lower production of CH_4 when they were compared to *Cynodon nlemfuensis.* Table 2 describes several studies that demonstrate the potential of condensed tannins for the reduction of CH_4 emissions under *in vitro* conditions.

Under *in vivo* conditions, Puchala *et al* (2005) found a reduction of 57.1% in CH_4 emissions in the rumen of goats consuming *L. cuneata* compared to goats that had consumed only grass (table 3). On the other hand Animut *et al* (2008) reported that goats consuming 200, 447and 613g DM of *L. striata* were able to reduce CH_4 emissions by 32.8, 47.3 and 58.4%; respectively. Dias-Moreira *et al* (2013) observed that when they included 40 g/kg DM of condensed tannins from *L. leucocephala* in the ration of Santa Inés sheep, emissions of CH_4 were reduced by 26%. Similar results were reported by Bhatta *et al* (2013[a]) when they added 1.8 g of CT + 3.8 g of HT (per kg DM) in the ration of goats, observing that methane production decreased by 22%, this effect being correlated with the reduction in the digestibility of organic matter (13.5%) and the lower concentration of acetic acid (17%) in the rumen.

In our laboratory, we fed cattle *Pennisetum purpureum* grass mixed with increasing levels of *L. leucocephala* (containing 1.5% CT) at levels of 40, 60 and 80% and using open-circuit respirations chambers, reductions in methane emissions of 18, 22 and 34% respectively have been obtained (Piñeiro-Vázquez, unpublished data). At this respect, Delgado *et al* (2012) observed that when they included 27% of *L. leucocephala* in the ration of sheep, CH_4 emissions decreased by 15.6%. Tiemann *et al* (2008) included 300 g/kg of DM as foliage of *C. calothyrsus* or *F. macrophylla* in the ration of growing sheep and recorded a reduction of 21 and 17.4% in the production of enteric CH_4, this reduction could have been caused by the decrease in organic matter digestibility of 8.3 and 11.5% respectively. Table 3 shows the effect of condensed tannins on the reduction of enteric CH_4 under *in vivo* conditions.

In a meta-analysis published by Jayanegara *et al* (2012), it was conclusively demonstrated that a clear relationship exist between the concentration of CT in the ration, and the reduction in the synthesis of enteric CH_4, which agrees with reports by several other authors (Bhatta *et al* 2013[a], Soltan *et al* 2013).

Even when the precise mechanism of action of CT on methanogenesis has not been clearly established both under *in vitro* as under *in vivo* conditions (tables 2 and 3), CT have demonstrated potential for the reduction of CH_4, part of this reduction in CH_4 synthesis is due to the formation of the tannin-protein and tannin-polysaccharide complexes. Huang *et al* (2011) found that the reduction in the emissions of CH_4 is closely related to the molecular weight of the CT involved. Naumann *et al* (2013) indicated that the difference in molecular weights does not affect CH_4 production, however they demonstrated that concentrations of CT had a direct effect on the reduction of CH_4 production in the rumen.

Still, Hatew *et al* (2014) evaluated different cultivars of *Onobrychis viciifolia and* found that the effect of this plant it is not only due to the concentration of CT, but rather to different associated components, among them the content of micronutrients and to the different molecular structures of tannins.

In the different experiments discussed in this review, several foliage of trees or shrubs have been employed as sources of secondary metabolites (condensed tannins), which have shown an effect on the reduction of methane emissions, in this context, it is important to know the potential of silvopastoral systems (Murgueitio *et al* 2013), as well as the inclusion of foliages and fruits which contain condensed and hydrolyzable tannins and saponins as an strategy for the mitigation of the emissions of greenhouse gases, specifically for the reductions of enteric CH_4. Additionally to the effect on the reduction of the emissions of greenhouse gases, it is important to assess the potential of silvopastoral systems to increase production of meat and milk in order to decrease rate of emission of GHG per unit product (g CH_4/kg milk or g of CH_4/kg live weight).

EFFECTS OF CONDENSED TANNINS ON ANIMAL PERFORMANCE

Condensed tannins exert a beneficial effect on daily weight gain probably due to the fact that they protect dietary protein from microbial degradation in the rumen which increases the absorption of amino acids from the small intestine (Min *et al* 2006, Soltan *et al* 2012).

