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REVIEW ARTICLE

Methane mitigation from ruminants using tannins and saponins

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Keywords Methane · Tannins · Saponins · Rumen fermentation

Abbreviations

BCM	Bromochloromethane
BW	Body weight
CT	Condensed tannins
DDMI	Digestible dry matter intake
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
GE	Gross energy
HT	Hydrolysable tannins
MR	Methane reduction on volume basis
MR_{TD}	Methane reduction on truly degraded substrate basis
OMD	Organic matter digestibility
PSM	Plant secondary metabolites
QSE	*Quillaja saponaria* extract
SCFAs	Short-chain fatty acids
TP	Total phenols
TT	Total tannins

Introduction

The ruminal methane production is a by-product of the microbial digestive process and represents a loss of 2–12% of the feed energy. Furthermore, emission of methane is considered as one of the most important global environmental issues (IPCC 2001). Therefore, decreasing methane production is desirable for reducing the greenhouse gas emission with improved efficiency of the digested energy utilization (Johnson and Johnson 1995). A previous report by Kurihara et al. (1999) indicated that methane energy loss in cattle fed on tropical forage diets was higher than in those fed on temperate forage diets, due to relative high levels of fibre and lignin and a low level of non-fibre carbohydrate in tropical forages. Also, the livestock in developing countries are predominantly maintained on a high-roughage diet with little or no concentrate resulting in increased ruminal methanogenesis. Therefore, the use of browse species containing secondary compounds as feed supplement rich in plant secondary metabolites (PSM) for ruminants in many parts of the tropics is increasing in order to improve animal performance and reduce methane (Abdulrazak et al. 2000). Tannins and saponins constitute the major classes of PSM that are currently under research in a number of laboratories. The antimicrobial action and effects on rumen fermentation of these compounds depend on their nature, activity and concentration in a plant or plant

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product. In this paper, in vivo studies available in literature wherein effects of tannins and saponins have been evaluated are presented, and their potential for mitigating methane from ruminant livestock is discussed. In addition, in vitro studies conducted in our laboratory on the effects of tannins and saponins on rumen methane production and their possible mode of actions are also discussed.

Tannins and saponins

Tannins are polyphenolic substances of diverse molecular weights and of variable complexity. They have the ability to bind proteins in aqueous solution. Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides. Tannins are classified into two classes—hydrolysable (HT) and condensed tannins (CT)—and are considered to have both adverse and beneficial effects depending on their concentration and nature besides other factors such as animal species, physiological state of the animal and composition of the diet (Makkar 2003). Saponins are natural detergents, chemically defined as high molecular weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety. These compounds result in cell death by forming complex with sterols in protozoal cell membranes (Cheeke 1999). They modify ruminal fermentation by suppressing ruminal protozoa and selectively inhibiting some bacteria. The symbiosis of protozoa with methanogenic bacteria in the rumen is well established, and selective suppression of protozoa has been suggested to be a promising approach to reduce the methane production. Plants rich in saponins have potential for enhancing flow of microbial protein from rumen, increasing efficiency of feed utilization and decreasing methanogenesis.

In vivo studies

Tannin-rich plants/extracts

Table 1 lists the in vivo studies on the evaluation of tannin-rich forages for their methane reducing effect. For tannin-containing plants, the anti-methanogenic activity has been studied mainly for condensed tannin-rich plants or extracts because of their lower risk of toxicity to the animal than hydrolysable tannins (Beauchemin et al. 2008). Tavendale et al. (2005) suggested two modes of action of tannins on methanogenesis: first, directly affecting activity or population of methanogens, resulting in lower methane emission and, second, indirectly by reduced hydrogen production by lowering feed degradation. Recently, Jayanegara et al. (2011) also supported this observation by evaluating a total of 30 experiments comprising 171 treatments with dietary tannins using meta-analysis and reported the direct effect of tannins on methanogens and indirect effect via a reduced ruminal nutrient degradation. Negative effects on ruminal fibre digestion may relate to decrease in number of cellulolytic bacteria (McSweeney et al. 2001), formation of tannin–cellulose complexes that are resistant to enzymatic digestion (Makkar et al. 1995) and/or impairment in substrate adhesion by fibrolytic microbes (Bento et al. 2005), which would reduce hydrogen availability and lessen methanogenesis (Carulla et al. 2005). Furthermore, tannins are known to decrease protozoal number (Makkar et al. 1995), and the decrease in methane production could also be mediated through decrease in protozoal number. Shift in short-chain fatty acid (SCFA) production is usually expected with inhibition of methane using condensed tannins; however, Tiemann et al. (2008) did not observe any change in proportions of SCFA or in protozoal population with reduction in methane production caused by condensed tannin-rich legumes. Condensed tannins from *Lotus* have been reported to reduce methane production (grammes per /kilogrammes of dry matter intake) by about 15% in sheep and by a similar amount in dairy cows (Waghorn and Woodward 2006).

