Effect of tea (Camellia sinensis) seed saponins on in vitro rumen fermentation, methane production and true digestibility at different forage to concentrate ratios

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ABSTRACT

The present study was conducted to evaluate the effect of tea (Camellia sinensis) seed saponins (TSS) on in vitro rumen fermentation with different forage to concentrate ratios in the in vitro gas production technique. Experimental treatments were a low forage diet (forage: concentrate = 30:70), a medium forage diet (forage: concentrate = 50:50) and a high forage diet (forage: concentrate = 70:30). TSS was added at levels of 0.0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of substrate. Protozoal count, ammonia-N production and methane production decreased linearly up to the dose level of 0.8% in all the substrates. The maximum reduction obtained was 54.6%, 57.2% and 60.6% for protozoal counts; 29%, 33% and 36% for methane production; and 36.6%, 36.6% and 33.8% for ammonia-N production at low, medium and high forage diets, respectively. Net 24 h gas production, short chain fatty acid production, metabolizable energy value increased and in vitro true dry matter and organic matter digestibility significantly decreased due to addition of saponins. Results suggest that TSS has the potential to reduce methane production and positively impact rumen fermentation across different forage to concentrate ratios.

1. Introduction

Excessive production of greenhouse gas (GHG) has been cited by many scientists world over as responsible for climate change. The adverse effects of climate change are enormous. The livestock sector is one of the important contributors of GHG. GHG emission from the global livestock sector was 7.1 gigatonnes CO\(_2\)- equivalent per annum, representing 14.5% of human-induced GHG emissions for the year 2005 (FAO 2013). The global cattle population is responsible for 65% of the GHG emissions from livestock. The rapid increase in demand for animal products in developing nations may result in higher emission shares and volumes over time. Methane production during enteric fermentation from ruminants is one of the important emission sources and 2.7 gigatonnes CO\(_2\)-equivalent per annum GHG emission was produced in the year 2005 (FAO 2013). Average loss of gross energy in the form of methane is reported to be 7.89% of intake for dry cows (Wilkerson et al. 1995). So there is an urgent need to reduce methane emission from ruminants.

Recent research suggests that plant secondary metabolites such as saponins may have potential to reduce methane emission, and improve N utilization by defaunating action in rumin (Wina et al. 2005; Gurbuz & Davies 2010). Jayanegara et al. (2014) observed in a meta-analysis study that despite large structural diversity, addition of saponin significantly reduced in vitro methane emission. They also reported that higher levels of saponin in the diet did not negatively influence digestibility. There are reports which suggest that forage to concentrate ratio is one of the primary factors which affect the effect of saponin supplementation (Goel et al. 2008; Gurbuz 2009; Manatbay et al. 2014) on rumen fermentation and animal production.

In view of this, the present experiment was conducted to test the effect of saponin extracts of tea (Camellia sinensis) seed on rumen fermentation and methane production by using the in vitro gas production technique (IVGPT) with three different forage to concentrate ratios and also to get the best level of saponin that should be added to improve rumen fermentation.

2. Materials and methods

The experimental protocols were reviewed and approved by the Institute Research Committee and Animal Ethics Committee.

2.1. Extraction of saponin from tea seeds

Saponin was extracted from tea seeds as per the method of Joshi et al. (2013). Dried and powered tea seeds were defatted with hexane in a percolator at room temperature. The defatted tea seed residue was suspended in 70% aqueous methanol. The methanolic extract was concentrated and dried into powder containing saponins. The powder was chromatographed on Diaion HP-20 eluting with H\(_2\)O→CH\(_3\)OH→CHCl\(_3\) to get H\(_2\)O, CH\(_3\)OH and CHCl\(_3\) fractions. The methanolic fraction was dried...
using rotar vapour followed by lyophilization into powder as crude saponins. Saponins were subjected to foam test and erythrocyte haemolysis test. Purity of the saponin was tested by the method of Joshi et al. (2013).

