Full Length Article

EFFECT OF SUPPLEMENTING PLANT METABOLITES THROUGH ACACIA NILOTICA PLANT EXTRACT ON METHANE MITIGATION AND RUMEN FERMENTATION CHARACTERISTICS FOR DAIRY CATTLE

A.Bharathidhasan*[1]

Post Graduate Research Institute in Animal Sciences
Tamil Nadu Veterinary and Animal Sciences University
Kattupakkam, Tamil Nadu, India

ABSTRACT

An experiment was conducted to study the effect of supplementing plant metabolites like tannin and saponin through Acacia nilotica plant extract on methane mitigation and rumen fermentation characteristics for dairy cattle by in vitro gas production technique (IVGPT) in forage based diet. A highly significant (P<0.01) reduction of methane was observed in Acacia nilotica plant extract supplemented groups than their respective controls. The significant (P<0.05) reduction of methane (ml) per 100 mg of truly digested substrate was found in 0.75 ml of Acacia nilotica extract supplemented group than other treatment groups after eliminating the error induced by the extractant. The rumen fermentation characteristics viz. ammonia nitrogen, in vitro true dry matter digestibility (IVTDMD), bacterial and protozoal population was significantly decreased in 0.75 ml and 1.0 ml Acacia nilotica plant extract added groups. The total volatile fatty acids (TVFA) and propionic acid were significantly (P<0.05) increased and acetic acid and acetate to propionate (A/P) ratio were significantly (P<0.05) decreased in 0.75 ml and 1.0 ml Acacia nilotica plant extract added groups than their respective control groups. It was concluded that the methane (ml) per 100 mg of truly digested substrate was significantly decreased at the inclusion level of 3.09 % of tannin and 2.34 % saponin through Acacia nilotica plant extract supplemented group than control.

Keywords: *Acacia nilotica*, Dairy cattle, Fermentation, Methane, Saponin and tannin

Received : 29.04.2022 Revised : 13.06.2022 Accepted : 26.07.2022

INTRODUCTION

The global estimated methane emission was 15–20 % from ruminants and produced under anaerobic fermentation as a path way

*Corresponding author Email Id: bdhasana@gmail.com
[1]Associate Professor

for the disposal of metabolic hydrogen ion in the rumen. It causes global warming and also represents a loss of feed energy by 8-12 %, thus lowers the animal production. Recently, animal nutritionists have been emphasized on the mitigation of methane emission by using various feeding strategies (Patra and Saxena, 2010). The use of plant metabolites is the most promising approach on reducing methane emission (Bharathidhasan *et al*., 2016). Further the development of resistance or food residue while using antibiotics/ chemical agents will compel the utilization of natural plants containing tannin and saponin on reduction of methane emission in dairy cattle. Tannins are combined together with proteins to form tannin protein complex due to the presence of phenolic hydroxyl 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 be reduced (Bharathidhasan, 2018). The majority of research on saponin has been employed to exploit it for inhibition of rumen ciliate protozoa, which might improve the efficiency of microbial protein synthesis by reducing microbial protein turnover and enhance the protein flow to the duodenum and finally reduce methane production (Patra and Saxena, 2010; Bharathidhasan *et al*., 2013). Hence the present study was carried out with an objective to assess the effect of supplementing plant metabolites like tannin and saponin through *Acacia nilotica* plant extract on methane mitigation and rumen fermentation characteristics by *IVGPT* in forage based diet for dairy cattle.

MATERIALS AND METHODS

The *IVGPT* was carried out as per the method of Menke and Steingass (1988) to evaluate the effect of supplementing tannin and saponin through *Acacia nilotica* plant extract at four graded levels in forage based substrate (Hybrid Cumbu Nappier (CO- CN4) grass (*Pennisetum purpureum* x *Pennisetum glaucum*) with six replicates on methane mitigation and rumen fermentation characteristics for dairy cattle as detailed in Table I. The tannin and saponin content of *Acacia nilotica* plant extract was estimated as per the standard procedure.

