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# Effects of tea saponins on rumen microbiota, rumen fermentation, methane production and growth performance—a review

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**Abstract** Reducing methane emission from ruminant animals has implications not only for global environmental protection but also for efficient animal production. Tea saponins (TS) extracted from seeds, leaves or roots of tea plant are pentacyclic triterpenes. They have a lasting antiprotozoal effect, but little effect on the methanogen population in sheep. There was no significant correlation between the protozoa counts and methanogens. The TS decreased methanogen activity. It seems that TS influenced the activity of the methanogens indirectly via the depressed ciliate protozoal population. The TS addition decreased fungal population in the medium containing rumen liquor in *in vitro* fermentation, but no such effect was observed in the rumen liquor of sheep fed TS. Tea saponins had a minor effect on the pattern of rumen fermentation and hence on nutrient digestion. When added at 3 g/day in diets, TS could improve daily weight gain and feed efficiency in goats. No positive associative effect existed between TS and disodium fumarate or soybean oil on methane suppression. Inclusion of TS in diets may be an effective way for improving feed efficiency in ruminants.

**Keywords** Tea saponins · Rumen microbiota · Rumen fermentation · Growth performance · Hydrogen acceptor

## Abbreviations

DF Disodium fumarate  
DGGE Denaturing gradient gel electrophoresis  
DM Dry matter

IPCC Intergovernmental Panel on Climate Change  
MCP Microbial protein  
*mcrA* Methyl-coenzyme M reductase  
SO Soybean oil  
TS Tea saponins  
TSDF Tea saponins plus disodium fumarate  
VFA Volatile fatty acids

## Introduction

Methane is one of the greenhouse gases. Ruminant animals are one of the largest sources of methane emission with 81–92 MT produced per year globally, equivalent to 23–27% of total anthropogenic methane (IPCC 2007). Methane produced during ruminal fermentation also represents a loss of 2–15% of gross energy intake (Johnson and Johnson 1995). Therefore, reducing methane emission from ruminant animals has implications not only for global environmental protection but also for efficient animal production. Saponins or saponinlike substances have been reported to suppress methane production, reduce rumen protozoa counts, and modulate rumen fermentation patterns (Lila et al. 2003; Wang et al. 2009). Saponins are found in various plants in different forms (Vincken et al. 2007), resulting in their different activities (Hassan et al. 2010). Both Yucca and Quillaja saponins are commercially available products that have been used as feed additives (Cheeke 2000), but little information is available on the use of tea saponins (TS) as ruminant feed additives.

China is one of the biggest producers of tea in the world. After oil extraction, tea seed meal is usually considered worthless and is disposed of. However, it contains large amount of saponins. Since 2001, a series of TS experiments

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was conducted in our group. To provide wider opportunities for the utilization of TS to enhance ruminant production, this review summarizes these experiments to discuss the effects of TS on rumen microbes, rumen fermentation, and ruminant production as well as the interaction between TS and hydrogen (H<sub>2</sub>) acceptors such as disodium fumarate and soybean oil.

### What are tea saponins?

Saponins are secondary compounds found in many plants. They form stable foam in aqueous solutions, as soap does, hence the name “saponins”. Saponins are structurally diverse molecules that are divided into two groups: triterpene and steroid glycosides (Vincken et al. 2007).

The TS extracted from seeds, leaves or roots of tea plant are pentacyclic triterpene. The basic structure of TS is composed of sapogenin (C<sub>30</sub>H<sub>50</sub>O<sub>6</sub>), sugars and organic acid. The main sapogenin is a derivative of oleanane. One or more sugar moieties containing galactose, xylose, arabinose or glucuronic acid are glycosidically linked to a sapogenin (aglycone). The organic acid is acetic, angelic, cevadic acid or cinnamic acid. The bioactive saponin constituents from tea plant (Theaceae) have been extensively studied. Japanese scientists reported the isolation and structure elucidation of theasaponins A1–A3, E1–E7, and F1–F3 from the seeds of Japanese tea plant [*Camellia sinensis* (L.) O. Kuntze (Theaceae)] (Kitagawa et al. 1998; Yoshikawa et al. 2005a; Morikawa et al. 2006), and of assamsaponins A–I from the seeds and leaves of Sri Lankan tea plant (*C. sinensis* L. var. *assamica* Pierre) (Murakami et al. 1999; Murakami et al. 2000). Studies were conducted on their antisweet, gastric emptying, and gastroprotective activities and accelerating effect on gastrointestinal transit.

