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Effect of feeding tanniferous diets on methane emissions in sheep

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Abstract
The present study was designed with the objective of *in vitro* and *in vivo* evaluation of methane emissions with tanniferaous feeds in Deccani ram lambs. *In vitro* studies were conducted to select one tanniferous diet based on *in vitro* dry matter digestibilities (IVDMD) and mean methane emissions for inclusion *in vivo* studies. During second phase *in vivo* studies, 12 Deccani ram lambs of uniform body weight (16.5±0.64 kg with130.11±3.00 days of age) were randomly allotted to 2 treatments in a completely randomized design. Metabolic studies conducted for nutrient digestibility studies and Respiratory chamber was used for estimation of methane emissions In *in vitro* studies observed that 3.6 per cent increase *in vitro* dry matter digestibilities and 3.2 per cent reduction in methane emissions with T1 (Horse gram) tannins over group average. In *in vivo* studies, the nutrient digestibilities increased (P<0.05) with horse gram meal inclusion. Mean enteric methane emissions (l/day) were significantly (P<0.01) lower (10.05± 0.39) with tanniferous horse gram meal than control (11.59± 0.70) lambs (Group I) and the reduction is 9.4 percent of daily methane emissions over control group. It may be concluded that inclusion of tanniferous protein source increased nutrient digestibilities and decreased enteric methane emissions, suggesting that the energy loss for ruminants in the form of methane emissions can be reduced efficiently.

Keywords: Tannins, horse gram, methane emissions, nutrients digestibility

1. Introduction
Increasing atmospheric methane emissions is having worldwide importance as the trends in CH4 showed a stabilization from 1999 to 2006, but CH4 concentrations have been increasing again starting in 2007 (Dlugokencky *et al*, 2009) [1], which has 25 times greater warming potential than that of CO_2 (Zhou *et al*, 2011) [2, 34]. Total anthropogenic sources contribute at present between 50 and 65% of the total CH4 sources (IPCC 2013) [3]. These enteric methane missions from ruminants varies based on the geographical location feed intake, feed composition and quality, processing of feed and animal breed (Hook *et al*, 2010) [4]. Apart from these environmental problems, the methane emission leads to loss up to 2–12 % of ingested energy from the rumen (Moss *et al*, 2000) [5]. This is because of low digestibility (<500 g digestible organic matter per kg dry matter), low crude protein content (<50 g/kg DM) and low content of available minerals and vitamins (Adugna *et al*, 2000) [6] in the available grazing resources and coarse crop residues. Such considerations have led to manipulate the ruminal fermentation in order to improve the efficiency of ruminant production in an ecologically sustainable way. More recently, the focus on manipulating methanogen numbers has been the reduction of greenhouse gas production from livestock. Techniques to manipulate methanogens have included the use of biological strategies, altering the dietary components, digestibility of feeds, chemicals and natural inhibitors like tannin containing feeds (Mathison *et al*, 1988) [7].

One major goal in increasing the efficiency of nutrient utilization is to alter molar proportions of ruminal volatile fatty acids, increase nutrient digestibility and reduce emission losses. Ideally such production strategies aim at limiting environmentally harmful enteric emissions in addition to the optimization of production potential in sustainable manner. Therefore the present study is designed to identify the best tanniferous feed on the basis of methane reducing capacity in *in vitro* and to evaluate the effect of same in *in vivo* on methane production in Deccani ram lambs.

2. Materials and Methods

2.1 *In vitro* studies

In vitro studies were conducted to identify the best tanniferous protein for further *in vivo* experimentation in Deccani ram lambs. The techniques used for the experiment were *in vitro* dry matter degradability (IVDMD) and *in vitro* gas production.

Suitable aliquot of gas collected from Gas-tight culture bottles (100ml capacity) consisting rumen contents and feed samples, was withdrawn from the tip of the incubation bottles using gas tight syringe and composition of gas in the headspace of bottles determined using gas chromatograph (450-GC, BRUKER Daltonics, Bremen, Germany).

2.2 *In vivo* studies

2.2.1 Animals, experimental design and management

Twelve growing Deccani ram lambs aged 130±3.0 d with average body weight of 16.5±0.64 kg were selected for conducting a growth trial for a period of 90 days at Central Research Institute for Dry land Agriculture (CRIDA) Livestock farm, Hyderabad. These animals were randomly divided in to two groups of six animals in each in a completely randomized design.