The *Acacia nilotica* plant extract was prepared by mixing the plant species with 50 % aqueous methanol in orbital shaker for 24 hrs and filtered and the filterant used for the *in vitro* study (Sirohi *et al.*, 2009). At the end of the incubation period, the total gas was measured and the methane was estimated in Gas Chromatography. The fermented fluid was collected for the estimation of rumen fermentation characteristics viz. ammonia nitrogen (Makkar and Becker, 1996), *IVTDMD* (Van Soest and Robertson, 1988), volatile fatty acids (Chase, 1990), Bacterial count (Gall e*t al.,* 1949) and protozoal population (Moir, 1951) using the standard procedure. Based on the results of *IVGPT* the actual reduction of methane and rumen fermentation characteristics were derived in a separate by subtracting the values of the extractant (50 % aqueous methanol) minus the values of *Acacia nilotica* extract for eliminating the error induced by methanol extractant. All data collected on various

parameters was statistically analyzed as per the method of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Methane mitigation

A highly significant (P<0.01) reduction of total gas was observed by 12.72 (P<0.05), 13.68, 20.48 and 19.95 % (P<0.01) in AN1, AN2, AN3 and AN4 respectively when compared to their respective controls (Table II). The lowered gas production was also observed by Ammar *et al*. (2005), when a mixture of leaves incubated by *in vitro*. The antibacterial and anti-protozoal effect of tannin and saponin decreases the fermentation leads to lowers the total gas production (Jayanegara *et al*., 2010). The gas production is an ideal technique to generate kinetics of fermentation by microorganism, which is used to predict the rate at which the feed is digested. There was a highly significant reduction (P<0.01) of methane and methane (ml) per 100 mg of truly digested substrate in all *Acacia nilotica* extract treated groups when compared to their respective controls. The percentage of methane reduction on total gas production was highly significant (P<0.01) in AN3 and significant (P<0.05) in AN4 than their respective controls (Table II). A significant (P<0.05) reduction on methane emission (ml) per 100 mg of truly digested substrate was observed in AN3 (3.09 % tannin and 2.34 % of saponin) when compared to other treatment groups after eliminating the error induced by the extractant (Table III).

The present finding was consistent with the earlier report by Hess *et al*. (2003) who observed that the *in vitro* evaluation of tropical fruit contained saponin (1.2 %) and condensed tannin (CT) (0.32 %) at 100 mg/g of substrate, decreased the methane emission by 20 %. The methane production was also decreased in *Lotus pedunculatus* (18.13 %) than *Medicago sativa* (15.19 %) due to the presence of CT at the level of 10.7 % in *Lotus pedunculatus* and 0.02 % in *Medicago sativa* respectively (Tavendale *et al*., 2005). Further the addition of CT and hydrolysable tannin (HT) at 100 g/kg reduced the percentage of methane emission on total gas production by 16.30 % and 15.85 % respectively when compared to control by *in vitro* (Pellikaan *et al*., 2011). They also reported that the methane emission per gram of organic matter was reduced by 24.48 % and 17.88 % respectively when the addition of CT and HT than control.

Tannin reduced the methane emission with two modes of action, first directly affecting activity/ population of methanogens, resulting lower methane emission and second, indirectly by reduced hydrogen production through lowering the feed degradation (Tavendale *et al*., 2005). The methane reduction was also due to the antiprotozoal activities of tannin, since a portion of methanogens attached with protozoa (Goel and Makkar, 2012). The symbiosis of protozoa with methanogenic bacteria is well established in the rumen and selective suppression of protozoa has been suggested to reduce the methane production (Cheeke, 1999). Saponin also causes a decrease in the number of protozoal population as a result of cell death by forming complexes with cell membranes (Cheeke, 1999) and it also modifies the ruminal fermentation, which inhibit some bacteria leads to lowers the methane emission. Hence, the tannin (3.08 %)