2.2. Preparation of inoculum and experimental protocol

The inoculum used for the *in vitro* studies was rumen fluids obtained from two fistulated steers fed on a maintenance diet consisting of wheat straw *ad lib* and concentrate mixture of 2 kg per day. The rumen fluid was sampled just before morning feeding (0 h) from these animals, strained through four layers of muslin cloth and transported in insulated flasks under anaerobic condition to the laboratory, pooled in equal proportions and used as a source of inoculum.

Tea seed saponin (TSS) was added at levels of 0.0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of substrate. Three different substrates varying in forage to concentrate ratios as 30:70 w/w (S1), 50:50 w/w (S2) and 70:30 w/w (S3) were used for the experiment. Forage used was oat fodder and concentrate mixture consisting of 34% maize grain, 28% soya bean meal, 35% wheat bran, 2% mineral mixture, 1% common salt. A total of 200 mg of substrate was used for the experiment. The incubation medium was prepared as described by Menke and Steingass (1988) and 30 ml of inoculum was dispensed anaerobically in each syringe. The IVGPT was completed in 6 runs (statistical replicates) with each treatment sample (9 doses × 3 forage concentrate ratios = 27 treatments) incubated in triplicate (analytical replicate) in 3 syringes in each run. The analytical replicates were averaged for each run prior to statistical analysis. Each run was considered as a statistical replicate. Each run also comprised three syringes of blank for correction of gas and methane produced from inoculums. After 24 h at 39°C, the incubation was stopped, total gas production was recorded and the contents were analysed for methane production, protozoa number, total gas production, TDMD and TOMD, the following general linear model was used: analysing the effect of different saponin levels and forage to concentrate ratios; i.e., the effect of the th treatment effect; the general mean effect; the effect of the th ratio; the peak was identified by comparison of the above standard and the peak area obtained was used to calculate the methane concentration in the gas sample. The volume of methane produced (ml) was calculated by multiplying the gas produced (ml) by the concentration of methane in the sample. The methane produced from the substrate after 24 h incubation was calculated by correcting the corresponding blank values.

The ammonia N in the syringe contents was estimated as per the procedure of Weatherburn (1967) and protozoa numbers were estimated as per the procedure of Kamra et al. (1991). True digestibility was estimated as per the method outlined by Van Soest and Robertson (1988). After 24 h of incubation in the Hohenheim IVGPT, the contents of the syringe were transferred quantitatively to a 500 ml spoutless beaker by repeated washing with 40 ml double strength neutral detergent solution. The contents in the beaker were refuxed for 1 h and filtered under vacuum through preweighed Gooch crucibles. The neutral detergent fibre (NDF) content of the residue was determined. The ash content of the NDF was also determined by burning the residue in a muffle furnace at 600°C. *In vitro* TDMD and TOMD of feed were calculated (Van Soest & Robertson 1988; Gurbuz 2007).

Short chain fatty acid (SCFA) concentrations were calculated according to Getachew et al. (2002) as

$$ SCFA (mmol/200 mg DM) = 0.0222 GP – 0.00425, $$

where GP is the 24 h net GP (ml/200 mg DM). Metabolizable energy (ME) values of substrates were calculated by a formula derived by Menke and Steingass (1988):

$$ ME (MJ) = 2.20 + 0.136 \times \text{gas (ml/200 mg DM)} + 0.0057 \times CP + 0.0029 \times EE. $$

2.4. Chemical analysis of substrates
The DM (ID number 930.15), EE (ID number 920.39), CP (ID number 954.01), ash (ID number 942.05) and ADL (ID number 973.18) were determined as per the protocols of AOAC (2000). The NDF and acid detergent fibre (ADF) were analysed as per the method of Van Soest et al. (1991; Gurbuz & Kaplan 2008). The NDF was estimated without amylase and NDF and ADF both were expressed inclusive of ash. Organic matter (OM), cellulose and hemicellulose were calculated by difference.

2.5. Statistical analysis
The statistical analysis was done by using factorial ANOVA. For analysing the effect of different saponin levels and forage to concentrate ratios on methane production, ammonia N, protozoa population, total gas production, TDMD and TOMD, the following general linear model was used:

$$ y_{ijk} = \mu + T_i + R_j + (TR)_{ij} + e_{ijk}, $$

where $y_{ijk}$ is the observed value of the response variable for the $i$th treatment and the $j$th ratio; $\mu$ the general mean effect; $T_i$ the $i$th treatment effect; $R_j$ the effect of the $j$th ratio; $(TR)_{ij}$ the
interaction effect of the $i$th treatment and the $j$th ratio; and $e_{ij}$ the error term.