Floratheasaponins A–C with antihyperlipidemic activities were also isolated from the flower part of the Japanese tea plant (Yoshikawa et al. 2005b). Figure 1 shows seven acylated oleananetype triterpene oligoglycosides and theasaponins from the seeds of Japanese tea plant (Yoshikawa et al. 2007).

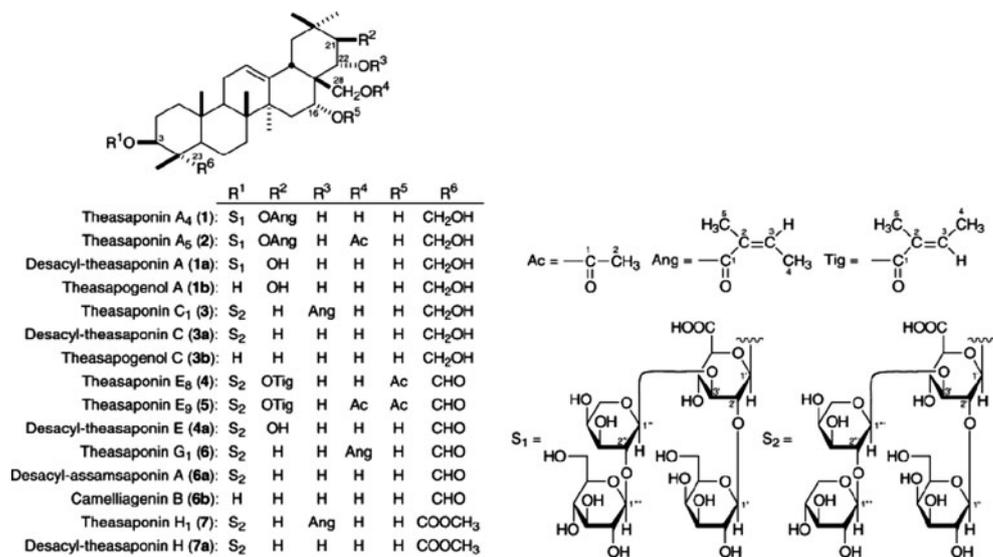
Based on their structure, TS may not only be effective in decontamination (Yuan et al. 2008), foam formation (Guo et al. 2009) and emulsification (Wu et al. 2007), but also function as agents for diminishing inflammation and resisting epiphyte growth (Sagesaka et al. 1996; Hayashi et al. 2000). The TS used in our experiments were obtained from Zhejiang Orient Tea Development Limited Liability Company affiliated to the Institute of Tea Science, Chinese Academy of Agricultural Sciences, China. They were a light yellow powder and easily soluble in water. The content of the triterpenoid saponins was above 600 g/kg, with foamability score of 160–190 mm and pH of 5.0–6.5.

### Effects on rumen microorganisms

#### Protozoa

Over 30 different genera (nearly 300 species) of protozoa from the rumen ecosystem have been described since their initial discovery nearly 150 years ago (Williams et al. 1991). Protozoa can be divided into two general groups: the “holotrichs” and “entodiniomorphs”. The holotrichs are important in utilizing soluble sugars and because their growth rate is slower and they are larger in size than bacteria, the holotrichs help control the rate of carbohydrate fermentation when large quantities of soluble carbohydrates are present in the diet. The entodiniomorphs are responsible for controlling starch digestion by engulfing whole starch

**Fig. 1** Seven acylated oleananetype triterpene oligoglycosides, theasaponins from the seeds of Japanese tea plant (Yoshikawa et al. 2007)



granules. This restricts bacterial access to the starch particles thus slowing down the rate of fermentation. Until recently, it has been difficult to study a single group of protozoa in isolation; consequently, most researchers refer to the faunated (with protozoa) and defaunated animals (without protozoa) (Rode 2000).

In *in vitro* gas production tests using equivalent grass meal and corn meal (50:50, w/w) as a substrate, TS significantly ( $P < 0.05$ ) decreased protozoa counts (Hu et al. 2005a; Guo et al. 2008). After 24-h incubations, protozoal counts were reduced by 19, 25, 45 and 79% when the TS was added at 10, 20, 30 and 40 g/kg substrate, respectively (Hu et al. 2005a) and the antiprotozoal effect of TS was later confirmed using a real-time PCR-based technique. At 0.4 mg TS/mL medium containing rumen fluid or 53 g TS/kg substrate, decreased protozoa count substantially (Guo et al. 2008).