All the experimental animals were housed in a well-ventilated animal shed with the provision for feeding and watering. The lambs were weighed individually at fortnight intervals before feeding and watering to observe the body weight changes for an experimental period of 90 days. After 60 days of growth trial, a seven days metabolic trial was carried out on lambs to study the digestibility of nutrients in experimental diets.

2.2.2 Experimental diets

The dietary treatments were *viz*., G-I: Basal diet (chopped sorgum stover as roughage source) (BD) + group 1 concentrate+ chopped green fodder (4kg), G-II: Basal diet + Group 2 concentrate (horse gram as tannin source selected from *in vitro* studies+ chopped green fodder (4kg). Deccani ram lambs were fed the respective diets *ad lib*.to meet the nutrient requirements (NRC, 2001) throughout 90 days of feeding trial. The ingredient and chemical composition of the experimental feeds is summarized in Table 1 & 2.

Table 1: Ingredient composition of concentrate mixtures (parts per 100) offered to Deccani ram lambs

Ingredients	Group 1 (control)	Group 2 (Horse gram inclusion)
Maize	40	40
Rice bran	32	29
Soya meal	25	00
Horse gram meal	00	28
Mineral mixture	02	02
Common Salt	01	01

Table 2: Chemical composition of experimental feeds (%DM) offered to Deccani ram lambs

Nutrient	Basal diet		Concentrate mixture	
	Green fodder (HN-CO4)	Dry fodder (Sorghum straw)	Group 1 (control)	Group 2 (Horse gram inclusion)
Proximate principles				
Dry matter	20.38	98.59	98.03	98.35
Organic matter	87.22	92.29	91.05	91.72
Crude protein	11.75	3.02	17.96	17.94
Crude fibre	35.86	40.45	14.96	14.94
Ether extract	2.64	2.49	6.34	6.63
NFE	36.97	46.34	49.61	48.93
Total ash	12.78	7.71	8.95	8.28
Cell wall constituents				
NDF	71.34	83.27	48.12	58.29
ADF	41.58	52.34	19.44	19.96
Hemicellulose	29.76	30.93	28.67	38.33
Cellulose	33.78	42.93	11.71	14.4
Anti-nutritional compounds				
Total Phenolic compounds	-	-	0.21	0.48
Condensed Tannins	-	-	0.09	0.14
Minerals				
Ca	0.40	0.34	1.12	1.18
P	0.16	0.24	0.82	0.78

2.2.3 Respiratory chamber

Enteric emissions from the animals were measured using closed respiratory chamber method. The respiration chamber was designed to enable accurate measurements of gaseous exchanges and provide a comfortable and safe environment for the animals.

Enteric emissions from the animals were measured using closed respiratory chamber method. The respiration chamber was designed to enable accurate measurements of gaseous exchanges and provide a comfortable and safe environment for the animals.

Respiratory chamber was made of 10 mm transparent acryl panels (0.602 m wide × 1.307 m length × 1.306 m tall, 1.028 m^3 volume) fixed to an iron angle frame (Fig 1). Three air pumps with 13 l/min capacity (AS16-1 Mini Air compressor piston type) each was equipped to draw air from chamber through the pipe and supply air to inside the chamber so that the rate of approximately 39 litres per minute flow was maintained. Based on our previous test, at this air flow rate, the carbon dioxide concentration in the chamber with a 25-kg goat did not exceed 0.5%, a suggested maximum concentration (Klein *et al*, 2006) [8]. Air samples from the chamber were collected from various heights at regular interval of 60 min in 24h duration in gas syringes, closed with airtight caps and sealed with parafilm. Composition of gas determined using gas chromatograph (450-GC, BRUKER Daltonics, Bremen, Germany) (Fig 2) with three detectors Thermal Conductivity Detector (TCD), Electron Capture

Detector (ECD) and Flame Ionization Detector (FID) with a 1041 PWOC Packed/Wide bore On-Column. Carrier gases were nitrogen (N2), helium (He), hydrogen (H2) and methane (CH4) at 500 kPa (max 1000kPa), H2 and air are detector fuel gases.

(a) **(b)**

Fig 1: Respiratory chamber for the measurement of enteric emissions in environmental controlled house (a & b)

Fig 2: Gas chromatography

2.3 Statistical analysis

The results obtained were csubjected to analysis through software (version 17.0: SPSS,2005) by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test (Duncan, 1955) [9] with a test of significance at $P<0.05$.. All the statistical procedures were done as per Snedecor and Cochran (1980) [10].