Sliwinski et al. (2002) reported that addition of 10 g of chestnut tree wood extract per kilogramme DM of the basal diet (0.2% tannin in diet) did not significantly decrease methane production. Beauchemin et al. (2007) also did not observe any inhibition of methane by feeding quebracho tannin extract up to 2% (1.8% CT) of the dietary dry matter. From these two observations, it is evident that 0.2% and 1.8% of tannins from chestnut tree wood extract and quebracho tannin extract, respectively, in the diets are below the threshold required to reduce methane in cattle. Although it is possible that higher amount of chestnut tree wood extract and quebracho tannin extract reduce methane production, high dietary concentrations of tannins could negatively affect digestibility of feed and the animal performance. Carulla et al. (2005) reported that supplementing the diet with 2.5% condensed tannin from *Acacia mearnsii* decreased methane production by approximately 12%, due in part to a 5% reduction in the total tract neutral detergent fibre digestion. Therefore, they suggested lower level of supplementations, to minimize negative effects of condensed tannins on fibre digestibility; however, this is also expected to affect methane suppression. Decrease in both methane production and in dry matter and organic matter digestibilities observed by other workers (Hess et al. 2006; Abdalla et al. 2007; Animut et al. 2008; Tiemann et al. 2008) also suggest that the observed methane mitigation, at least partially, is due to decrease in feed digestibility. From these limited studies conducted so far, it appears that substantial reduction in methane emission would be difficult to achieve without decreasing the feed digestibility

Table 1 Effects of tannin-rich plants or extracts on ruminal methanogenesis in vivo

Tannin source, composition	Animal and feeding levels of diets	Control diet offered to the animal	Tannin-containing diet	Methane reduction	Decrease in digestibility	Reference
LP (CT 5.3% by butanol–HCl method, information on standard not provided)	Sheep (800–900 g DM/day)	Ryegrass-based pasture	LP	28.5% as g/kg DDMI compared to ryegrass	Not reported	Woodward et al. (2001)
		Lucerne		23.6% as g/kg DDMI compared to lucerne		
LC (CT 2.59% by butanol–HCl method, information on standard not provided)	Cow (feeding ad libitum)	Ryegrass silage	LC silage	23.7% g/kg DMI		
CS tannin extract (Prodex, tree wood extract, source: Valorex Prodex SA, France)	Lamb (1.16 kg/day)	Hay/concentrate (1:1)	1 and 2 g tannin/kg DM or 5 and 10 g of the extract per kg DM	No effect	No effect	Sliwinski et al. (2002)
LC (CT 2.62% by butanol–HCl method, information on standard not provided)	Cattle (40 kg DM)	Ryegrass pasture	LC	17.7% as g/DMI compared to ryegrass	Not reported	Woodward et al. (2004)
LeC (CT 17.7% by butanol–HCl method, CT extracted and purified using Sephadex LH-20 from LeC used as a standard)	Goat (feeding ad libitum)	Pasture containing Crabgrass and Kentucky-31 tall fescue	LeC pasture	30% as g/day 50% as g/kg DMI	Not reported	Puchala et al. (2005)
AM extract (source: Weibull Black, Tanac S.A., Montenegro, Brazil) 61.5% CT by butanol–HCl method, information on standard not provided	Sheep (75 g of forage DM per kg metabolic BW	Rye grass	41 g of crude extract/kg dietary DM	15% as kJ/MJ of GE intake	Decrease in DE intake^{a};	Carulla et al. (2005)
		Rye grass + red clover (1:1)	41 g of crude extract /kg dietary DM	13% as kJ/MJ of GE intake	decrease in apparent digestibility of all nutrients except hemicellulose^{a}	
		Rye grass + alfalfa (1:1)	41 g of crude extract /kg dietary DM	11% as kJ/MJ of GE intake		
SQ extract (CT 90% by butanol–HCl method; source: Unitan Saica Chaco, Argentina)	Cattle (feeding ad libitum)	Forage–concentrate diet (70:30)	2% extract (1.8% CT in diet)	No effect	No effect	Beauchemin et al. (2007)
MC (CT 7.2% by butanol–HCl method, leucocyanidine equivalent)	Sheep (1.3 kg DMI/day)	Forage–concentrate diet (66:34)	12.7% MC (CT 0.91% in diet)	28% as L/day	Decrease^{a}	Abdalla et al. (2007)
LS (CT 15.1% using butanol–HCl method, CT extracted and purified using Sephadex LH-20, from LeC used as a standard)	Goat (feeding at 1.3 times metabolisable energy)	Sorghum Sudan grass (SG)	SG + 33 g of LS	32.8% as L/day	7% in OMD	Animut et al. (2008)
			SG + 67 g of LS	47.3% as L/day	18% in OMD	
			SG + 100 g of LS	58.4% as L/day	25.7% in OMD	
CC (CT 17.5%) and FM (CT 11.5%) by butanol–HCl method, information on standard not provided)	Lamb 6 treatments (combinations of grass, grass + legume, grass + legume + Tannin-rich diet); fed at 60 g DM/ kg metabolic BW	BB (100)	BB:V:CC (55:30:15), CT 2.23%	7.8% as L/day compared to BB:V	8.3% in OMD compared to BB:V	Tiemann et al. (2008)
		BB/V (55:45)	BB:V:CC (55:15:30), CT 3.28%	21% as L/day compared to BB	8.3% in OMD compared to BB	
			BB:V:FM (55:30:15), CT 1.42%	7.8% as L/day compared to BB:V	9.1% in OMD compared to BB:V	
			BB:V:FM (55:15:30), CT 2.88	21.5% as L/day compared to BB	8.3% in OMD compared to BB	