Wallace et al. (2002) indicated that saponins might kill or damage protozoa by forming complexes with sterols in the protozoal membrane surface. The membrane may become impaired and eventually disintegrate. However, several reports showed no effect of saponin on protozoa, and some showed an increase (Wina et al. 2005a). Some saponins have a negative effect on protozoa, but the effect was not persistent after several days of feeding (Teferedegne et al. 1999; Ivan et al. 2004).

Focusing on this transient effect, we conducted two *in vivo* trials lasting 21 and 72 days. The diet was made of 60% Chinese wild ryegrass (*Aneurolepidium Chinese Kitagawa*) and 40% concentrate mixture with 3 g/day TS. After 21 days of feeding, the protozoa decreased significantly from 4.68 to 2.66% of total bacterial 16S rRNA gene (Table 1, Zhou et al. 2011). The protozoa count relative to

bacteria was reduced by 41% after 72 days of feeding (Table 2, Mao et al. 2010). The number of denaturing gradient gel electrophoresis (DGGE) bands and the Shannon diversity index of rumen protozoal DGGE profiles showed significantly lower diversity after 21 days of feeding of TS ( $P < 0.05$ , Fig. 2, Zhou et al. 2011) suggesting that the effect of TS on protozoa might not transient.

### Methanogens

Methanogenic archaea have been observed on the exterior surface of rumen ciliate protozoa (Vogels et al. 1980) and as endosymbionts within the ciliates (Finlay et al. 1994). Protozoa also provide some advantage to methanogens by quenching oxygen through their oxygen-tolerant hydrogenosomes, or they simply provide a vehicle for retention of slower-growing methanogens in the rumen (Müller 1993; Zinder 1993). Because 10 to 20% of methanogens live in association with protozoa (Tokura et al. 1999), it is expected that reducing protozoa would also reduce methanogens, thus decreasing methane production.

From *in vitro* (Guo et al. 2008) and *in vivo* (Mao et al. 2010; Table 2) experiments, it has been observed that TS addition had little effect on the methanogen population. No significant correlation existed between the protozoa counts and methanogens. A weak association between protozoal suppression and methanogens was obtained by Goel et al. (2008) who also reported that *Sesbania* saponins were more inhibitory to methanogens (78%) than *Fenugreek* and *Knautia* saponins (22 and 21%), while the reductions in protozoal numbers were 36, 39 and 25%, respectively. Guo et al. (2008) found that addition of TS at 0.4 mg/mL inhibited the expression of the methyl-coenzyme M

**Table 1** Effects of tea saponins on relative abundance of marker genes for specific microbial populations (% of total bacterial 16S rDNA) in sheep under defaunated and refaunated status ( $n=3$ ) (Zhou et al. 2011)

	RfN	RfTs	DfN	DfTs	SEM	P-value <sup>a</sup>		
						Df	TS	Df×TS
Methanogens ( <i>mcrA</i> ) <sup>b</sup>	0.61	0.57	0.34	0.35	0.064	**	NS	NS
Protozoa	4.68	2.66	6.75E-04	1.84E-04	0.364	**	*	*
Fungi, ×10 <sup>-3</sup>	15.19	14.70	0.34	0.63	0.004	**	NS	NS
<i>Ruminococcus albus</i> , ×10 <sup>-2</sup>	4.85	5.49	3.42	3.07	0.022	NS	NS	NS
<i>Ruminococcus flavefaciens</i> , ×10 <sup>-2</sup>	14.74	9.61	7.04	4.60	0.024	*	NS	NS
<i>Fibrobacter succinogen</i>	1.28	0.27	3.78E-04	4.23E-04	0.047	**	**	**
<i>Butyrivibrio fibrisolvens</i>	1.03	0.88	2.27	2.05	0.295	**	NS	NS

All values were determined as pooled rumen fluid samples (before feeding and at 3, 6 and 12 h after feeding over 2 days)

\*  $P < 0.05$

\*\*  $P < 0.01$

<sup>a</sup> Df defaunation effect, TS tea saponins effect, Df×TS interactive effect, NS  $P > 0.05$

<sup>b</sup> *mcrA*, methyl-coenzyme M reductase

**Table 2** Effects of addition of tea saponins, soybean oil or tea saponins plus soybean oil on microbial populations (% of total bacterial 16S rDNA) (Mao et al. 2010)

