Full Length Articles

EFFECT OF STANDARD TANNIN ON *IN VITRO* RUMEN METHANE REDUCTION AND RUMEN FERMENTATION CHARACTERISTICS

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ABSTRACT

Methane is normally emitted by ruminants and represents a loss of feed energy by 8-12 %. Various feeding strategies are used to reduce the methane emission from ruminants for sustainable animal production. In this context an experiment was conducted to find out the effect of a standard tannin on rumen methane reduction and rumen fermentation characteristics for dairy cattle by *in vitro* gas production technique (*IVGPT)*. The *IVGPT* was carried out by incubating the Cumbu Nappier hybrid (CO4) grass and rumen liquor with standard tannin at varying level viz. 0, 1.03, 2.06, 3.09 and 4.12 % of substrate in six replicates in shaking water bath for a period of 24 hours. After 24 hours the total gas production and pH were measured and methane was estimated in Gas Chromatography. The *in vitro* true dry matter digestibility (*IVTDMD*) was estimated and methane emission (ml) per 100 mg truly digested substrate was calculated. The rumen fermentation characteristics were also studied. The total gas production was significantly ($p<0.05$) decreased in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented groups than other treatment groups. The methane production was significantly ($p<0.05$) decreased by 11.98 %, 29.34 %, 33.47 % and 34.71 % in 1.03 %, 2.06 %, 3.09 % and 4.12 % tannin levels, respectively than control. A highly significant ($p<0.01$) reduction of methane was observed at minimum level of standard tannin (2.06 %) per 100 mg of truly digested substrate, when compared to control. The rumen ammonia nitrogen, total bacteria and total protozoa were significantly reduced in tannin treated groups when compared to control. The other fermentation characteristics viz. *IVTDMD*, pH, TVFA, acetic acid, propionic acid, butyric acid and acetate propionate ratio were not differed among treatment groups. It was concluded that at minimum concentration of 2.06 % of standard tannin significantly reduced the methane emission, percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate ($p<0.01$) than control without any adverse effect on rumen fermentation characteristics by *IVGPT*.

Key words: Tannin, methane, *in vitro* rumen fermentation, dairy cattle

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Introduction

The estimated methane (CH_4) emission from rumen fermentation was 15–20 % (Moss *et al*. 2000) causes global warming. Methane produced under anaerobic fermentation as a path way for disposal of metabolic carbon and hydrogen ion produced during microbial fermentation. The methane production is represents a loss of feed energy by 8-12 % leads to lower animal production. Therefore, decreasing methane production is desirable for reducing methane emission with improved efficiency of the digested energy utilization (Johnson and Johnson 1995). A decrease in methane emission is also desirable for increasing animal production in terms of growth, milk production, are also reducing green house gas emission in the environment, which decreases the global warming. There are many more feeding strategies used to reduce the methane emission. Changes in the feeding pattern and feeds can help to mitigate methane emission. Further, the use of antibiotics like ionophore compounds such as monensin, lasolocid and many other chemical feed additives have been shown to decrease methane emission in ruminants. Plants also produce a diverse array of plant metabolites such as tannins, saponins, organic acids, essential oils and organosulphur compounds that have been shown to selectively modulate the rumen microbial populations resulting in an improvement of rumen fermentation and nitrogen metabolism, and a decrease in methane production (Patra and Saxena, 2011). Further, a novel approach using the plant metabolites like saponin (Bharathidhasan *et al.*, 2013), tannin (Bharathidhasan *et al.,* 2014) and organic acids (Bharathidhasan *et al.,* 2016) modulates the rumen microbial populations and reduces the methane production. Tannins have the capacity to form complexes mainly with proteins due to the presence of a large number of phenolic groups and have been found to be toxic for some of the rumen microbes, especially ciliate protozoa, fiber degrading bacteria and methanogenic archaea, and as a result methanogenesis in the rumen can also be reduced. Ramirez-Restrepo and Barry (2005) reported that the condensed tannin containing leguminous forages reduced methane. Hess *et al*. (2006) also reported that reduction in methane emissions when feeding high tannins. Hence, the present experiment was conducted to study the effect of standard tannin on reduction of methane emission and rumen fermentation characteristics by *in vitro* gas production technique in forage based diet of dairy cattle.