The multiple comparisons between saponin levels, forage to concentrate ratios and interaction effects of saponin levels with forage to concentrate ratios were done using the Tukey HSD test at 5% level of significance. The statistical analysis was done by using JMP 8.0 software.

3. Results and discussion

3.1. Saponin yield, purity and activity

Crude saponin powder obtained was light brown in colour and was easily soluble in water. Tea seeds yield on dry matter basis was 6.5–25.1% of crude saponins having purity of 73.6%. TSS produced 46 ml of foam after 30 min and also showed good haemolytic activity; 100 µg of TSS produced complete haemolysis of 3% sheep RBC.

3.2. Effect of addition of TSS on in vitro rumen fermentation profile and true digestibility

Chemical composition of different substrates used for the present study is given in Table 1. Since substrates with varying forage concentrate ratios were used, as expected there was variation in crude protein (CP) and fibre contents among the substrates used for in vitro studies.

Volume of gas produced in different treatments is given in Table 2. Gas production (ml/200 mg of substrate) was significantly higher in S1 as compared to S2 in which it was higher

Concentration of ammonia-N in different treatments is given in Table 3. Ammonia-N concentration was significantly decreased with increasing level of saponins up to a certain dose level, that is, 15.89 mmol/L at 0% TSS to 10.34 mmol/L at 0.9% TSS (35% reduction). Concentration of ammonia-N was significantly higher in S1 (17.13 mmol/L) as compared to S2 (16.05 mmol/L) in which it was higher than S3 (14.48 mmol/L). A lower concentration of ammonia-N indicated that addition of TSS improved efficiency of microbial protein synthesis in all substrates. The lowest value in S1 may be due to reduced in vitro digestibility. Results are in agreement with Hu, Liu et al. (2005), and Hu, Wu et al. (2005). Hu, Wu et al. (2005) reported decreased ammonia-N concentration from 15 to 14.39, 12.77, 12.31 and 11.41 mmol/L when tea saponin was added at the levels of 1%, 2%, 3% and 4% of the basal substrate. Decreased ammonia-N concentration from 11.6 to 10.4, 8.9 and 8.4 mmol/L was reported when tea saponin was added at 0.27%, 0.53% and 1.07% of the basal substrate (Hu, Liu et al. 2005). Reduced ammonia-N may be due to reduced protozoal population, which is responsible for degradation of bacterial proteins, thereby increasing rumen ammonia levels and reducing efficiency of microbial protein synthesis. It is also well known that saponin adsorbs ammonia. Out of the total rumen nitrogen some 10–40% was contributed by protozoa (Van Soest 1994).

Protozoal number in different treatments is given in Table 3. Protozoal population (10⁷/ml) decreased from 2.19 at 0% TSS to 0.99 at 1.0% TSS (54.8% reduction). Protozoal population was significantly higher in S1 (1.56) as compared to S2 (1.52) and S3 (1.51). A number of studies have demonstrated that pure saponins and saponin-containing plants have an inhibitory effect on protozoa (Patra & Saxena 2009). Higher protozoal number in S3 may be due to more forage portion in it which results in higher pH of the in vitro medium as compared to S1 and S2 (protozoa are highly sensitive to reduced pH). Hu, Wu et al. (2005) reported that after 24 h incubation, protozoal counts were reduced by 19%, 25%, 45% and 79% when the tea saponin was added at 1%, 2%, 3% and 4% of the substrate, respectively. It has been postulated that as saponins have the property of binding with lipids, they may form complexes with the sterol moiety of protozoal cell membrane and disrupt it, leading to leakage of cell contents (Wallace et al. 2002), which leads to reduced protozoal population.