Table 1 (continued)

Tannin source, composition	Animal and feeding levels of diets	Control diet offered to the animal	Tannin-containing diet	Methane reduction	Decrease in digestibility	Reference
AM tannins (CT 60.3%) using a purified extract of AM as standard	Cattle (14 days) Cattle (35 days)	Grazed on pasture with 4.5 kg grain/ day	8.6 g AM tannins/kg DMI 14.6 g AM tannins/kg DMI	14% as g/day 22.4% as g/kg DM digested	Decrease[a] Decrease[a]	Grainger et al. (2009)
TC seed pulp (CT 0.11% DM) information on standard not provided	Sheep (47.2 g DM/kg BW)	Forage/concentrate (50:50)	10 g TC seed pulp/kg DMI	24% as L/kg digested DM intake	Increase[a]	Patra et al. (2011)

LP, *Lotus pedunculatus*; *LC*, *Lotus corniculatus*; *CS*, *Castanea sativa*; *LeC*, *Lespedeza cuneata*; *AM*, *A. mearnsii*; *SQ*, *Schinopsis quebracho-colorado*; *MC*, *Mimosa caesalpineaefolia*; *LS*, *Lespedeza striat*; *CC*, *Calliandra calothyrsus*; *FM*, *Flemingia macrophylla*; *TC*, *Terminalia chebula*; *BB*, *Brachiaria brizantha*; *V*, *Vigna unguiculata*; *DMI*, dry matter intake; *DDMI*, digestible dry matter intake; *BW*, body weight; *DE*, digestible energy

[a] Value not provided

and productivity using tannins. However, more in vivo studies with a wide range of tannins sources (and types) need to be conducted to evaluate the full potential of tannins. Since the effects of tannins depend on their nature, there is a need to find 'ideal' tannins that are specific in decreasing methanegenesis but do not adversely affect the feed digestibility.