Materials and methods

The *in vitro* gas production technique (Menke and Steingass, 1988) was aimed to evaluate the effect of standard tannin at different levels viz. 1.03, 2.06, 3.09 and 4.12 % of substrate in six replicates on rumen methane production (Table 1). The standard tannin was procured from Sigma chemicals. The substrate Hybrid Cumbu Nappier (CN- CO4) grass (*Pennisetum purpureum* x P*ennisetum glaucum*) was used for this study. The *in vitro* gas production study was carried out with rumen fluid collected by using rumen extraction pump from three cattle maintained on grazing and it was squeezed through four layers of muslin cloth in to an Erlenmeyer flask under continuous

flushing with CO_2 and it was maintained at the temperature of 39 °C. Then rumen fluid was mixed with buffer as described by Menke and Steingass (1988). The substrate Hybrid Cumbu Nappier grass (CN-CO4) was dried and milled to pass through 1 mm sieve and 200 mg was weighed and taken in 100 ml calibrated syringes and weighed quantity of tannin at 0, 2.06 mg (1.03 %), 4.12 mg (2.06 %), 6.18 mg (3.09 %) and 8.24 mg (4.12 % of substrate) were added to the substrate in the syringes in six replicates. Then 30 ml of rumen inoculum was anaerobically transferred to glass syringe and it was incubated in a shaking water bath at 39 °C for 24 hrs. At the end of the incubation period the total gas was measured and pH also determined in fermentation fluid.

Table 1. Experimental design to identify the level of standard tannin needed to reduce methanogenesis

Treatment*	Inclusion level of standard tannin (% of substrate)	Quantity of standard tannin included to 200 mg of substrate inoculated
1 (Control)	0	0 mg
2	1.03	2.06 mg
3	2.06	4.12 mg
4	3.09	6.18 mg
5	4.12	8.24 mg

*Each treatment was carried out with six replicates

The gas samples were collected in vacuotainer for estimation of methane and fermented fluid was collected for the estimation of ammonia nitrogen, volatile fatty acids, true dry matter digestibility, bacterial and protozoal count.

Estimation of methane

Methane concentration was estimated using Gas Chromatography (Perkin Elmer, Claurus 500 model) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer diameter). Helium was used as carrier gas with oven temperature at 60° C, injector temperature at 100°C and detector temperature at 110°C. Methane concentration in samples (%) was calculated using the following formula.

$$\text{Methane concentration (\%)} = \frac{\text{Peak area of sample gas}}{\text{Peak area of standard gas}} \times \text{Methane concentration in standard (\%)}$$

$$\text{Methane emission (ml)} = \frac{\text{Methane concentration (\%)}}{100} \times \text{Net gas production (ml)}$$

In vitro true dry matter digestibility (_IVTDMD_) estimation

The fermented fluid was centrifuged and the residue was transferred into sintered glass crucible and fitted in Fibretec and 100 ml of Neutral Detergent Solution (NDS) was added and it was refluxed for one hour after which the residue was recovered. The true digestibility was calculated as the weight of substrate incubated minus the weight of the residue after NDS treatment (Van Soest and Robertson, 1988).

Ammonia nitrogen

The ammonia nitrogen was estimated by steam distillation as per the method of Makkar and Becker (1996).

Estimation of total volatile fatty acids (TVFA)

The volatile fatty acids were estimated as per the method of Chase (1990). 2.5ml of fermented medium from each syringe was mixed with 0.5ml of 25 % meta phosphoric acid and centrifuged at 20000g for 30 minutes at 4°C and clear supernatant was collected in GC vials. The 1µl of supernatant was injected into Gas Chromatography (Perkin Elmer, Claurus 500 model) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer diameter). Helium was used as carrier gas with oven temperature at 175° C, injector temperature at 220°C and detector temperature at 240°C.