In the present study, methane production (ml/gm OM degraded) decreased by 32.5%, that is, from 65.26 (0% TSS) to

### Table 1. Chemical composition of substrates used for in vitro trials (on %DM basis).

| Attributes       | FC = 30:70 (S₁) | FC = 50:50 (S₂) | FC = 70:30 (S₃) |
|------------------|-----------------|-----------------|-----------------|
| Organic matter   | 87.45           | 86.78           | 87.90           |
| Crude protein    | 18.64           | 17.03           | 15.41           |
| Ether extract    | 1.93            | 1.96            | 1.98            |
| Total ash        | 12.55           | 12.33           | 12.10           |
| NDF              | 47.27           | 51.34           | 55.41           |
| ADF              | 17.87           | 22.75           | 27.63           |
| Cellulose        | 13.97           | 18.59           | 23.20           |
| Hemicellulose    | 29.39           | 28.59           | 27.79           |

Note: NDF: neutral detergent fibre; ADF: acid detergent fibre; FC: forage to concentrate ratio.

### Table 2. Effect of addition of tea seed saponin (TSS) on 24 h gas production (ml/200 mg).

| TSS levels (%) | Forage to concentrate ratio | Level | Mean ± SE | P-value |
|---------------|----------------------------|-------|-----------|---------|
|               | 30:70 (S₁) | 50:50 (S₂) | 70:30 (S₃) | Ratio | <0.0001 |
| 0.0           | 42.3 ± 0.21 | 39.9 ± 0.28 | 37.2 ± 0.66 | 39.8 ± 0.56 | <0.0001 |
| 0.3           | 47.0 ± 0.21 | 44.0 ± 0.46 | 41.2 ± 0.32 | 44.1 ± 0.81 | <0.0001 |
| 0.4           | 48.5 ± 0.18 | 45.2 ± 1.12 | 44.2 ± 0.28 | 45.9 ± 0.58 | <0.0001 |
| 0.5           | 50.1 ± 0.76 | 47.0 ± 0.38 | 43.0 ± 0.11 | 46.7 ± 0.76 | <0.0001 |
| 0.6           | 49.7 ± 0.97 | 47.3 ± 0.21 | 45.8 ± 0.28 | 47.6 ± 0.50 | <0.0001 |
| 0.7           | 52.8 ± 0.32 | 49.1 ± 0.11 | 48.0 ± 0.59 | 50.0 ± 0.54 | <0.0001 |
| 0.8           | 52.8 ± 0.53 | 50.7 ± 0.21 | 47.5 ± 0.66 | 50.3 ± 0.60 | <0.0001 |
| 0.9           | 51.3 ± 0.59 | 50.2 ± 0.38 | 45.8 ± 0.28 | 49.1 ± 0.62 | <0.0001 |
| 1.0           | 54.2 ± 0.59 | 50.8 ± 0.38 | 48.3 ± 0.56 | 51.1 ± 0.64 | <0.0001 |
| Mean ± SE     | 49.9 ± 0.50 | 47.1 ± 0.49 | 44.6 ± 0.49 |         |         |

Notes: SE: standard error. Means bearing different superscripts (A–F) in a column differ significantly; means bearing different superscripts (a–c) in a row differ significantly.
44.05 ml/g OM degraded (0.8% TSS), due to addition of TSS but no further reduction was observed beyond the 0.8% level (Table 4). Low methane production was observed in all substrates with addition of TSS and the lowest value from S3 may be due to reduced in vitro digestibility. Hu, Liu et al. (2005) reported inclusion of 0.27% and 0.53% tea saponin of the total substrate (composed of 50% corn meal and 50% grass meal) decreased methane production by 12.7% and 14.0%, respectively, in the faunated rumen fluid. Wang et al. (2000) also reported that the methane production was 15% lower from Yucca saponin-supplemented substrate compared to the control. Reduction in methane may be partially due to suppression of protozoal population as their numbers were decreased by 13.1% and 16.4% on addition of 0.27% and 0.53% tea saponin of total substrate, respectively, in the faunated rumen fluid but not in the defaunated rumen fluid (Hu, Liu et al. 2005). This may be due to the fact that protozoa act as H+ ion donors to methanogenic archaea which are associated with them as ectosymbionts and endosymbionts (Finlay et al. 1994; Tokura et al. 1997; Hess et al. 2003). Hess et al. (2003) reported that though methane production was reduced by 20%, methanogen population was unaffected when Sapindus saponaria fruit was used. Hu, Wu et al. (2005) also reported that tea saponin deceased in vitro methane production by 13% and 22% at a concentration of 1% and 2% of the substrate, respectively; whereas at greater concentrations (3% and 4% of the substrate) it had no further inhibitory effect on methane production as in the present study.