3. Results and Discussion

3.1 *In vitro* studies

Five tannin treatments horse gram meal (T1), groundnut meal (T2), *Acacia* pod meal (T3), *Leucaena leucocephala* (Subabul) leaf meal (T4) and coconut meal (T5) were used to study the effect of tanniferous diets inclusion on IVDMD and *in vitro* methane emissions with sorghum stover as basal diet and the results are presented in Table 3.

Table 3: *In vitro* methane emissions (g/kg IVDMD) and *in vitro* dry matter degradability (%) from *in vitro* coarse crop residues fermentation with different probiotics and tanniferous diets

S.No	Experimental diet	Treatment	CH4 g/kg IVDMD	IVDMD (%)	Tannins (%)	Methane (ml)*	Total gas (ml)*
01	Horse gram meal	T1	16.72 ± 0.20^a	64.18 ± 0.22^a	1.53 ± 0.13	11.8± 0.51^b	42.6± 0.42^b
02	Ground nut meal	T2	17.48 ± 0.42^b	62.76 ± 0.32^b	0.05± 0.01	12.3± 0.75^a	44.5± 0.30^a
03	*Acacia* pod meal	T3	17.76 ± 0.54^b	59.62 ± 0.16^b	1.98± 0.15	12.5± 0.64^a	45.2± 0.45^a
04	*Leucaena leucocephala* meal	T4	17.22 ± 0.38^b	61.28 ± 0.36^b	0.14± 0.01	12.1± 0.48^a	43.8± 0.26^a
05	Coconut meal	T5	17.16 ± 0.26^b	62.02± 0.26^b	2.3± 0.21	12.1± 0.72^a	43.7± 0.42^a

***In vitro** methane emissions (ml/0.5g) and total gas (ml/0.5g) from *in vitro* coarse crop residues fermentation with different probiotics and tanniferous diet

Significantly ($P<0.05$) higher *in vitro* dry matter degradability (%) was observed with T1 Tannin (64.18 ± 0.22) than other tanniferous protein rich feeds. Inclusion of this tanniferous horse gram meal resulted in 3.6 per cent increase in IVDMD over group averages. This could be due to precipitation of proteins by tannins through hydrogen bonding and hydrophobic interactions to form stable complexes at rumen pH, which adversely affect protein and fibre degradation in the rumen (Ramana, 2000) [11]. Further, negative effect of tannins on cellulose digesting bacteria might be the cause for decreased IVDMD.

Barman *et al*. (2008) [12] reported no difference in *in vitro* digestibility of dry matter (IVDMD) up to 4% tannins in goats fed with total mixed rations (TMR) containing 4, 6, 8, 10, 12% tannins through *Accacia nilotica* pods but decreased thereafter. Contrary to this, Getachew *et al*. (2008) [13] reported tannic acid significantly ($P<0.05$) reduced *in vitro* true degradability of DM (IVTD DM) with tannic acid (TA) sprayed on chopped alfalfa at three concentrations (30, 60 and 90 g TA per kg DM).

3.2 *In vitro* gas and methane production

Typical relationship between head-space gas pressure and gas volume from 20 bottles read on 8 occasions during a 24h incubation period for sorghum stover are presented in Figure 3. Total gas and mean methane emissions were lower ($P<0.01$) with T1 tannins.

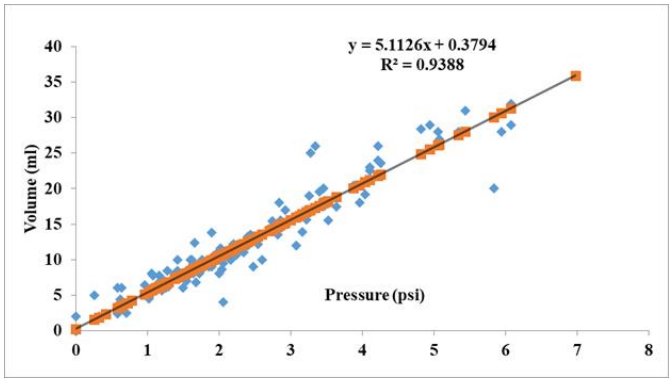

Fig 3: Typical relationship between head-space gas pressure and gas volume from 20 bottles read on 8 occasions during a 24h incubation period