Table I. Levels of tannin and saponin inclusion in different treatments

Treatments		Inclusion level of tannin + saponin (%) through *Acacia nilotica* plant extract	Quantity of 50 % aqueous methanol solution (or) *Acacia nilotica* plant extract for 200 mg of CO- CN4 grass based substrate
Control 1	C1	0	0.25 ml of 50 % aqueous methanol solution
Treatment 1	AN1	1.03 + 0.78	**0.25 ml of** *Acacia nilotica* plant extract in 50 % aqueous methanol solution
Control 2	C2	0	0.5 ml of 50 % aqueous methanol solution
Treatment 2	AN2	2.06 + 1.56	0.50 ml of *Acacia nilotica* plant extract in 50 % aqueous methanol solution
Control 3	C3	0	0.75 ml 50 % aqueous methanol solution
Treatment 3	AN3	3.09 + 2.34	0.75 ml of *Acacia nilotica* plant extract in 50 % aqueous methanol solution
Control 4	C4	0	1.00 ml 50 % aqueous methanol solution
Treatment 4	AN4	4.12 + 3.12	1.00 ml of *Acacia nilotica* plant extract in 50 % aqueous methanol solution

AN1 –Acacia nilotica plant extract 1, AN2 –Acacia nilotica plant extract 2, AN3 –Acacia nilotica plant extract 3 and AN4 –Acacia nilotica plant extract 4.

Table II. Effect of supplementing plant metabolites like tannin and saponin through *Acacia nilotica* plant extract on methane mitigation and rumen fermentation characteristics (Mean# ± S.E)

Parameters	Treatments							
	C1	AN1	C2	AN2	C3	AN3	C4	AN4
Total gas (ml)	12.03 ± 0.29 [b]	10.50 ± 0.29 [a]	11.70 ± 0.31 [b]	10.10 ± 0.06 [a]	11.57 ± 0.23 [b]	9.20 ±0.17 [a]	11.03 ± 0.29 [b]	8.83 ±0.20 [a]
Significance	P<0.05		P<0.01		P<0.01		P<0.01	
Methane (ml)	2.37 ±0.03 [b]	1.93 ±0.05 [a]	2.23 ±0.09 [b]	1.77 ±0.04 [a]	2.20 ±0.06 [b]	1.51 ±0.04 [a]	2.17 ±0.13 [b]	1.45 ±0.03 [a]
Significance	P<0.01		P<0.01		P<0.01		P<0.01	
% of methane on Total gas production	19.70 ±0.72	18.39 ±0.04	19.11 ±0.88	17.51 ±0.52	19.02 ±0.41 [b]	16.41 ±0.21 [a]	19.61 ±0.78 [b]	16.40 ±0.21 [a]
Significance	NS		NS		P<0.01		P<0.05	
Methane (ml) per 100 mg of truly digested substrate	1.85 ±0.05 [b]	1.64 ±0.04 [a]	1.75 ±0.02 [b]	1.49 ±0.05 [a]	1.79 ±0.04 [b]	1.29 ±0.04 [a]	1.82 ±0.12 [b]	1.23 ±0.02 [a]
Significance	P<0.05		P<0.01		P<0.01		P<0.01	
Ammonia Nitrogen (mg/100ml)	19.83 ± 0.48	19.42 ± 0.52	19.67 ± 0.67	18.98 ± 0.18	19.33 ± 0.42 [b]	18.28 ±0.07 [a]	19.17 ± 0.48 [b]	17.95 ±0.20 [a]
Significance	NS		NS		P<0.05		P<0.05	
IVTDMD (%)	64.05 ±0.30 [b]	58.84 ±0.20 [a]	63.71 ±0.33 [b]	59.40 ±1.81 [a]	61.45 ±0.68 [b]	58.52 ±1.01 [a]	59.61 ±0.75	58.94 ±1.04
Significance	P<0.01		P<0.05		P<0.05		NS	
Bacterial count (X 10^8)	4.79 ±0.03	4.72 ±0.08	4.71 ±0.04 [b]	3.86 ±0.03 [a]	4.65 ±0.07 [b]	3.68 ±0.06 [a]	4.58 ±0.06 [b]	3.63 ±0.05 [a]
Significance	NS		P<0.01		P<0.01		P<0.01	
Protozoal count (X 10^5)	3.60 ±0.08	3.37 ±0.09	3.57 ±0.08 [b]	3.13 ±0.07 [a]	3.53 ±0.04 [b]	2.61 ±0.08 [a]	3.51 ±0.10 [b]	2.58 ±0.07 [a]
Significance	NS		P<0.01		P<0.01		P<0.01	
pH	6.67 ± 0.09	6.70 ± 0.10	6.73 ± 0.03	6.90 ± 0.06	6.97 ± 0.07	6.80 ± 0.17	6.80 ± 0.17	6.73 ± 0.12