Saponin-rich plants/extracts

As for tannins, a limited number of in vivo studies have been conducted with saponin (Table 2). Using Yucca as source of saponins, Sliwinski et al. (2002) did not record methane reduction while Santoso et al. (2004) observed a decrease of 6.7%. In the former study, the maximum level of Yucca saponins used was 0.003% in diet, and in the latter, the level of Yucca extract was 0.012% in diet. On the other hand, Holtshausen et al. (2009) at 1% of Yucca extract in the diet (saponin content 0.06% in diet) did not observe reduction in methane production. The saponin content in the Yucca extract used in the study of Santoso et al. (2004) is not given. Although Yucca extracts used in the studies of Santoso et al. (2004) and Holtshausen et al. (2009) were obtained from the same commercial company, the products used could be different. However, it may be noted that Holtshausen et al. (2009) used 100-fold higher amount of the extract than that used by Santoso et al. (2004), and also in the former study, saponins used were fivefold higher than the amount of the extract, but no methane reduction was observed. Although these results are difficult to explain, the difference in effects could be due to different diets used; effects might be higher for silage based diet used by Santoso et al. (2004). Hess et al. (2004) used dried fruits of *Sapindus saponaria* and recorded a decrease in methane production. The level of saponins in the diet in this study was 0.75%, which is much higher than the levels of Yucca extracts/saponins that elicited methane reduction. Since information on the content of saponins in Yucca product and the methods used for saponin determination is lacking, it is difficult to compare the efficacy of these saponins. Different methods for determination of saponins can result in different values (Makkar et al. 2007). The reduction in feed digestibility was observed in only one study (Table 2). Unlike tannins, saponins could have wider applicability in mitigating methane production. The other in vivo studies using saponins from *Sapindus rarak* (Wina et al. 2004), *Sapindus saponaria* (Abreu et al. 2004; Hess et al. 2004), *Sesbania sesban* (Teferedegne et al. 1999) and Yucca commercial sarsaponin (Hristov et al. 1999) indirectly suggest reduction in methane production, since increase in propionate production and decrease in protozoal number have been recorded in these studies.

Table 2 Effects of saponin-rich plants or extracts on ruminal methanogenesis in vivo

Saponin-rich source and content	Animal and feeding level	Treatments	Methane reduction	Decrease in digestibility	Reference
YS extract (saponin content: 30%; source: micro-aid or Sevarin, Distributors Processing Inc., USA)	Lamb (1.16 kg/day)	Hay/concentrate (1:1)+2 mg saponin /kg DM Hay/concentrate (1:1)+30 mg saponins /kg DM	No effect	No effect	Sliwinski et al. (2002)
YS extract (product: DK Sarsaponin 35, source: Desert King International, Saponin content: not given)	Sheep (fed at 55 g DM per kg metabolic BW)	Grass silage/concentrate (70:30)+120 mg YS extract/kg DM	6.7% as L/kg BW	No effect	Santoso et al. (2004)
SP dried fruits (saponin 12%)	Lamb (fed at 60 g DM per kg metabolic BW)	Grass hay+0.6 g/kg metabolic weight of crude saponin from fruits of SP	10.5% as L/day	5.3% in OMD	Hess et al. (2004)
		Grass/CA (1:2)+0.6 g/kg metabolic weight of crude saponin from fruits of SP	5.7% as L/day	3.7% in OMD	
		Grass/CA (2:1)+0.6 g/kg metabolic weight of crude saponin from fruits of SP	No effect	3.6% in OMD	
TS (triterpenoid saponins >60%, source: Hu et al. 2005)	Sheep (1 kg DM)	Hay/concentrate (3:2)+5 g/kg TS	8.7% as L/kg DMI	Not reported	Yuan et al. (2007)
QS extract (saponin 5–7%) or YS extract (saponin 8–10%) source: Mitsuba Trading Co., Ltd. Tokyo, Japan)	Sheep (fed at 55 g DM per kg metabolic BW)	Concentrate and Italian ryegrass hay (2:3)+0.8–1.13 g QS extract/day or 1.31–1.64 g of *Yucca* saponins/day	No effect	No effect	Pen et al. (2007)
YS powder (saponins 6%) or QS powder (Saponins 3%) (source: Desert King International, USA)	Cow (feeding ad libitum)	Hay/concentrate (51:49)+10 g/kg of DM of each powder	No effect	No effect	Holtshausen et al. (2009)
YS extract (Desert King International Product, USA)	Sheep (1.72 kg DM)	Hay/concentrate (3:1) + extract (170 mg/day)	15.5% as g/day	No effect	Wang et al. (2009)
TS (triterpenoid saponins >60%, source: Hu et al. 2005)	Lamb (at maintenance requirement for digestible energy)	60:40 Wild rye/concentrate + TS 3 g/day	27.2%	Not reported	Mao et al. (2010)
TS (600 g triterpenoid saponins/kg DM), Zhejiang Orient Tea Development Co. Ltd. (Hangzhou, Zhejiang, China)	Sheep (at maintenance requirement for digestible energy)	60:40 Wild rye/concentrate + TS 3 g/day	10.6%	Not reported	Zhou et al. (2011)

YS, *Yucca schidigera*; *SP*, *Sapindus saponari*; *CA*, *Cratylia argentea*; *TS*, *Tea saponins*; *QS*, *Q. saponaria*; *DM*, dry matter; *DMI*, dry matter intake; *OMD*, organic matter digested

In vitro studies

In order to understand better the mechanism of action and to identify new plant materials with potential to mitigate methane production, we conducted various in vitro studies on tannin-rich plants, purified tannins, pure simple phenolics, saponin-containing plants and saponin-rich fractions.