Results were not uniform but in general for all substrates with addition of TSS, mean TDMD as well as TOMD decreased significantly (Tables 5 and 6). TDMD decreased from 77.3% (0% TSS) to 74.5% (1.0% TSS) and TOMD decreased from 79.7% (0% TSS) to 75.5% (1.0% TSS). Digestibility reduced by 3 to 5 units on addition of TSS more than 0.8% of the basal substrate. Both TDMD% as well as TOMD% was significantly higher in S1 (TDMD 76.9, TOMD 78.5) and S2 (TDMD 77.1, TOMD 78.5) as compared to S3 (TDMD 75.6, TOMD 77.3) in most treatments.

Calculated values of SCFA produced and ME of substrates in different treatments are given in Tables 7 and 8, respectively. Both SCFA production (mmol/g substrate) and ME values (MJ/kg substrate) were significantly higher for S1 as compared to S2 for which it was higher than S3. SCFA production and ME value increased linearly with increasing level of TSS. As in the present study, Jayanegara et al. (2014) also reported linear increase in SCFA production with increasing saponin level. However, Hu, Wu et al. (2005) observed no effect of tea saponins on total volatile fatty acids production in vitro. The difference observed in effective saponin levels in the above studies may be due to different chemical structure and purity of the saponins used.

The total gas production and methane gas production were significantly higher and the in vitro digestibility, ME values, ammonia N and SCFA production were significantly lower in S1 as compared to S2 for which it was higher than S3. SCFA production and ME value increased linearly with increasing level of TSS. As in the present study, Jayanegara et al. (2014) also reported linear increase in SCFA production with increasing saponin level. However, Hu, Wu et al. (2005) observed no effect of tea saponins on total volatile fatty acids production in vitro. The difference observed in effective saponin levels in the above studies may be due to different chemical structure and purity of the saponins used.

The addition of TSS in all the three substrates with different forage concentrate ratios reduced methane emission and protozoal count, ammonia N concentration and improved SCFA
### Table 4. Effect of addition of tea seed saponin (TSS) on in vitro methane production (ml/gm OM degraded).

| TSS levels (%) | Forage to concentrate ratio | Mean ± SE | Level | P-value | Ratio | Level | Ratio × Level |
|----------------|-----------------------------|-----------|-------|---------|-------|-------|---------------|
|                | 30:70 (S₁) | 50:50 (S₂) | 70:30 (S₃) |          |       |       |               |
| 0.0            | 68.18 ± 0.53 | 67.55 ± 0.61 | 60.06 ± 0.62 |          |       |       |               |
| 0.3            | 55.40 ± 0.71 | 48.95 ± 0.56 | 43.52 ± 0.83 |          |       |       |               |
| 0.4            | 51.71 ± 0.84 | 50.31 ± 0.78 | 43.06 ± 0.59 |          |       |       |               |
| 0.5            | 61.00 ± 1.93 | 48.11 ± 0.80 | 44.30 ± 0.71 |          |       |       |               |
| 0.6            | 57.07 ± 0.09 | 45.64 ± 0.91 | 42.74 ± 0.79 |          |       |       |               |
| 0.7            | 61.23 ± 1.07 | 46.82 ± 0.70 | 38.84 ± 0.74 |          |       |       |               |
| 0.8            | 48.55 ± 1.56 | 45.36 ± 0.65 | 38.23 ± 0.58 |          |       |       |               |
| 0.9            | 50.67 ± 0.18 | 50.95 ± 1.17 | 40.52 ± 1.13 |          |       |       |               |
| 1.0            | 55.30 ± 0.74 | 51.90 ± 0.64 | 41.41 ± 0.60 |          |       |       |               |
| Mean ± SE      | 55.46 ± 1.25 | 50.62 ± 0.91 | 43.63 ± 0.87 |          |       |       |               |

Notes: OM: organic matter; SE: standard error. Means bearing different superscripts (A–D) in a column differ significantly; means bearing different superscripts (a–c) in a row differ significantly.