Total Bacterial count

Total bacterial count was carried out using gram's staining as per the method of Gall e*t al.* (1949). One ml of fermented fluid was diluted to 5 ml using 10 % formal saline. One ml of diluted fluid was again diluted with 10 % formal saline to 100 ml. This fluid of 0.01ml was spread over marked area of 1 square centimeter on a clean glass slide. The smear was air and flame dried and stained with gram's staining. The bacteria were counted in 30 fields from all over the smear. The total bacterial load/ml effluent was calculated as follows,

Area of each field: πr^2
'r' – Radius of the microscopic fluid measured by using micrometer
Area of the smear: 1 square cm (or) 100 square mm

No. of fields/100 square mm: 100/ πr^2 = H
Total nos. field counted: 30
Average number of bacteria/field: N
Volume of fermented fluid used: 0.00002 ml
No. of bacteria in 1 ml of fermented fluid =

$$\frac{(H \times N \times 10^5)}{2}$$

Total Protozoal count

Total protozoal count was calculated using Moir (1951) technique. One ml of fermented fluid was diluted to 5 ml with 10 percent formal saline. Two percent Eosin stain was added to the diluted fluid at the rate of one drop per 5 ml. Five to ten minutes time was given for the protozoa to take up the stain and the contents were

mixed thoroughly and the haemocytometer was charged. The protozoa were counted in all the eight WBC chambers.

The total protozoal count/ml effluent was calculated as follows.

Dilution ratio: 1:5

Volume of 1 WBC chamber: 0.0001 cmm

0.0001 cmm of diluted effluent contained: A number of protozoa

Number of protozoa in 1 ml of fermented fluid =

$$\frac{A \times 5}{0.0001} = A \times 5000$$

The data collected on various parameters was statistically analyzed as per the method of Snedecor and Cochran (1989).

Results and Discussion

The effect of standard tannin on total gas (ml), methane (ml), percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate are presented in Table 2. The total gas production was significantly ($p<0.05$) decreased in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented group than control. The total gas was lowered by 21.68 %, 25.59 % and 25.75 % in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented groups respectively than control. Similar decrease in total gas production was also observed by Getachew *et al*. (2008), who reported that the addition of purified quebracho tannins to alfalfa hay on *in vitro* gas production technique decreased the rate of gas production significantly with increased level of tannin from 0 to 150 mg/kg DM. Further they observed that the addition of gallic acid and tannic acid reduced the rate of gas production but increased the potential gas production.

Table 2 Effect of standard tannin on total gas (ml), methane (ml), percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate (Mean# ± S.E)

Treatment	Inclusion level of standard tannin (% of substrate)	Total gas (ml)*	Methane (ml)**	Percentage of methane on Total gas production**	Methane (ml) per 100 mg of truly digested substrate **
1	0 (Control)	12.27 ± 0.19 ^{b}	2.42 ± 0.01^{b}	19.75 ± 0.37^{b}	2.02 ± 0.01^{b}
2	1.03	10.87 ± 0.32 ^{ab}	2.13 ± 0.12^{b}	19.64 ± 0.65^{b}	1.89 ± 0.11^{b}
3	2.06	9.61 ± 0.32 ^{a}	1.71 ± 0.07^{a}	17.86 ± 0.31^{a}	1.51 ± 0.10 ^{a}

4	3.09	9.13 ± 0.32 [a]	1.61 ± 0.07[a]	17.62 ± 0.25[a]	1.41 ± 0.02[a]
5	4.12	9.11 ± 0.21[a]	1.58 ± 0.06[a]	17.24 ± 0.33[a]	1.36 ± 0.06 [a]

[#] Mean of six observations; [NS] Not significant, Means bearing different superscripts in the same column differ significantly (*p<0.05), (**p<0.01)

The decrease in the rate of gas production and concomitant increase in asymptotic gas production when gallic acid and tannic acid was used might suggest that rumen microbes are capable of degrading gallic acid and tannic acid or are able to tolerate the effects of tannic acid. On the other hand condensed tannin such as quebracho tannin was largely resistant to microbial degradation. This is the reason which was responsible for the decrease in the total gas production recorded in the present study. Vieira and Borba (2011) also reported that the effect of tannin in the form of *Quebracho* extracts from the plant extract of *Trifolium repens, Lotus corniculatus* and *Lolium perenne* at 2.5 % and 5.0 % levels significantly decreased the total gas production than control. On the contrary to the present findings Pellikaan *et al*. (2011) reported that the addition of condensed tannin (CT) and hydrolysable tannin (HT) at 100g/kg feed did not affect the total gas production by *in vitro*. They also observed that the condensed tannin rich sources like grape seed, Quebracho had lower gas production and higher hydrolysable tannin sources like green tea and myrabolan tended to increase the gas production.