Tannin-rich plants/extracts

We evaluated different polyphenol-containing plants, pure simple phenols in the form of phenolic acids and purified tannins from chestnut, mimosa, quebracho and sumach. These studies were conducted using the in vitro Hohenheim gas production method (Menke and Steingass 1988) as modified by Makkar et al. (1995). In a study with 17 plants containing polyphenols, statistically significant and negative relationships were observed between total phenols (TP), total tannins (TT) or tannin activity and methane production, whereas the relationship between condensed CT and methane production was non-significant (Jayanegara et al. 2008). Highest correlation was found between tannin activity determined by the tannin bioassay and methane decrease. Between TP and TT, the correlations between TP and decrease in methane or increase in methane on addition of polyethylene glycol (a tannin-inactivating agent) were higher, which indicated that non-tannin phenols also contribute to the methane reduction. It is of interest to note that non-tannin phenols could be better alternative to tannins since non-tannin phenols are not likely to decrease the utilization of proteins and other nutrients, and could also have beneficial effects (antioxidant, anticarcinogenic) associated with phenolic compounds (Makkar 2003; Makkar et al. 2007). We then evaluated six simple phenols (benzoic, cinnamic, phenylacetic, caffeic, p-coumaric and ferulic acids), as representatives of non-tannin phenols. The addition of simple phenols decreased gas production, although for most of them the reduction was not significant and the effect was higher at higher concentration (2 and 5 mM were evaluated). Cinnamic, caffeic, p-coumaric and ferulic acids decreased methane production significantly ($P<0.05$) when added at 5 mM. The order of simple phenols to decrease methane was: p-coumaric >ferulic >cinnamic. Caffeic acid at 5 mM decreased methane by 6.3% on absolute basis and 9.4% when expressed as per unit organic matter digested (Jayanegara 2009). These results suggest that phenolics with higher number of hydroxyl groups are expected to elicit higher methane reduction effect. The effect of phenolic acids on methanogenesis could be expected since they affect the activities of rumen microbes, inhibiting fibre degradation and decreasing protozoal population. The decrease in methane due to anti-protozoal activities of phenolic acids would decrease methane production since a portion of methanogens is attached to protozoa; however, this does not hold always true as a weak association between protozoal numbers and methanogenesis was observed with saponin-containing plants (discussed in saponin section below).

To exclude the role of other confounding components present in tannin-containing plants and to study the specific role of tannins, tannins were purified from chestnut, mimosa, quebracho and sumach and their effects studied at concentrations of 0.5, 0.75 and 1.0 mg/ml in the in vitro rumen fermentation system (Jayanegara et al. 2009) (Table 3). Chestnut and sumach tannins represented the hydrolysable tannins, whereas mimosa and quebracho tannins represented the condensed tannins. The addition of purified chestnut and sumach tannins at 1 mg/ml to hay/concentrate (70:30) diet significantly decreased ($P<0.05$) methane production by 6.5% and 7.2%, respectively. The addition of mimosa and quebracho tannins (condensed tannins) did not significantly decrease methane production, even at the highest concentration. These sources of condensed tannin decreased organic matter digestibility (OMD) and total SCFA production much greater than hydrolysable tannins. Only the addition of sumach tannins at 1.0 mg/ml increased significantly the partitioning of nutrients to gas partition factor (PF), expressed as milligrammes of truly degraded substrate per millilitre of gas produced, a measure of efficiency of microbial protein synthesis. The changes in the microbial population as estimated by the comparative delta Ct method (Denman and McSweeney 2006) showed a reduction in methanogens by 24%, 29% and 37% by quebracho, mimosa and chestnut tannins as 1 mg/ml. The condensed tannins decreased gas production and OMD more than the hydrolysable tannins. The results suggested that the hydrolysable tannins are more effective in decreasing methane emissions than the condensed tannins, while at the same time the hydrolysable tannins did not significantly decrease OMD. The condensed tannins appear to decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins act more through inhibition of the growth and/or activity of methanogens- and/or hydrogen-producing microbes (direct effect). Other changes in fibre-degrading microbes as determined by the delta Ct method were 80%, 80% and 93% reduction in anaerobic fungi; 63%, 74% and 86% reduction in *Fibrobacter succinogenes* and 24% (non-significant from control), 10% (non-significant from control) and 33% reduction in *Ruminococcus flavefaciens* at 1 mg/ml of quebracho, mimosa and chestnut tannins. Although it was evident from our studies that polyphenols in soluble forms reduce ruminal methane production significantly, it should be noted that we used in vitro experiments to measure the effects, and in in vivo situations where tannins are a part of