### Table 5. Effect of addition of tea seed saponin (TSS) on in vitro true dry matter digestibility (%).

| TSS levels (%) | Forage to concentrate ratio | Mean ± SE | Level | P-value | Ratio | Level | Ratio × Level |
|----------------|-----------------------------|-----------|-------|---------|-------|-------|---------------|
|                | 30:70 (S₁) | 50:50 (S₂) | 70:30 (S₃) |          |       |       |               |
| 0.0            | 77.9 ± 0.12 | 77.2 ± 0.09 | 76.8 ± 0.13 |          |       |       |               |
| 0.3            | 78.0 ± 0.12 | 77.1 ± 0.34 | 75.5 ± 0.32 |          |       |       |               |
| 0.4            | 78.3 ± 0.17 | 77.1 ± 0.60 | 75.3 ± 0.29 |          |       |       |               |
| 0.5            | 77.3 ± 0.10 | 78.0 ± 0.50 | 75.4 ± 0.17 |          |       |       |               |
| 0.6            | 77.3 ± 0.32 | 77.8 ± 0.37 | 75.7 ± 0.21 |          |       |       |               |
| 0.7            | 76.1 ± 0.03 | 76.2 ± 0.24 | 76.7 ± 0.19 |          |       |       |               |
| 0.8            | 77.2 ± 0.23 | 77.2 ± 0.16 | 76.8 ± 0.10 |          |       |       |               |
| 0.9            | 75.1 ± 0.09 | 78.0 ± 1.58 | 75.2 ± 1.18 |          |       |       |               |
| 1.0            | 75.0 ± 0.11 | 75.4 ± 0.38 | 73.2 ± 0.33 |          |       |       |               |
| Mean ± SE      | 76.9 ± 0.17 | 77.1 ± 0.22 | 75.6 ± 0.20 |          |       |       |               |

Notes: SE: standard error. Means bearing different superscripts (A–C) in a column differ significantly; means bearing different superscripts (a and b) in a row differ significantly.

### Table 6. Effect of addition of tea seed saponin (TSS) on in vitro true organic matter digestibility (%).

| TSS levels (%) | Forage to concentrate ratio | Mean ± SE | Level | P-value | Ratio | Level | Ratio × Level |
|----------------|-----------------------------|-----------|-------|---------|-------|-------|---------------|
|                | 30:70 (S₁) | 50:50 (S₂) | 70:30 (S₃) |          |       |       |               |
| 0.0            | 81.4 ± 0.38 | 79.0 ± 0.16 | 78.8 ± 0.14 |          |       |       |               |
| 0.3            | 79.8 ± 0.27 | 78.7 ± 0.71 | 77.0 ± 0.20 |          |       |       |               |
| 0.4            | 78.8 ± 0.12 | 79.8 ± 0.52 | 77.2 ± 0.32 |          |       |       |               |
| 0.5            | 78.8 ± 0.45 | 79.6 ± 0.47 | 77.2 ± 0.24 |          |       |       |               |
| 0.6            | 77.3 ± 0.06 | 77.7 ± 0.28 | 78.3 ± 0.29 |          |       |       |               |
| 0.7            | 78.6 ± 0.33 | 78.9 ± 0.18 | 78.4 ± 0.14 |          |       |       |               |
| 0.9            | 76.2 ± 0.05 | 77.2 ± 0.76 | 77.0 ± 1.36 |          |       |       |               |
| 1.0            | 76.1 ± 0.21 | 76.8 ± 0.45 | 74.2 ± 0.43 |          |       |       |               |
| Mean ± SE      | 78.5 ± 0.24 | 78.5 ± 0.20 | 77.3 ± 0.23 |          |       |       |               |

Notes: SE: standard error. Means bearing different superscripts (A–D) in a column differ significantly; means bearing different superscripts (a and b) in a row differ significantly.