Tannins at 15 mg/0.5 g sorghum stover had significant influence on the total gas production. A similar finding was observed with different additives for soyabean hulls (Pellikaan *et al*, 2011) [14]. Inclusion of tanniferous horse gram meal (T1) resulted in 3.2 per cent reduction in methane, over group averages. The effect of tannin on suppressing

methanogenesis was mostly attributed to decreasing the methanogenic population in rumen fluid (Bhatta *et al*, 2009) [15] and forming tannin-macromolecule complex which inhibits microbial enzyme activities (Mcsweeny *et al*, 2001) [16] which resulting in decreased methane production. Tabacco *et al.* (2006) [17] showed that high tannin concentration in the diet may be a cause for reduction in microbial enzyme activities like cellulase. Bento *et al.* (2005) [18] reported that mimosa tannin depressed gas production rate and concluded that this reduction might bind tannin with microorganisms or their enzymes. Reza Maleki Baladi *et al.* (2014) [19] report significantly (P<0.01) lower gas production was recorded from soya bean meal treated with 4.5% and 6% of tannins extracted from pomegranate pomace. Rira *et al*., (2014) [20] reported Methane production, VFA concentration and fermented organic matter decreased with increased proportions of Tannin-rich plants. Similar results were also reported by Gemeda *et al* (2015) [21] and Barros-Rodríguez *et al* (2014) [22]. Contrary to this Seresinhe *et al.* (2012) [23] reported no significant effect of CT level on methane production.

3.3 Nutrient intake and digestibilities

Horse gram meal inclusion had no effect on Dry matter intakes (DMI) and Organic matter intake (OMI) in ram lambs (Table 4). This indicated that relatively low level of condensed tannins (CT) in experimental concentrate mixtures had no effect on intake. These findings are in agreement with earlier observations of Pathak *et al*., 2013 [24].

Table 4: Effect on Intake and digestibilities of DM, OM and NDF of experimental rations fed to Deccani ram lambs.

Indicators	Group I	GroupII	SEM	P
Intake				
DMI kg/day	1.049 ± 0.01	0.996 ± 0.03	0.01	0.01
DMI % LW	4.80 ± 0.32	4.63 ± 0.36	0.01	0.01
OMI kg/day	0.961 ± 0.01	0.908 ± 0.02	0.01	0.02
Apparent digestibilities				
DM %	64.32^{b} ± 0.08	70.48^{a} ± 1.19	0.93	0.01
OM %	67.95^{b} ± 0.67	73.2^{a} ± 1.07	0.81	0.02
CP %	66.94^{b} ± 0.68	73.25^{a} ± 0.98	0.93	0.05
NDF %	68.19^{b} ± 0.71	73.4^{a}± 1.16	0.84	0.001

Horse gram meal inclusion had increased digestibilities of DM (P<0.01), OM (P<0.02), CP (P<0.01) and NDF (P<0.01) in ram lambs. Increased nutrient digestibilities due to horse gram inclusion could be due to escape of more liable proteins from degradation in the and consequently complete digestion in lower part of the digestive tract rumen (Mangan, 1988). Tannins present in the feed decreases ruminal protein degradation and increases duodenal protein flow when provided at moderate doses (Min *et al*., 2003).

The results are in accordance with the findings of Hart *et al.* (2011) who reported increased DM and OM (P<0.01) digestibility in lambs fed on low-tannin diets. Similarly Barros-Rodríguez *et al* (2014) [22] reported increased nutrient digestibility in sheep. Beauchemin *et al.* (2007) [28] also reported increased CP digestibility in cattle supplemented with 1 and 2% Quebracho CT extract. Likewise increase in NDF digestibility is supported by Bengaly *et al.* (2007) [29] with tannins supplementation in Nguni and Boer goats. Rajei Sharifabadi *et al.* (2014) [30] reported no effect on DM, OM digestability in lambs with inclusion of pistachio by-products aqua extract (PBE) as a source of tannin. Similar results were also reported by Pathak *et al*., (2013) [24], Dentinho *et al.* (2014) [31] and Avijit Dey A *et al* (2014) [32].

3.4 Enteric methane emissions

Enteric methane emissions for 24 h sampling plotted standard curve showing linearity are presented in Fig 04.