Items	Diets <sup>a</sup>				SEM	P-value		
	NTNS	TS	SO	TS–SO		TS	SO	TS×SO
Methanogens	0.34 <sup>b</sup>	0.36 <sup>b</sup>	0.20 <sup>c</sup>	0.24 <sup>c</sup>	0.044	0.57	0.02	0.78
Protozoa	9.71 <sup>b</sup>	5.72 <sup>c</sup>	4.71 <sup>c</sup>	5.42 <sup>c</sup>	1.192	0.20 <sup>d</sup>	0.05 <sup>c</sup>	0.08
Fungi, ×10 <sup>-2</sup>	5.43	4.03	3.49	4.20	0.972	0.72	0.39	0.31
<i>Ruminococcus flavefaciens</i> , ×10 <sup>-1</sup>	1.43 <sup>b</sup>	0.70 <sup>bc</sup>	0.28 <sup>c</sup>	0.76 <sup>bc</sup>	0.287	0.67	0.08	0.06
<i>Fibrobacter succinogen</i>	1.00 <sup>bc</sup>	1.29 <sup>b</sup>	0.68 <sup>c</sup>	0.81 <sup>bc</sup>	0.182	0.26	0.05	0.68

<sup>a</sup> Diets: NTNS no tea saponins nor soybean oil, TS tea saponins, SO soybean oil, TS–SO tea saponins plus soybean oil

<sup>b,c</sup> Within a row, means with different superscripts differ at  $P < 0.05$

<sup>d</sup> In case of the tendency of interaction between TS and SO, a secondary statistical test indicated the efficacy of factor SO within factor TS: without SO,  $P = 0.04$ , with SO,  $P = 0.68$

<sup>e</sup> In case of the tendency of interaction between TS and SO, a secondary statistical test indicated the efficacy of factor TS within factor SO: without TS,  $P = 0.02$ , with TS,  $P = 0.86$

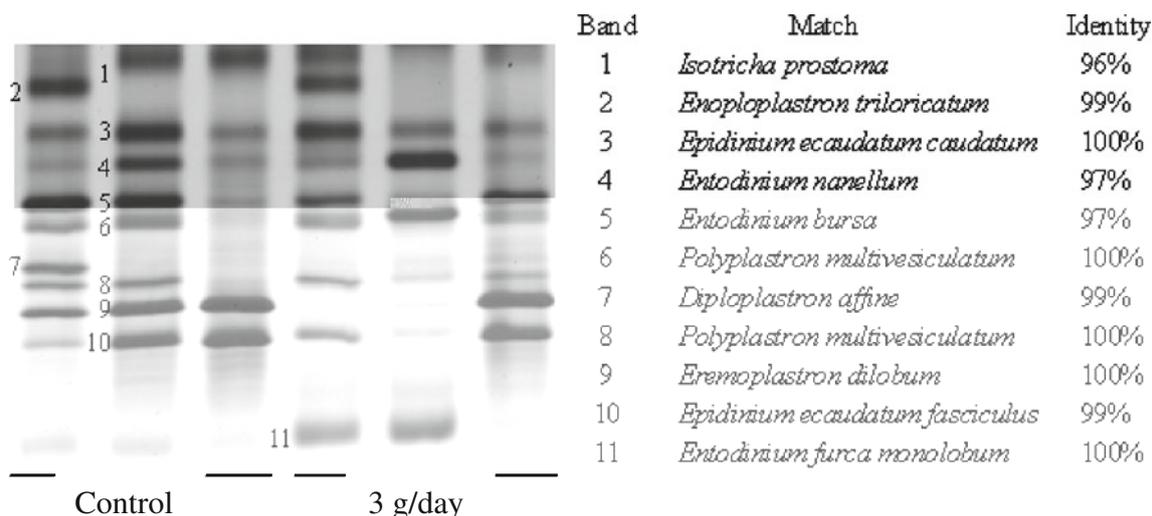
reductase (*mcrA*) gene by 76% ( $P < 0.01$ ) at 24 h of in vitro incubation. The *mcrA* is crucial to the final step of methanogenesis where it is involved in the reduction of the methyl group bound to coenzyme M. Expression of the *mcrA* gene is closely related to the activity of methanogens, indicating that methanogen activity was decreased by addition of TS.