Significance	NS		NS		NS		NS	
TVFA (mg/dl)	66.15 ± 0.34	66.47 ± 0.41	66.18 ± 1.08	67.18 ± 0.29	66.20 ± 0.67	68.13 ±0.25	66.23 ± 0.71	68.67 ±0.44
Significance	NS		NS		P<0.05		P<0.05	
Acetic acid (%)	67.25 ±0.36	66.67 ±0.80	67.22 ±0.60	65.67 ±0.50	67.20 ±1.10	64.83 ±0.75	67.23 ±0.88	64.38 ±0.57
Significance	NS		NS		P<0.05		P<0.05	
Propionic acid (%)	23.17 ±0.33	23.60 ±0.38	23.13 ±0.63	24.07 ±0.10	23.15 ±0.40	24.57 ±0.32	23.16 ±0.32	24.67 ±0.38
Significance	NS		NS		P<0.05		P<0.05	
Butyric acid (%)	9.58 ±0.42	9.73 ±0.20	9.65 ±0.91	10.27 ±0.60	9.65 ±0.78	10.60 ±1.01	9.61 ±1.04	10.95 ±1.06
Significance	NS		NS		NS		NS	
A/P ratio	2.91 ± 0.05		2.83 ± 0.07		2.92 ± 0.08		2.73 ± 0.03	
Significance	NS				NS			

[#]Mean of six observations; [NS] Not significant, Means bearing different superscript between the control and treatment groups (C1 & AN1, C2 & AN2, C3 & AN3 and C4 & AN4) differed significantly for each parameter, AN1 –Acacia nilotica plant extract 1, AN2 –*Acacia nilotica* plant extract 2, AN3 –*Acacia nilotica* plant extract 3 and AN4 –*Acacia nilotica* plant extract 4

Table III. Effect of supplementing plant metabolites like tannin and saponin through *Acacia nilotica* plant extract on reduction/increase of rumen methane emission and rumen fermentation characteristics based on the derived values from Table II by eliminating the error induced by the extractant (Mean[#] ± S.E)