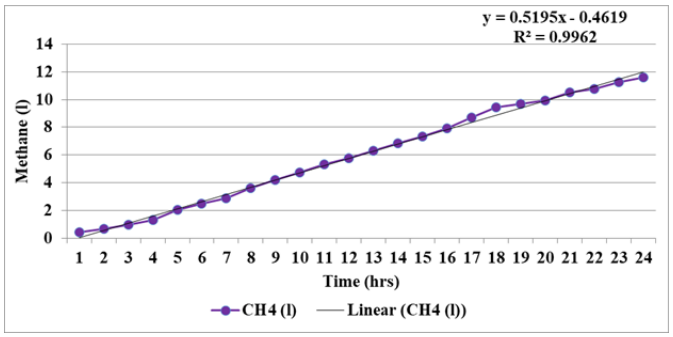

Fig 4: Enteric methane emissions curve for 24 hrs sampling in Deccani ram lambs

A significantly (P<0.01) lower methane emissions were observed from lambs fed with horse gram than control group lambs in terms of lower methane in (g)/day (10.50± 0.39 Vs 11.59± 0.70 g/day), methane emissions l/day (7.50 ± 0.28 Vs 8.28 ± 0.50 l/day) and methane emission in l/DMI/day (0.68 ± 0.01 Vs 0.79 ± 0.01 l/DMI/day) (Table 05). Over all it was observed that the horse gram inclusion reduced (P<0.01) daily methane emissions by 9.4 per cent in Deccani ram lambs over control group.

Table 5: Methane (CH_4) emissions as affected by feeding experimental rations in Deccani ram lambs

Indicators	Group I	Group II	SEM	P
B.Wt	22.73 ± 1.32	24.29 ± 0.92	0.82	0.83
M.bwt	10.39 ± 0.45	10.94 ± 0.31	0.28	0.81
DMI/day	1.05^{a} ± 0.01	1.00^{b} ± 0.03	0.01	0.11
DM Digest %	64.32^{b} ± 0.08	70.48^{a} ± 1.19	0.93	0.01
OM Digest %	67.95^{b} ± 0.67	73.2^{a} ± 1.07	0.81	0.02
CH_4 gm/Day	11.59^{a} ± 0.70	10.50^{b} ± 0.39	0.38	0.01
CH_4 gm/L.WT	11.07^{a} ± 0.72	10.57^{b} ± 0.46	0.38	0.02
CH_4 gm/M.BWT	0.51^{a} ± 0.00	0.43^{b} ± 0.00	0.02	0.00
CH_4 gm/Kg DMI	1.11^{a} ± 0.02	0.96^{b} ± 0.01	0.03	0.00
CH_4 L/Day	8.28^{a} ± 0.50	7.50^{b} ± 0.28	0.27	0.01
CH_4 L/L.WT	7.91^{a} ± 0.51	7.54^{b} ± 0.33	0.27	0.02
CH_4 L/M.BWT	0.35^{a} ± 0.00	0.31^{b} ± 0.01	0.01	0.00
CH_4 L/Kg DMI	0.79^{a} ± 0.01	0.68^{b} ± 0.01	0.02	0.00

DMI: Dry matter intake; CH_4: Methane; Means with the different superscripts along the row are significantly different; SEM, standard error of the mean

Effect of tannins on suppressing methanogenesis was mostly attributed to decreasing the methanogenic population in rumen fluid (Bhatta *et al*., 2009) [15]. The results of the present investigation are in agreement with the findings of Bhatta *et al* (2013) [33] who reported natural tannins, even at a low concentration (2-8 g/kg DM of the diet), reduce CH_4 emissions and Similarly Liu *et al*., (2011) [34] reported sheep fed with chestnut tannins (CT) significantly decreased methane emissions. Similar reports were reported by Archimède (2016) [35] and Malik *et al* (2017) [36] Whereas Wischer *et al* (2014) [37] reported tannin-rich extracts temporary affect processes in the rumen but did not alter methane release over a longer period. Moreira *et al* (2013) [38] suggested that tropical tanniniferous legumes may have

potential to reduce CH_4 emission in sheep, but more research is warranted to confirm these results.

4. Conclusions

Based on the results of the present study, it may be concluded that inclusion of tanniferous protein source in sheep diets, increased the nutrient digestibilities and decreased enteric methane emissions, suggesting that the energy loss for ruminants in the form of methane emissions can be reduced efficiently.

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