The methane was significantly (p<0.01) decreased in 2.06 %, 3.09 % and 4.12 % standard tannin added group than control and 1.03 % tannin added group. The reduction in methane was 29.34, 33.47 and 34.71 % respectively in treatment 3, treatment 4 and treatment 5 when compared to control. The minimum level that reduced maximum methane was 2.06 %. The significant reduction in methane emission was observed in this experiment is in confirmed with earlier reports by Jayanegara *et al*. (2010), who also reported a significant negative relationship between total tannins and methane production. They also observed that simple phenolics like cinnamic, caffeic, p-coumaric and ferulic acids decreased methane production significantly when added at 5 mM and addition of purified chestnut and sumach (hydrolysable tannin) at 1mg/ml to *in vitro* rumen fermentation system containing hay: concentrate (70:30) decreased methane production by 6.5 and 7.2 %, respectively. Bhatta *et al*. (2009) observed that the addition of *quebracho* tannin (7.62 % hydrolysable tannin and 1.33 % condensed tannin) at 5, 10, 15, 20 and 25 % of substrates in timothy hay (65): concentrates (35) decreased the methane production by 10.2 to 41.7 % in in *vitro* gas production technique.

The highly significant (p<0.01) decrease in methane (%) on total gas production was observed in 2.06 %, 3.09 % and 4.12 % standard tannin supplemented groups than control. The minimum level of 2.06 % of standard tannin was able to

reduce the methane on total gas production by 9.57 % when compared to control. Similarly Pellikaan *et al*. (2011) also reported that the addition of condensed tannin and hydrolysable tannin at 100g/ kg reduced *in vitro* methane emission on total gas production by 16.30 % and 15.85 % respectively when compared to control by *in vitro* study. The authors also opined that the addition of poly ethylene glycol increased the total methane emission on total gas production equivalent to control.

The methane per 100 mg of truly digested substrate was also significantly ($p<0.01$) reduced in 2.06 %, 3.09 % and 4.12 % tannin added groups than control. Standard tannin at 2.06 % level was reduced the methane per 100 mg of truly digested substrate by 25.25 % when compared to control. The present study was in agreement with the earlier findings of Castro –Montoya *et al*. (2011) who reported that the purified condensed tannin like *quebracho* tannin and mimosa tannin at 0.5, 0.75 and 1.0 mg/ ml decreased the methane emission per 100 mg true dry matter digestibility by 25, 30.77 and 36.54 % in *quebracho* tannin and 23.08, 32.69 and 40.38 %, respectively in mimosa tannin than control. They also found that the purified hydrolysable tannin like sumach tannin and chestnut tannin at 0.5, 0.75 and 1.0 mg/ml decreased the methane emission (ml) per 100 mg true dry matter digestibility by 17.31, 23.08 and 30.76 % respectively in sumach tannin and 13.46, 17.31 and 21.25 % respectively in chestnut tannin than control. Similarly the addition of condensed tannin and hydrolysable tannin at 10 % reduced *in vitro* methane emission per gram of organic matter by 24.48 % and 17.88 % respectively than control (Pellikaan *et al*., 2011). The decrease in methane emission might be due to the effect of tannin on suppression of protozoa and methanogenic bacteria. The effects of tannin on ruminal fibre digestion may be attributed to decrease in number of cellulolytic bacteria (Mc Sweeney *et al*., 2001), formation of tannin cellulose complexes that are resistant to enzymatic digestion (Makkar *et al*.1995) 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 tannin is known to reduce the protozoal numbers which might decrease in methane production (Makkar *et al*., 1995).

The ammonia nitrogen content in all tannin treated groups was significantly ($p<0.01$) reduced than control. Similarly, Alexander *et al*. (2008) reported that the addition of 2 mg/ ml of *Moringa oleifera* aqueous methanol extract which contained 1.11 % of HT decreased the total ammonia nitrogen by 13.63 % than control. Pellikaan *et al*. (2011) also reported that the ammonia nitrogen was significantly decreased by 35.18 and 33.38 % in CT and HT addition at 100g/kg substrate than control. The decreased ammonia nitrogen might be due to tannins binds with protein, which resisted the rumen degradation and reduced ammonia nitrogen in the rumen (Vieira and Borba, 2011). The reduced ammonia concentrations in the rumen are typical when protozoal growth is inhibited, presumably as a result of depressed bacterial lysis (Pen *et al*., 2006).