To separate the effect of TS on protozoa and methanogens, defaunated and faunated (refaunated) sheep were used in a recent study (Zhou et al. 2011). The abundance of methanogen *mcrA* genes relative to total bacterial 16S rRNA gene was reduced by defaunation ( $P < 0.05$ ), whereas additional TS had no effect on *mcrA* gene abundance in either refaunated or defaunated sheep. Therefore, tea saponin appeared to reduce methane production by inhibit-

ing protozoa and presumably lowering methanogenic activity of protozoal associated methanogens.

#### Fungi

Although anaerobic fungi comprise only a small proportion of the total mass of the rumen microflora, they are important in the rumen for digesting fiber (Bauchop 1979). In our experiment, we observed that TS lowered fungal concentration relative to bacteria in the medium containing rumen liquor in in vitro fermentation (Guo et al. 2008), but not in the rumen liquor of sheep when fed with 3 g/day TS for 21 days (Table 1, Zhou et al. 2011) or 72 days (Table 2, Mao et al. 2010). Similar observations have been reported with addition of *Sapindus rarak* extract



**Fig. 2** Denaturing gradient gel electrophoresis profile of rumen protozoal community in Hu sheep showed significantly lower diversity after 21 days of feeding with tea saponins at 3 g/day ( $P < 0.05$ ) (Zhou et al. 2011)

(Wina et al. 2005b; Wina et al. 2006). This may be due to the adaptation of fungi during long-term feeding. Fungi produce certain carbohydrases (Mountfort and Asher 1989), which may degrade saponins. Further study is needed to confirm this.

#### Bacteria

Methanogens are intimately associated with other rumen microbes through  $H_2$  supply and utilization. Hydrogen is one of the major end products of fermentation by protozoa, fungi and some bacteria. Methanogens live by consuming  $H_2$  in the rumen and have to compete with propionate-producing microbes that consume  $H_2$  to form propionate (Zinder 1993).

There are several studies on the effects of saponins on specific rumen bacteria, but they have demonstrated inconsistent results. For example, Wang et al. (1998) found that the number of cellulolytic bacteria decreased by 30% when 0.5 mg/mL *Yucca* extract was added to 5 g chopped lucerne hay and 5 g concentrate in an in vitro fermentation. Cellulolytic bacteria were more susceptible to *Yucca* extract than amylolytic bacteria (Wang et al. 2000a). However, Diaz et al. (1993) observed a significant increase in cellulolytic and total bacteria in the rumen of sheep fed *Sapindus saponaria* fruit. A similar observation was made by Thalib et al. (1996) who reported that total cellulolytic bacteria increased when sheep were fed a diet containing saponins from *S. rarak*. Increases in total bacteria and the population of *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* relative to total bacteria were also observed in vitro on supplementation with *Sesbania*, *Fenugreek* and *Knautia* saponins (Goel et al. 2008).

In our in vitro experiment, 0.4 mg/mL TS had no effect ( $P>0.05$ ) on the relative abundance of *R. flavefaciens*, but increased ( $P<0.05$ ) the relative abundance of *F. succinogenes* (Guo et al. 2008). However, in vivo addition of TS had no effect on the population of *R. flavefaciens* or *F. succinogenes* (Table 2, Mao et al. 2010). Defaunation decreased the relative abundance of *R. flavefaciens* ( $P<0.05$ ) and *F. succinogenes* ( $P<0.01$ ), but increased the abundance of *Butyrivibrio fibrisolvens* ( $P<0.01$ ), and had no effect on *Ruminococcus albus*. There was a negative interaction effect between TS and defaunation ( $P<0.01$ ) on the abundance of *F. succinogenes* (Table 1, Zhou et al. 2011).

Klita et al. (1996) explained the susceptibility of rumen protozoa and lack of susceptibility of rumen bacteria to saponins by the presence of cholesterol in eukaryotic membrane (including protozoa) but not in prokaryotic bacteria cells. Vinogradov et al. (2001) reported that the presence of 2-aminoethylphosphoric acid and glycolipid in the membrane enhanced the membrane stability of *F. succinogenes*.

#### Effects on rumen fermentation

##### Ammonia and microbial protein

Ammonia in the rumen is produced from both feed degradation and microbial lysis and tends to decline after feeding due to a reduced substrate degradation and/or to an enhanced utilization of ammonia by bacteria (Owens and Bergen 1983). In a review by Wina et al. (2005a), out of 51 reports, 14 showed no effect and 17 showed a negative effect of saponins on rumen ammonia concentration. Wina et al. (2005a) suggested that the observed decrease in rumen ammonia concentration was an indirect result of the decreased protozoal number caused by the added saponins. Fewer protozoa would mean less predation and lysis of bacteria, hence less release of the products of protein breakdown. Protozoa contribute some 10–40% of the total rumen nitrogen (N) (Van Soest 1994).