Rumen fermentation characteristics	Treatments			
	AN1	AN2	AN3	AN4
Reduction in total gas (ml) [NS]	1.53 ±0.32	1.60 ±0.26	2.73 ± 0.23	2.20 ± 0.10
Reduction in Methane (ml) [NS]	0.44 ± 0.09	0.46 ± 0.12	0.69 ± 0.10	0.72 ± 0.11
Reduction in % of methane on total gas production [NS]	1.31 ± 0.74	1.60 ± 1.01	2.61 ± 0.54	3.21 ± 0.77
Reduction in Methane (ml) per 100 mg of truly digested substrate*	0.21 ± 0.07 [a]	0.26 ± 0.05[a]	0.50 ± 0.02[ab]	0.59 ± 0.11[b]
Reduction of Ammonia Nitrogen [NS] (mg/100ml)	0.42 ± 0.90	0.68 ± 0.73	1.05 ±0.48	1.22 ± 0.51
Reduction of *IVTDMD (%)**	5.20 ± 0.35 [b]	4.31 ± 1.75 [b]	2.93 ± 0.92 [a]	0.66 ± 0.65[a]
Reduction of Bacterial count (X 10^8) **	0.08 ± 0.08 [a]	0.85 ± 0.06 [b]	0.97 ± 0.72 [b]	0.96 ± 0.14 [b]
Reduction of Protozoal count (X 10^5)**	0.23 ± 0.12 [a]	0.43 ± 0.08 [ab]	0.92 ± 0.10 [bc]	0.93 ± 0.12 [c]
Reduction/increase of pH [NS]	-0.03 ± 0.01	-0.20 ± 0.03	0.18 ± 0.09	0.07 ± 0.05
Increase of TVFA (mg/dl) [NS]	0.32 ± 0.19	1.00 ± 0.81	1.93 ± 0.70	2.43 ± 0.73
Reduction of acetic acid (%) [NS]	0.58 ± 0.84	1.55 ± 0.45	2.37 ± 0.82	2.85 ± 1.06
Increase of propionic acid (%) [NS]	0.43 ± 0.57	0.93 ± 0.69	1.42 ± 0.34	1.51 ± 0.60
Increase of Butyric acid (%) [NS]	0.15 ± 0.81	0.62 ± 0.75	0.95 ± 0.54	1.34 ± 1.41
Reduction of A/P ratio [NS]	0.08 ± 0.09	0.19 ± 0.09	0.27 ± 0.07	0.29 ± 0.06

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

AN1 –*Acacia nilotica* plant extract 1, AN2 –*Acacia nilotica* plant extract 2, AN3 –*Acacia nilotica* plant extract 3 and AN4 –*Acacia nilotica* plant extract 4

and saponin (2.33 %) in the *Acacia nilotica* plant extract reduced the methane emission in the present study.

Rumen fermentation characteristics

The ammonia nitrogen was significantly (P<0.05) reduced by 5.43 and 6.36 % in AN3 and AN4 groups when compared to their respective control groups (Table II). The addition of *Moringa oleifera* aqueous methanol extract contained 1.11 % of HT and 4.09 % saponin, decreased the total ammonia nitrogen by 13.63 % than control (Alexander *et al*., 2008). The ammonia nitrogen reduction in the rumen are typical when protozoal growth is inhibited, presumably as a result of depressed bacterial lysis by tannin and saponin and also due to the formation of tannin protein complex reduced the ammonia nitrogen production (Vieira and Borba, 2011). There was a significant difference on *IVTDMD* while supplementing the *Acacia nilotica* plant extract in AN1 (P<0.01), AN2 and AN3 (P<0.05) when compared to their respective control (Table II). Alexander *et al*. (2008) also reported a significant (P<0.05) reduction on apparent dry matter digestibility (ADMD) by 7.94 to 13.53 % when the addition of HT and saponin through *Moringa oleifera* aqueous methanol extract by *in vitro*. The anti microbial effect of tannin and saponin lowers the rumen fermentation and digestibility as in the present study.

The bacterial and protozoal count were significantly (P<0.01) decreased by 18.05 and 12.32 % in AN2, 20.86 and 26.06 % in AN3 and 20.76 and 26.5 % in AN4 respectively than their respective control (Table II). The inhibitory activity of tannin against bacteria has been implicated due to the ability of tannins to form complexes with the cell wall and membrane bacteria causing morphological changes of the cell wall and the extracellular enzymes secreted (Smith *et al*., 2005). The addition of saponin with tannin in the present finding further agrees well with Wallace *et al*. (1994) who reported that saponins, inhibit the growth of rumen bacteria. It seems that saponins show a more marked antibacterial activity against Gram positive than Gram negative bacteria (Patra and Saxaena, 2010). Hess *et al*. (2003) reported that the saponin (1.2%) and CT (0.32 %) decreased the protozoal population significantly (P<0.05) by 53.97 % than control. Antiprotozoal activities of tannins and saponin through various plant metabolites have been reported earlier (Feng *et al*., 2012; Bharathidhasan *et al.*, 2013). One possible mechanism of saponin on protozoa is a change in cell membrane permeability (Klita *et al*., 1996) as they form complexes with cholesterol in protozoal cell membranes and result in cell lysis.