The relatively lower degradability of dietary protein in the rumen has been widely demonstrated in plants which contain high concentrations of condensed tannins. In this sense, Soltan *et al* (2012) found that with *L. leucocephala* in a concentration of 46 eq-g of tannic acid/kg DM of condensed tannins compared to a grass without tannins, rumen protein degradation was reduced from 614 to 117 g/kg of DM, while rumen undegradable protein was increased from 386 to 888 g/kg DM. These authors also showed that digestible protein in the small intestine increased from 416 to 464 g/kg DM, this increase in undegradable protein was in turn correlated with the increase in the excretion of nitrogen in the faeces. The influence of CT on the nitrogen balance has been reported. In this regard, Soltan *et al* (2013) determined that with rations containing 8.8 eq-g tannic acid/kg DM, the amount of nitrogen in feces increased by 70.19%, while urinary nitrogen decreased by 12.9%. Similar results were reported by Carulla *et al* (2005) when they fed sheep with extracts from *A. mearsii* observing an increase in nitrogen content of faeces, as well as a reduction of 9% in the proportion of total nitrogen excreted in urine. A similar trend was also observed by Chung *et al* (2013) in steers fed sainfoin containing 2.4%

Table 2. Effect of condensed tannins on methane emissions under *in vitro* conditions.
Efecto de taninos condensados sobre las emisiones de metano bajo condiciones *in vitro*.

Test system	CT Sources	Dose	Methane reduction	Propionic acid Increase	Protozoa reduction	Reference
In vitro	Extracts of *L. leucocephala* (100%)	MW: 1348.80, 857.01, 730.06, 726.00 494.56	66.1% 53.7% 35.5% 19.8% 21.4%	ND	ND	Huang *et al* 2011
In vitro	Extracts of *Acacia cornigera, Albizia lebbekoides* and *L. leucocephala*	240.0 mg	15.0%, 20.5% and 9.5%	5.2%, 6.7% and 2.6%	ND	Rodríguez *et al* 2011
In vitro	Extracts of *L. leucocephala*	10.0 15.0 20.0 25.0 and 30.0 mg	33.0%, 47.0%, 57.0%, 59.0%, and 63.0%	0.77% 1.9% 1.2% 4.8% and 6.8%	86.0%, 83.0%, 62.0%, 55.0% and 55.0%	Tan *et al* 2011
In vitro	*Mimosa diplotricha* L. (4.3% of CT) *Tagetes erecta* (1.3% of CT)	0.5%, 1.5% and 2.5% of CT/0.9999 g DM	25.6%, 33.80% and 23.4%. 38.9%, 30.3% and 13.4%	ND	ND	Andrade-Rivero *et al* 2012
In vitro	*Acacia saligna* and *L. leucocephala* with a concentration of CT of 6.3 and 4.6% respectively	100 mg foliage of the plants	36.0% 38.0%	NE	9.0% 23.0%	Soltan *et al* 2012
In vitro	*Alpinia galanga* (7.5 g of CT/kg of DM) *vs* *Mentha citrata* (0 g of CT/kg of DM)	200 mg of sample	79.3%	ND	6.3%	Bhatta *et al* 2013b
In vitro	Extracts of *S. balansae* (90.4% of CT)	100, 150 and 200 g/kg of CT	23.0, 34.0 and 40.0%	9.7%, 10.7% and 13.2%	ND	Hassanat and Benchaar 2013

MW = Molecular weight; CT = Condensed tanins; NE = No effect; ND = Not determined.

Table 3. Effect of condensed tannins on methane emissions under *in vivo* conditions.

Efecto de taninos condensados sobre las emisiones de metano bajo condiciones *in vivo*.

Species	CT Sources	Dose	Methane reduction	Protozoa reduction	Reference
Goats	Fed forage *L. striata* (151 g CT/kg of DM)	200, 447 and 613 of DM intake (g/day)	32.8% 47.3% and 58.4%	42.0%, 56.0%, 69.0%	Animut *et al* 2008
Cattle	Extracts of *A. mearnsii* (603 g CT/kg of DM)	166 and 266 g CT/day	14.0% and 29.0%	ND	Grainger *et al* 2009
Cattle	140 g of *Vaccinium vitis idaea*	2 g CT/ kg of DM	11.3%	26.9%	Cieslak *et al* 2012
Goats	*L. cuneata* with 153 g CT/kg of DM	frequency of feeding: daily every 2nd day every 4th day	 46.3% 39.7% 17.6%	 20.0 % 18.4% 12.3%	Puchala *et al* 2012[a]
Goats	*L. cuneata* (20 % of CT/kg of DM)	*Ad libitum* (DM intake 907 g/d)	40.9%	70.8%	Puchala *et al* 2012[b]
Goats	CT of *Mimosa spp* containing 37 g CT/kg of DM and 76 g HT/kg of DM.	1.9 HT + 5.8 CT (g/kg of food)	22.4%	ND	Bhatta *et al* 2013[b]
Sheep	*L. leucocephala*	40 g CT /kg of DM	25.7%	ND	Dias-Moreira *et al* 2013
Sheep	*L. leucocephala* (8.8 eq-g leucocyanidin/kg of DM)	35% of the ration	14.1%	ND	Soltan *et al* 2013