### Table 7. Effect of addition of tea seed saponin (TSS) on in vitro SCFA production (mmol/g DM).

| TSS levels (%) | Forage to concentrate ratio | Mean ± SE | Level | P-value | Ratio | Level | Ratio × Level |
|----------------|-----------------------------|-----------|-------|---------|-------|-------|---------------|
|                | 30:70 (S₁) | 50:50 (S₂) | 70:30 (S₃) |          |       |       |               |
| 0.0            | 4.68 ± 0.02 | 4.40 ± 0.03 | 4.10 ± 0.07 |          |       |       |               |
| 0.3            | 5.20 ± 0.02 | 4.86 ± 0.05 | 4.55 ± 0.04 |          |       |       |               |
| 0.4            | 5.36 ± 0.02 | 4.99 ± 0.12 | 4.88 ± 0.03 |          |       |       |               |
| 0.5            | 5.54 ± 0.08 | 5.19 ± 0.04 | 4.75 ± 0.01 |          |       |       |               |
| 0.6            | 5.49 ± 0.11 | 5.23 ± 0.02 | 5.07 ± 0.03 |          |       |       |               |
| 0.7            | 5.84 ± 0.04 | 5.43 ± 0.01 | 5.30 ± 0.07 |          |       |       |               |
| 0.8            | 5.84 ± 0.06 | 5.60 ± 0.02 | 5.25 ± 0.07 |          |       |       |               |
| 0.9            | 5.68 ± 0.07 | 5.55 ± 0.04 | 5.07 ± 0.03 |          |       |       |               |
| 1.0            | 5.99 ± 0.07 | 5.62 ± 0.04 | 5.34 ± 0.06 |          |       |       |               |
| Mean ± SE      | 5.51 ± 0.06 | 5.21 ± 0.05 | 4.92 ± 0.05 |          |       |       |               |

Notes: SE: standard error. Means bearing different superscripts (A–F) in a column differ significantly; means bearing different superscripts (a–c) in a row differ significantly.
production and improved ME values of the diet. It is clear from the results that the potential of TSS to improve rumen fermentation and reduce methane emission was not affected by different forage to concentrate ratios.

Methane emission reduction and improving animal production without using antibiotics are the major goals of the animal industry. In general, the forage to concentrate ratios of animal ration varies depending on type of animal, age, physiological status and production level. In this regard, the current findings indicate that TSS may have the potential to reduce methane emission and improve rumen fermentation and thereby animal production across diets containing different forage to concentrate ratios.

4. Conclusion

From these findings, it may be inferred that TSS may have the potential to manipulate rumen fermentation and to reduce protozoal count and methanogenesis across different forage to concentrate ratios. The TSS dose levels up to 0.8% could be further explored in in vivo experiments.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 8. Effect of addition of tea seed saponin (TSS) on ME (MJ/kg DM) value of the substrate.

| TSS levels (%) | 0.5  | 0.7  | 0.9  | 1.0  | 1.2  | 1.5  | 2.0  | 2.5  | 3.0  | 4.0  |
|---------------|------|------|------|------|------|------|------|------|------|------|
| 30:70 (S,)    | 9.19 ± 0.03 | 9.19 ± 0.03 | 9.13 ± 0.05 | 9.13 ± 0.05 | 9.29 ± 0.08 | 9.29 ± 0.08 | 9.25 ± 0.09 |
| 50:50 (S,)    | 9.49 ± 0.04 | 9.49 ± 0.04 | 9.50 ± 0.07 | 9.50 ± 0.07 | 9.50 ± 0.07 | 9.50 ± 0.07 | 9.50 ± 0.07 |
| 70:30 (S,)    | 9.82 ± 0.08 | 9.82 ± 0.08 | 9.89 ± 0.01 | 9.89 ± 0.01 | 9.89 ± 0.01 | 9.89 ± 0.01 | 9.89 ± 0.01 |
| Mean ± SE    | 9.09 ± 0.07 | 9.09 ± 0.07 | 9.09 ± 0.07 | 9.09 ± 0.07 | 9.09 ± 0.07 | 9.09 ± 0.07 | 9.09 ± 0.07 |

Notes: SE: standard error. Means bearing different superscripts (A–F) in a column differ significantly; means bearing different superscripts (a–c) in a row differ significantly.
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