The TVFA (2.83 and 3.55 %) and propionic acid (5.78 and 6.12 %) were significantly (P<0.05) increased and the acetic acid (3.53 and 4.24 %) and acetate to propionate ratio (9.28 and 10.31 %) were significantly (P<0.01) decreased in AN3 and AN4 when compared to their respective control (Table II). The tannin and saponin addition through forages, were significantly increased TVFA and propionic acid from 13.83 % to 19.64 % than control as reported early (Alexander *et al*., 2008; Feng *et al*., 2012). The decrease in acetic acid and increase in propionic acid in the present study suggest that the nutrients were partitioned more towards microbial

protein synthesis in the presence of tannins (Makkar *et al*.**,** 1995). Further, the decrease in acetic acid may also be due to the stronger inhibitory effect over acetate producing bacteria **(***Ruminococcus albus, Butyrivibrio fibrisolvens***)** than others, either by directly inhibiting them or by inhibiting the production of their preferred substrate (Castro-Montoya *et al*.**,** 2011). The strong inverse relationship between the propionic acid and methane production can be predicted from knowledge of interactions among ruminal microbial populations and compounds like tannin and saponin that promote higher production of propionic acid in the rumen may also decrease methane production (Tavendale *et al*.**,** 2005).

CONCLUSION

It was concluded that the minimum concentration of *Acacia nilotica* plant extract at 0.75 ml which had equivalent of 3.09 % of tannin and 2.34 % of saponin, significantly reduced the methane emission per 100 mg of truly digested substrate ($P<0.01$) by 27.93 % when compared to their respective control by *IVGPT.* The energy saved through decrease in methane emission could be used for sustainable animal production and may also curtail global warming.

REFERENCES

Alexander, G., Sing, B., Sahoo, A. and Bhat, T.K. (2008). *In vitro* screening of plant extracts to enhance the efficiency of utilization of energy and nitrogen in ruminant diets. *Animal Feed Science and Technology*, **145:**229-244.

Ammar, H., Boubaker, A., Kayouli, C. and Lopez, S. (2005). Gas production technique to evaluate the nutritive value of tannin-containing shrub species from a mountain area in northern Tunisia. *CIHEAM*, 361-366.

Bharathidhasan, A. (2018). Effect of standard tannin on *in vitro* rumen methane reduction and rumen fermentation characteristics. *Indian Journal of Veterinary and Animal Sciences Research*, **47**(4):1379-1391.

Bharathidhasan, A., Karunakaran, R., Pugazhenthi, T.R. and Ezhilvalavan, S. (2016). The effect of supplemental organic acid on methane reduction to decrease the global warming from dairy cattle. *International Journal of Advanced Chemical Science and Applications,* **3**(4):60-64.

Bharathidhasan, A., Viswanathan, K., Balakrishnan, V., Valli, C., Ramesh, S. and Sethilkumar, T.M.A. (2013). Effects of purified saponin on rumen methanogenesis and rumen fermentation characteristics studied using *in vitro* gas production technique. *International Journal of Veterinary Science,* **2**(2):44-49.

Castro-Montoya, J.M., Makkar, H.P.S. and Becker, K. (2011). Chemical composition of rumen microbial fraction and fermentation parameters as affected by tannis and saponins using an *in vitro* rumen fermentation systems. *Canadian Journal of Animal Science*, **91:**433-448.

Chase, LE. (1990). Analysis of fatty acids by packed column gas chromatography. G.C., Bulletin 856, Division of Rohand Has, Supelcom, pp: 1-12.