In our studies, ammonia-N concentration in vitro decreased with increasing level of TS ( $P<0.01$ ), and was 8, 18, 21 and 27% lower at 24-h incubation when the TS were added at levels of 10, 20, 30 and 40 g/kg, respectively (Hu et al. 2005a). To determine the effect of protozoa on ammonia concentration, we measured ammonia-N concentration in rumen liquor on addition of TS to faunated and defaunated rumen fluid. In vitro, a 19% reduction in ammonia concentration was observed for 0.4 mg/mL TS in defaunated medium, a little higher than the effect on defaunation rumen fluid (Hu et al. 2005b). In an in vivo study, feeding of TS at 3 g/day to defaunated animal decreased ammonia concentration in the rumen fluid by 4%, which was much lower than the reduction in ammonia concentration observed by only defaunation (31%, Zhou et al. 2011). These differences between in vitro and in vivo experiments may be attributed to the higher TS concentration in vitro. Nevertheless, these observations demonstrate the impact of protozoa on ammonia concentration.

Jouany (1996) assumed that ciliated protozoa contributed significantly to intraruminal cycling of microbial N and reduced the efficiency of microbial protein (MCP) synthesis, thus reducing protozoal populations could improve dietary N utilization and increase MCP flow to the intestine. This was confirmed by Santoso et al. (2007), who reported that protozoal numbers decreased with the increase in the concentration of *Biophytum petersianum* Klotzsch saponins. There was a linear decrease in urinary N ( $P<0.01$ ) and total N excretion ( $P=0.05$ ) per unit of N intake. There was a linear decrease in retained N as a proportion of N digested and the efficiency of microbial N synthesis ( $P<0.01$ ) in response to saponin feeding. Addition of 40 g/kg of TS resulted in the lowest ammonia-N concentration and the highest MCP yield (Hu et al. 2005a). Hussain and Cheeke (1995) considered that the reduction in

ammonia-N by saponins from *Yucca scidigera* was due to the reduced urease activity. However, using glycofractions of the Yucca plant, Headon et al. (1991) suggested that this effect was due to ammonia-binding properties of glycofractions.

#### Short chain fatty acid and H<sub>2</sub> balance

Volatile fatty acids (VFA) represent the rumen fermentation pattern and efficiency of nutriment digestion (France and Dijkstra 2005). Santoso et al. (2007) reported that there were linear decreases ( $P<0.05$ ) in total VFA concentration and proportions of butyrate and *iso*-acids in response to feeding increased *B. petersianum* Klotzsch saponin. In our work, VFA were significantly reduced by defaunation ( $P<0.05$ ), but the concentrations of acetate, propionate and butyrate were not greatly affected by TS inclusion in either faunated or defaunated rumen fluid (Hu et al. 2005b). Addition of 30–40 gTS/kg diet tended to increase the propionate, but had little effect on acetate and butyrate suggesting that TS had only a minor effect on the pattern of rumen fermentation and hence on nutrient digestion (Hu et al. 2005a).

In the rumen, the formation of acetate and butyrate results in production of hydrogen gas (H<sub>2</sub>), a substrate that methanogenic archaea use to reduce carbon dioxide resulting in the production of methane (Hegarty 1999; Moss et al. 2000). Propionate production, on the other hand, serves as a competitive pathway for H<sub>2</sub> utilization and is accompanied by a decrease in overall methane production (Hegarty 1999; Moss et al. 2000). In our studies, rumen fermentation was not significantly affected by TS; therefore, H<sub>2</sub> production, utilization and recovery were not influenced by TS. This may be due to the self-regulation of microflora in the rumen because overall rumen fermentation appears to have been relatively homeostatic.

Two major H<sub>2</sub> producers, protozoa and fungi, were decreased with addition of TS and the activity of methane-producing archaea that are considered the major H<sub>2</sub> users was also decreased. In order to maintain H<sub>2</sub> balance, the number of H<sub>2</sub>-producing and H<sub>2</sub>-utilizing bacteria such as propionate-producing microbes might increase. Additionally, the activity of protozoa and fungi might change under the TS treatment, which should be investigated further.