Table 3. Effect of standard tannin on Ammonia nitrogen, (mg/100ml), Bacterial count (per ml), Protozoal count (per ml), *In vitro* true dry matter digestibility (*IVTDMD*) and pH (Mean[#] ± S.E)

Treatment	Inclusion level of standard tannin (% of substrate)	Ammonia Nitrogen (mg/100ml)	Bacterial count (X 10^8) (per ml)	Protozoal count (X 10^5) (per ml)	*In vitro* true dry matter digestibility [NS] (*IVTDMD*)	pH [NS]
1	0 (Control)	35.29 ± 0.40 [b]	4.37 ± 0.05 [c]	3.47 ± 0.03 [c]	59.93 ± 0.43	7.05 ± 0.08
2	1.03	34.56 ± 0.38 [a]	3.98 ± 0.06 [b]	2.97 ± 0.04 [b]	56.73 ± 1.36	6.90 ± 0.11
3	2.06	33.44 ± 0.34 [a]	3.76 ± 0.04 [a]	2.88 ± 0.03 [ab]	57.07 ± 1.53	6.77 ± 0.03
4	3.08	33.27 ± 0.25 [a]	3.73 ± 0.03 [a]	2.79 ± 0.03 [a]	57.17 ± 1.52	7.03 ± 0.07
5	4.11	33.52 ± 0.57[a]	3.77 ± 0.03[a]	2.78 ± 0.03 [a]	57.27 ± 1.13	7.10 ± 0.10

[#] Mean of six observations; [NS] Not significant, Means bearing different superscripts in the same column differ significantly ($p<0.01$)

There was no significant difference on *in vitro* dry matter digestibility due to supplementation of tannin in forage based diet. Sliwinski *et al*. (2002) also observed that the addition of *Castanea sativa* wood extracts containing the hydrolysable tannin at 0.5 and 2.5 g per kg dry matter to the basal diet with grass silage, barley grain and grass hay did not influence the organic matter degradation among the treatment groups by *RUSITEC*. Similarly, Castro –Montoya *et al*. (2011) reported that the purified CT like quebracho tannin and mimosa tannin and HT like sumach tannin and chestnut tannin at 0.5, 0.75 and 1.0 mg/ml of *in vitro* rumen fermentation study did not influence the true dry matter digestibility in all treatment groups.

The bacterial count in all tannin treated groups was significantly ($p<0.01$) reduced than control. The minimum dose with maximum reduction of bacterial load by 13.96 % was observed in 2.06 % tannin treated group than control. Tannins are generally regarded inhibitory to the growth of the rumen micro organisms. The early work of Tagari *et al*. (1965) showed that the growth of cellulolytic and proteolytic bacteria was inhibited by carob tannins in an artificial rumen. It has been stated that tannins from carob pod extract changed the morphology of bacteria to produce antimicrobial activity (Heins *et al*., 1964). Hence, inhibitory activity of tannins against bacteria has been implicated due

to the ability of tannins to form complexes with the cell wall and membrane causing morphological changes of the cell wall and the extracellular enzymes secreted (Smith *et al*., 2005). Sliwinski *et al*. (2002) observed that the addition of *Castanea sativa* wood extracts containing the hydrolysable tannin at 0.5 and 2.5 g per kg dry matter to the basal diet with grass silage, barley grain and grass hay reduced the bacterial count numerically decreased by 3.14 and 11.55 % in 0.5 and 2.5 g HT added groups than control in *Rusitec.*

The protozoal count was significantly ($P<0.05$) reduced in all tannin supplemented groups than control. The reduction in protozoal count was 14.4 %, 17.00 %, 19.59 % and 19.88 % in 1.03, 2.06, 3.09 and 4.12 % of tannin supplemented groups, respectively than control. Makkar *et al*. (1995) reported that the quebracho tannin significantly reduced the numbers of total protozoa, Entodiniomorph and holotichs, the effect being higher in Holotrichs which may increase the efficiency of microbial protein synthesis in the rumen. Briceno *et al*. (2005) screened the defaunating properties of 15 tree fodders containing tannins but inhibitory effect on protozoa was observed in *Acacia farnesiana, Calliandra calothyrsus and Lysiloma latisiliquum.* Patra *et al*. (2006) observed that the tannin extracted with ethanol and methanol from *Terminalia chebula* decreased the numbers of total protozoa. Anti protozoal properties of tannins from different plants have been reported in many studies with *Lotus striata* and *Lotus cuneata* (Animut *et al*., 2008) Quebracho and mimosa tannin (Bhatta *et al.,* 2009).