Cheeke, P.R. (1999). Actual and potential application of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition*, Proceedings of the American Society of Animal Science*, pp: 1–10.

Feng, Z.H., Yu-Feng, CaO., Yan-Xia, Gao., Qiu-Feng, Li. and Jian-Guo, Li. (2012). Effect of biological action of saponins in animal systems: a review. *British Journal of Nutrition*, **88:**587-605.

Gall, L.S., Burroughs, W., Gerlaugh, P. and Edgington, B.H. (1949). Special methods for rumen bacterial studies in the field. *Journal of Animal Science*, **8:**433-440.

Goel, G. and Makkar, H.P.S. (2012). Methane mitigation from ruminants using tannins and saponins. *Tropical Animal Health and Production*, **44:**729–739.

Hess, H.D., Kreuzer, M., Diaz, T.E., Lascano, C.E., Carulla, J.E., Soliva, C.R. and Machmuller, A. (2003). Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated fluid. *Animal Feed Science and Technology,* **109:**79-94.

Jayanegara, A., Goel, G., Makkar, H.P.S. and Becker, K. (2010). Reduction in methane emissions from ruminants by plant secondary metabolites: effects of polyphenols and spaonins. In: N.E. Odongo, M. Gracia and G.J. Viljoen (eds), 'sustainable improvement of animal production and health. FAO, Rome, **2010:**151-157.

Klita, P.T., Mathison, G.W., Fenton, T.W. and Hardin, R.T. (1996). Effects of alfalfa root saponins on digestive function in sheep. *Journal of Animal Science,* **74:**1144–1156.

Makkar, H.P.S. and Becker, K. (1996). Effect of *Quillaja saponaria* on *in vitro* rumen fermentation. *Advances in Experimental Medicine and Biology*, **405:** 387-394.

Makkar, H.P.S., Blummel, M. and Becker, K. (1995). *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. *Journal of the Science of Food and Agriculture,* **69:**481-493.

Menke, K.H. and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development,* **28:**7–55.

Moir, R.J. (1951). The seasonal variation in the ruminal microorganism of grazing sheep. *Australian Journal of Agricultural Research*, **27**:322.

Patra, A.K. and Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry*, **71:**1198-1222.

Pellikaan, W.F., Stringano, E., Leenaars, J., Bongers, D.J.G.M., Van schuppen, S.V.L., Plant, J. and Harvey, I.M. (2011). Evaluating effects of tannins on extent and rate of *in vitro* gas and CH_4 production using an automated pressure evalution system (APES). *Animal Feed Science and Technology,* **166-167**:377-390.

Sirohi, S.K., Pandey, N., Goel, N., Mohini, M., Pandey, P. and Chouthry, P.P. (2009). Microbial activity and ruminal methanogenesis as affected by plant secondary metabolites in different Plant extracts. In: Proceedings of International Conference on Energy and Environment, March **19-21:**722-728.

Smith, A.H., Zoetendal, E.G. and Mackie, R.I. (2005). Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microbial Ecology*, **50:**197–205.

Snedecor, G.W. and Cochran, W.C. (1989). Statistical Methods 8th edn. Iowa State University Press, Ames, Iowa.

Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attawood, T.G. and Sivakumaran, S. (2005). Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa* and effects of extra condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology*, **123-124:**403-419.

Van Soest, P.J. and Robertson, (1988). A Laboratory manual for animal science, 612, Ithaca, New York, Cornell University.

Vieira, S.C. and Borba, A.E.S. (2011). Effects of condensed tannins from Quebracho extract on the kinetic of *in vitro* gas production on *Trifolium repens*, *Lotus corniculatus* and *Lolium perenne*. *Journal of Agricultural Science Technology*, **B1:**982-988.

Wallace, R.J., Arthaud, C.J.L. and Newbold, C.J. (1994). Influence of *Yucca schidigera* extracts on ruminal ammonia concentration and ruminal microorganisms. *Applied and Environmental Microbiology*, **60:**1762-1767