#### Methane

Methane produced during enteric fermentation in ruminants leads to a substantial loss of feed energy for the animals, and increased ecological problems through greenhouse gas emissions. Therefore, reducing methane production has significant economic and environmental benefits. Methane

production was reduced by the inclusion of TS in the faunated rumen fluid and by defaunation (Hu et al. 2005b). Inclusion of 0.2 and 0.4 mg/mL TS decreased methane production by 13.3 and 14.3%, respectively, in the faunated rumen fluid in agreement to the results of Wang et al. (2000b) who observed that the methane production was 15% lower in Yucca saponin-fed group compared to the control. The methane suppressing effects of saponins were presumably a direct action against the rumen microbes involved in methane formation including methanogens and protozoa. In our research, defaunation was demonstrated as an efficient way to inhibit methane production. After 24-h incubation, methane production was reduced by 57.2% with defaunation (Hu et al. 2005b). Inclusion of TS significantly reduced methane production in the faunated rumen fluid, but not in the defaunated rumen fluid, suggesting that inhibition of methanogenesis by TS could be due to their antiprotozoal activity. Hess et al. (2003) noted a 54% decrease in protozoa counts and 20% decline in in vitro methane production with no effect on methanogens and suggested that defaunation reduced methane production because of a lower H<sub>2</sub> supply, thus reducing activity per methanogen.

The relative quantity of methanogens to total bacteria increased slightly, while methane production decreased, indicating the lack of correlation between methane production and methanogens (Guo et al. 2008). Soliva et al. (2003) reported the apparent lack of correlation between methane release and counts of microbes involved in methanogenesis. A weak relationship between methanogenesis and the methanogen population expressed as a proportion of total anaerobes was observed by Nollet et al. (1998) in vitro and in vivo and by Goel et al. (2008) in vitro. The lack of correlation between methane production and methanogens caused by TS in our study could be explained by their effects on the activity of methanogenic archaea, as mentioned above. It seems that TS influenced the activity of the methanogens via the depressed ciliate protozoa population.

#### Effects on growth performance and serum biochemical parameters

Growing Boer goats were used to investigate the effects of addition of TS on growth performance and serum biochemical parameters (Hu et al. 2006). Addition of 3 gTS/day increased dry matter intake, although not significantly. This result was different from those of Lovett et al. (2006), who observed a decreased dry matter intake in the Yucca extract-added diets. Other researchers reported little effects of saponin on feed intake (Singer et al. 2008; Nasri et al. 2011). From the information available on microbes and fermentation parameters, the increased dry matter intake

might be attributed to the increased efficiency of MCP synthesis and decreased degradability of feed protein from the reduced protozoa.

The goats fed a diet containing 3 g of TS/day had higher average daily gain and feed conversion ratio than those on 0 and 6 g of TS/day during the whole period (Hu et al. 2006), whereas Nasri et al. (2011) showed that the administration of 30, 60 or 90 mg *Quillaja saponarie* per kg dry matter (DM) intake had a defaunation effect but failed to improve feed digestibility, growth performance and meat quality in Barbarine lambs. Concentrations of total protein and albumin in the goats receiving 3 g of TS/day were higher than those receiving 0 and 6 g of TS/day (Hu et al. 2006). Urea N concentration was lower in the TS-fed goats than in the control. Higher concentrations of serum Ca, P and alkaline phosphatase were observed in the group receiving 3 g of TS/day. Serum cholesterol level was decreased and the high density lipoprotein cholesterol increased in the TS-fed animals. The concentration of glucose and activities of glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase were not affected by the addition of TS, suggesting that TS have no adverse effect on hepatic metabolism. There was no hemolytic phenomenon during the preparation of the serum of the animals. A number of studies have suggested that saponins from different sources lowered serum cholesterol levels in a variety of animals (Matsuura 2001; Afrose et al. 2010; Owolabi et al. 2010). Oakenfull and Sidhu (1990) reported that saponins act either directly, by inhibiting absorption of cholesterol from the small intestine, or indirectly, by inhibiting reabsorption of bile acids. Where direct inhibition of cholesterol absorption occurs, saponins prevented absorption of not only a high proportion of dietary cholesterol, but also of a high proportion of the cholesterol derived from bile and desquamation of mucosal cells.