Table 3 Effects of purified condensed and hydrolysable tannins on rumen fermentation parameters (Jayanegara 2009a)

Treatment	Partition factor	MR (%)	MR_{TD} (%)	Total SCFA (mM)
Control	3.15a	0.0a	0.0a	52.16e
Chesnut tannins (mg/ml)				
0.50	3.20a	1.3a	2.9ab	49.67bcde
0.75	3.21a	3.2abc	5.0ab	49.19bcde
1.0	3.19a	6.5bc	7.5abc	47.95abcde
Mimosa tannins (mg/ml)				
0.50	3.18a	1.9ab	2.8ab	46.64abc
0.75	3.22a	2.5abc	4.6ab	46.58abc
1.0	3.26ab	3.0abc	6.3ab	45.82ab
Quebracho tannins (mg/ml)				
0.50	3.24ab	1.6a	4.4ab	51.21cde
0.75	3.22a	2.3abc	4.4ab	47.34abcd
1.0	3.32ab	3.3abc	8.3bc	43.82a
Sumach tannins (mg/ml)				
0.50	3.31ab	1.9ab	6.7abc	51.58de
0.75	3.30ab	3.4abc	7.9bc	51.72de
1.0	3.41b	7.2c	14.2c	50.41bcde
SEM	0.015	0.41	0.72	0.724

Values in the same column with different letters are different at $P<0.05$

MR methane reduction, MR_{TD} methane reduction per unit truly degraded substrate

the feed, the effects might be different. Nevertheless, the in vitro studies give insight into the mechanism of action of various tannins, their comparative effects and possible in vivo effects.

Translation of the in vitro results to in vivo responses is always challenging. Flachowsky and Lebzien (2009) proposed a three-step program to assess the methane reduction potential of feed additives or feeding measurements because of the poor relationship between methane produced in vivo and in vitro ($r^2=0.264$). The three-step program includes in vitro screening of substances, short-term in vivo experiments in target animals and finally in vivo long term recording of methane production together with other animal performance parameters. Such a three-step program will substantially increase the relevance of such studies to the industry and potential users. In the three-step program, the short-term in vivo studies could also be replaced by continuous fermentation studies as conducted by Goel et al. (2009). The continuous fermentation experiment was conducted for 15 days with 6 days of adaptation period and 9 days of experimental period using bromochloromethane (BCM). The results obtained using the continuous fermentation were similar to those obtained in the batch process, although the degrees of changes observed were different. Using the continuous fermentation system, a persistent effect of BCM on methane reduction (85–90%) was obtained throughout the study with no effect on gas production, SCFA production, acetate/propionate proportion, true degradability and efficiency of microbial mass synthesis. The batch fermentation is an attractive tool for initial screening of plants and plant products for anti-methanogenic activity, while the continuous fermentation could be used to evaluate the persistency of the effects. The use of continuous fermentation system could replace the intermediatory step involving short-term in vivo studies, thereby saving resources and time.