OMD = Organic matter digestibility; DMI = Dry matter intake; DM = Dry matter; ND = Not determined; CT = Condensed tannins; HT = Hydrolyzable tannins.

of condensed tannins in which protein digestibility was reduced by 7%.

Some studies have indicated that CT exert a beneficial effect on daily weight gain; Min *et al* (2006) fed steers with 2% condensed tannins from quebracho (Chemtan Company Inc., Exeter, NH; extract contains approximately 75% CT in the dry matter) and obtained an increase of 20.8% in live weight gain; similarly Ayala-Monter (2013) reported that when they added 2.5% condensed tannins in the ration, daily weight gain increased by 5.5%. This could be due to the effect of condensed tannins on the reduction of rumen degradable protein (Soltan *et al* 2012), promoting its pass to the abomasum, where proteins are digested, and the amino acids released are absorbed further down the alimentary tract in the small intestine.

Burke *et al* (2014) obtained an increase in daily weight gain of 26.1% in grazing sheep supplemented with 900 g of *L. cuneata* compared to a group of sheep supplemented with 900 g of a concentrate with 16% crude protein. Min *et al* (2012) included 1.5% of CT from *A. mearnsii* in the ration of cattle and they did not find differences in weight gains between treatments. Nonetheless, some studies have reported reductions in daily weight gain of sheep (135 vs 48 g/day), when they were fed corn and 2.5% CT in the ration (Priolo *et al* 2000). This reduction in daily weight gain may have been due to the reduction in voluntary feed intake, digestibility of organic matter and the fibrous components of the ration.

The weight loss of the animals consuming CT may be related to the increase of 45% in the energy loss in feces and the reduction of 30% in nitrogen retention, when goats were fed 5.7 g of CT per kilogram of DM (Bhatta *et al* 2013[b]).

On the other hand an increase in milk production has been recorded in several experiments. Anantasook *et al* (2014) showed an increase of 10% in milk yield of cows fed 88 g of CT per kg DM. And Dey *et al* (2014) with crossbred cows fed 1.5% condensed tannins per kg DM were able to increase milk yield by 24.7% compared to cows without CT in the ration. In this sense, under tropical conditions the widely available tropical shrub *L. leucocephala* provided at levels of 30 and 45% on the DM of ration was able to increase milk yield up to 6.64 and 7.76 kg per cow per day respectively compared to cows (5.11 kg milk/day) fed only a grass ration (*P. purpureum*) (Ruiz-González 2013).

In spite of a positive effect of CT on milk production, some findings have been reported a reduction in milk production. At this respect Grainger *et al* (2009) found that when they supplemented cows with 244 g of CT per day, milk production was decreased by 29.7%. This reduction was related to a decrease on 26.4% in feed intake induced by CT. On the other hand, Fagundes *et al* (2014) evaluated the inclusion of 25% of *F. macrophylla* with a concentration of 105 g/kg DM of CT in the ration of goats, and found a reduction of 10.8% in voluntary feed intake; however, they did not find any difference in milk yield.

Due to the variation in the effect of CT both under *in vitro* as under *in vivo* conditions, on rumen microorganisms, patterns of rumen fermentation, emissions of CH_4 and productive performance, it is advisable to continue performing experiments on the effect of CT, specific doses given, and to explore the effect of molecular weight of condensed tannins on the production of CH_4 in the rumen as well as to determine the effect of CT on energy retention in the body and on the possible increase in added value of ruminant products.

CONCLUSION

Even when the specific effect of condensed tannins on the reduction of CH_4 production in the rumen is still relatively unclear, it has been well established that condensed tannins have the ability to reduce the population of protozoa and methanogens in the rumen, furthermore, they reduce digestibility and alter the pattern of volatile fatty acids in the rumen, reducing the molar proportion of acetic acid and increasing propionic acid. In general, both under *in vitro* as under *in vivo* conditions, condensed tannins have the ability of reduce the emissions of enteric CH_4, and improve the use of feed energy, which in turn may increase ruminant performance (i.e. weight gain and milk yield).

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