No significant difference was observed in pH among the treatment groups. The result of this study was in agreement with the earlier findings such as the addition of condensed tannin through *Medicago sativa* and *Lotus pedunculatus* did not influence the pH by *in vitro* gas production technique (Tavendale *et al*., 2005). But, Alexander *et al*. (2008) and Pellikaan *et al*. (2011) reported that the pH significantly decreased by addition of tannin. The tannin supplementation decreased the number of methanogens which leads to reduced methane production and increased the accumulation of hydrogen ions. The unchanged pH in the present study was due to the carbohydrate fermenting bacteria utilize other mechanism of reducing equivalent particularly elimination of hydrogen ions and there by the pH is unaltered (Kessel and Russel, 1996).

The TVFA, acetic acid, propionic acid, butyric acid and A/P ratio were not differed among tannin supplemented groups. Sliwinski *et al*. (2002) observed that the addition of *Castanea sativa* wood extracts containing the hydrolysable tannin at 0.5 and 2.5 g per kg dry matter to the basal diet with grass silage, barley grain and grass hay did not influence TVFA, acetic acid, propionic acid, butyric acid and A/P ratio among the treatment groups in *Rusitec*.

Similarly, Tavendale *et al*. (2005) reported that the addition of condensed tannin through *Lotus pedunculatus* (10.7% CT) and *Medicago sativa* (0.02% CT) did not influence the total VFA, acetic acid, propionic acid, butyric acid and acetate propionate ratio (A/P ratio) levels by *in vitro* fermentation study. Similarly, acetic acid,

propionic acid, butyric acid and acetate propionate ratio were also significantly not differed in tannin supplemented groups by *in vitro*. Pellikaan *et al*. (2011) also reported that the propionic acid production and butyric acid production were not affected by the addition of CT (20.2 %). Hess *et al*. (2006) observed that the acetate to propionate ratio was not influenced by supplementation with increasing proportion of low tannin legume *Cratylia argentea* and rich tannin legume *Calliandra calothyrsus* supplement or in combination between of these forages in *Rusitec.*

Table 4. Effect of standard tannin on Total volatile fatty acid (mg/dl), Acetic acid, Propionic acid, Butyric acid and A/P ratio (Mean# ± S.E)NS

Treatment	Inclusion level of standard tannin (% of substrate)	TVFA (mg/dl)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	A/P ratio
1	0 (Control)	64.98 ± 0.34	65.33 ± 0.42	23.33 ± 0.49	11.33 ± 0.72	2.81 ± 0.06
2	1.03	64.94 ± 0.25	65.00 ± 0.37	23.33 ± 0.33	11.67 ± 0.33	2.79 ± 0.05
3	2.06	65.08 ± 0.31	64.50 ± 0.99	24.00 ± 0.52	11.50 ± 0.62	2.70 ± 0.10
4	3.08	65.09 ± 0.24	67.00 ± 0.36	24.17 ± 0.48	10.83 ± 0.31	2.70 ± 0.06
5	4.11	65.44 ± 0.59	66.33 ± 0.56	22.83 ± 0.48	10.84 ± 0.17	2.91 ± 0.08

Mean of six observations; NS Not significant

It was concluded that the minimum concentration of 2.06 % of tannin significantly caused a maximum reduction in the total gas production, methane emission, percentage of methane on total gas production and methane (ml) per 100 mg of truly digested substrate. The rumen ammonia nitrogen, total bacteria and protozoa were significantly reduced in tannin treated groups when compared to control. The other fermentation characteristics viz. *IVTDMD*, pH, TVFA, acetic acid, propionic acid, butyric acid and A/P ratio were not differed among tannin treated groups. The energy saved through decrease in methane emission will be utilized for milk or meat production and the global warming also reduced.

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