## The interaction between tea saponins and H<sub>2</sub> acceptor

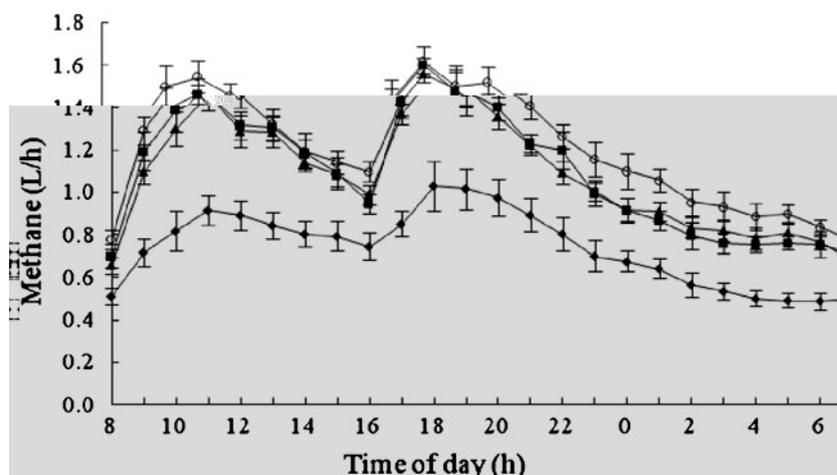
### Tea saponins and disodium fumarate

Diverting H<sub>2</sub> away from methane formation would promote alternative electron sink metabolic pathways to dispose of the reducing power (Ungerfeld et al. 2003). Hydrogen could be disposed of through fumarate reduction to succinate (Newbold et al. 2005).

However, addition of disodium fumarate (DF) to the TS-added diet did not have higher inhibitory effects on methane production in our in vivo experiment compared to the addition of TS alone. Methane production was measured using simple open-circuit respiratory chambers (Yuan et al. 2007). Addition of TS (5 g/day) and TSDF (TS 5 g/day plus DF 20 g/day, TSDF) resulted in a similar diurnal pattern of methane production as the control (Fig. 3). Methane production rapidly increased to a maximum after 2–3 h of feeding, and then decreased gradually until the next feeding. The diets with TS and TSDF have no effects ( $P>0.05$ ) on methane production during the day, but methane production was significantly decreased ( $P<0.001$ ) at night. Machmüller et al. (2003) also observed a similar diurnal pattern, which may be associated with the difference in animal activity and digestion between day and night times. Further study is needed to clarify this.

In the Rusitec, 6.25 mmol fumarate caused a 1.2 mmol fall in methane production (Lopez et al. 1999). In our in vivo study (Yuan et al. 2007), daily methane production was reduced by 9.6% on addition of TSDF, but if only DF was used, almost 2 mol (more than 300 g) of DF was needed to achieve the same level of methane reduction. In our study, the daily dose of fumarate was 20 g/day per sheep. Consequently, the contribution of fumarate on methane inhibition was limited. Newbold et al. (2005) concluded

**Fig. 3** Diurnal pattern of methane production from sheep in a chamber fed diets with different additives: control (unfilled circle, no additive), tea saponins [filled square, 5 g TS/kg dry matter (DM)], tea saponins plus disodium fumarate (filled triangle, 5 g TS/kg DM plus 20 g disodium fumarate/kg DM) and coconut oil (filled diamond, 7% DM). The vertical bars indicate standard error of mean at selected times (Yuan et al. 2007)



that although it might be possible to use fumarate to decrease ruminal methane production, the quantities required to make a major impact on daily methane production might be impractical.

### Tea saponins and soybean oil

Unsaturated fatty acids may provide an alternative metabolic H<sub>2</sub> acceptor to carbon dioxide, so it may be another way of diverting H<sub>2</sub> away from methane formation (Ungerfeld et al. 2003). Soybean oil (SO) contains more than 50% of linoleic acid (Zhang et al. 2008) and may be an effective H<sub>2</sub> acceptor.

Daily methane production was reduced by 27.7, 13.9 and 18.9%, respectively in lambs fed the diets with TS (3 g/day), SO (3% of DM), or TS–SO (TS, 3 g/day plus SO, 3% of DM) (Mao et al. 2010). However, no positive associative effect existed between TS and SO on methane suppression, while a negative associative effect was observed between TS and SO on the protozoa population. In our study, when SO or TS were added separately, their inhibitory effect on the protozoa population was significant, but the population of protozoa was not influenced when they were added together.

### Conclusions

Addition of tea saponins (TS) in animal diets may be an effective way to inhibit methanogenesis and hence has implications not only for global environmental protection but also for efficient animal production. A systematic evaluation is needed to confirm the active structural components of TS, and their interaction with the microbial community, the host animal and the diet, and to clarify the mechanism by which TS or their metabolites exert effects on the rumen microbes.

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