Saponin-rich plants/extracts

The saponin-containing plant materials—leaves from *Sesbania* (*Sesbania sesban*) or seeds of Fenugreek (*Trigonella foenum-graecum* L.)—when supplemented to hay- or concentrate-based diets did not produce substantial reduction in methane production. These supplements resulted in reduction in protozoal population which did not accompany the decrease in methane production in the incubations using rumen liquor from hay-fed animals, whereas a small reduction in methane was produced in incubations with rumen liquor from concentrate-fed animals. Diet-dependent effects of saponins were also reported by Hegarty (1999) where studies have shown slight effect of defaunation on methane production when animals were fed with roughages and no effect with a diet rich in starch. The supplements tested did not adversely affect the degradability of the basal feeds, hay or concentrate–hay mixture, and these possess the potential to partition higher proportion of the substrate to microbial mass production and to elicit some methane reduction per unit of substrate degraded (Goel et al. 2008a). Since there is a limit beyond which these supplements cannot be incorporated into an animal diet due to adverse effect on feed intake of the basal diet, saponin-rich fractions were prepared from leaves of *Sesbania*, *Knautia* (*Knautia arvensis*) and seeds of Fenugreek and evaluated in vitro (Goel et al. 2008b). These fractions could be added as additives into animal diets, without constraining intake of the basal diet. Saponins have been reported to alter the rumen fermentation by affecting the digestibility (either increase or no effect) and increasing microbial protein synthesis (Makkar et al. 1998). However, in the study of Goel et al. (2008b), the saponin-rich fractions did not affect digestibility and a trend towards slightly higher gas production was observed, which might be due to the saponin-mediated increase in fibre-degrading bacteria (discussed below). These fractions did not show any increase in the partitioning of nutrients to gas (Table 4), while increased PF values were observed on supplementation of the plant materials from which these saponins were isolated. It may be noted that the amount of saponin-rich fractions added in the in vitro system corresponded to the amount of saponin-containing plants incubated in the earlier study Goel et al. (2008a). This difference in the response could either be due to the absence of active saponins responsible for methane reduction effect in the fractions studied or inactivation of saponins while isolating them from the plants.

Saponin-rich fractions did not result in any methane reduction although they lowered protozoal population by 36–39% (Table 3). A weak association of protozoal number and methanogenesis was evident in both studies, with original plant material as well as their saponin-rich fractions. A weak association between the protozoal suppression and methanogenesis has been reported by Dohme et al. (1999) and Pen et al. (2007) wherein they used coconut oil and QSE, respectively. Goel et al. (2008a) reported no differences in total SCFA (Table 3), but they observed a trend towards higher propionate and lower ammonia on supplementation of fenugreek seed and Sesbania leaf saponins. Similar results have been observed for *Quillaja saponin* (Makkar and Becker 1996), *Yucca* extract (Wang et al. 1998), *Quillaja saponaria* fruit (Hess et al. 2003) and *Sesbania pachycarpa* (Muetzel et al. 2003).

The saponin-rich fractions from different sources tended to change the microbial population differently as estimated by the comparative delta Ct method (Denman and McSweeney 2006). *Sesbania* saponins decreased methanogen population by 78%. Decrease in ruminal fungal population (20–60%) and increase in *F. succinogenes* (21–45%) and *R. flavefaciens* (23–40%) were observed. The increase in total bacterial population was indicated by decrease in absolute Ct value that was expected due to decrease in protozoal numbers since there is no predation of bacteria by protozoa. The increase in population of *F. succinogenes* can be attributed to their resistance against saponins as observed by Wang et al. (2000) and Vinogradov et al. (2001).

We did not observe any decrease in methane production while a decrease in protozoal number and methanogen population was noticed (Goel et al. 2008b). These observations indicated that the association between methanogens and protozoa is not obligatory, and the different groups of methanogens are not equally associated with ciliate protozoa. A weak relationship between methanogenesis and the methanogen population expressed as a proportion of total anaerobes was observed by Nollet et al. (1998) under both in vitro and in vivo conditions. In our study, no inhibition of methane production with decrease in methanogens could have been caused by (1) slow rate of association between protozoa and methanogens due to higher generation time of protozoa as compared to methanogens, (2) an increased metabolism of methanogenic microbes independent of species remained after addition of saponins and/or (3) by an altered composition of methanogenic community (Machmüller et al. 2003) and their increased efficiency of methane production. Additionally, it could be suggested that on inhibition of protozoa, the species belonging to Methanobacteriaceae (living in association with protozoa) declined with an increase in the number of free-living Methanobacteriales. The reduced rate of association of protozoa and methanogens could result in higher interspecies hydrogen transfer between increased population of both

Table 4 Effect of saponin-rich fractions of test plants on rumen fermentation parameters

Substrate[a]	Partition factor	MR (%)	MR_{TD} (%)	Protozoa[b] ($\times 10^4$/ml)	Total SCFA (μmol/ml)
S	3.25			19.54	871.6
S+F 5.62	3.12	1.82	−1.59	16.60 (15)	1014.0
S+F 11.54	3.07	1.97	−4.47	11.93 (39)	837.2
S+Se 10.9	3.14	4.69	1.54	16.80 (14)	849.1
S+Se 21.8	3.08	6.14	1.71	12.41 (36)	911.9
S+K 3.88	3.16	5.50	3.23	16.83 (14)	866.3
S+K 7.76	3.16	6.43	3.94	14.66 (25)	1035.2
SEM	0.122	1.821	1.112	1.224	10.11

[a] S: hay/concentrate (1:1), saponin-rich fractions (in milligrammes) from F (fenugreek), Se (*Sesbania*) and K (*Knautia*)

[b] Values in parentheses are the percent reduction in protozoal number

hydrogen-producing bacteria (*R. flavefaciens* and *F. succinogenes*) and free-living Methanobacteriales indicating no effect on methane production.

Conclusions

The concentration window for tannins, at which in vivo anti-methanogenic effects without decreasing organic matter digestibility and productivity of animals have yet to be observed, is expected to be narrower than for saponins. Furthermore, for tannins, substantial decrease in methane reduction would be difficult to achieve without compromising production; however, simultaneous benefits that could be accrued, for example decrease in rumen protein degradability and increase in post-rumen protein availability, partitioning of excreted nitrogen more towards faeces and lesser towards urine, and increase in efficiency of microbial protein synthesis recorded in earlier studies (Makkar 2003), might make the use of tannins attractive.

Among the tannin assays, tannin bioassay (a reflection of tannin activity) is the best predictor of the methane reduction potential of a plant. Total phenols and total tannins are also good predictors of the methane reduction potential. For screening a large number of tannin-containing plants and plant products, these assays could provide useful information on the potential candidates for further studies. In in vitro, methane decrease by addition of phenolic acids is relatively small, and the effect of phenolic acids on methane reduction depends on their concentration and number of hydroxyl groups on them. The higher the number of hydroxyl groups, the higher the potential methane reduction. Hydrolysable tannins appear to decrease methane production, and methane production per unit organic matter is digested to a greater extent than condensed tannins. The condensed tannins decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins appear to act more through inhibition of the growth and/or activity of methanogens- and/or hydrogen-producing microbes (direct effect). In vitro, the saponin-containing plants did not produce substantial reduction in methane production but showed the potential to partition higher proportion of the substrate to microbial mass production. The saponins tested possessed anti-protozoal activity but did not always result in methane inhibition suggesting that the uni-directional relationship between protozoal numbers and methanogenesis, as affected by saponins, is not obligatory.

A way forward

Since the effects of tannins are a function of their nature, which varies from source to source, generalization on the concentration-dependent anti-methanogenic effects of tannins must be avoided. The same holds true for saponins.

Different methods for determination of tannins and saponins are used by different workers, making comparison of anti-methanogenic effects difficult. There is a need to harmonise methods and to come to an agreement of using the same set of methods. Methods based on the activity of tannins and saponins must be included in the battery of methods used in the studies. For tannins, use of a bioassay based on the increase in gas production from an in vitro rumen fermentation system by the addition of polyethylene glycol could be a useful method in this context, and for saponins, there is a need to develop a suitable activity-based method. At present, haemolytic method for saponin quantification could be considered as an activity-based method; however, a method based on rumen anti-protozoal effect will be better. Work on development of such an assay is required. Even for the same genotype, nature of tannins and saponins changes with the maturity of the plant and with environmental factors; proper recording of these factors for the materials used and their proper characterization using a battery of assays based on chemical and biological properties must be conducted and described in the studies. These characterization studies would also help to minimize batch to batch variation and to bring a reliable product in the market for livestock users.

Not all hydrolysable tannins are toxic. More in vivo studies should be conducted with hydrolysable tannins since they appear to be more promising as far as decrease in methane production without decreasing feed digestibility is concerned. Yucca and tea saponins have potential for reducing methane emission in vivo; however, the products used need to be well characterized for saponin levels and activities.

For most of the additives, including plants and plant products, the effects elicited depend on the diets as well. Comparisons of the studies should take in account this variable as well.

There is a need to integrate life cycle analysis approaches in order to evaluate the overall benefits of using a methane mitigation strategy. If a strategy decreases methane but increases another green house gas (GHG), the overall aim of reduction of GHG is not served. This can only be captured if life cycle analysis is done. Furthermore, the mitigation of methane from ruminants should be included in the Clean Development Mechanism, and for this, an approach to verify methane reduction is also required.

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