Effect of feeding tanniferous diets on methane emissions in sheep

T Parthasarathi, A Sarat Chandra, DBV Ramana and J Raju

Abstract
The present study was designed with the objective of in vitro and in vivo evaluation of methane emissions with tanniferous 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 with 130.11±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 1) 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 CO2 (Zhou et al, 2011) [2, 34]. Total anthropogenic sources contribute at present between 50 and 65% of the total CH4 sources (IPCC 2013) [1]. 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) [3]. This is because of low digestibility (<0.50 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 into 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 sorghum 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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Group 1 (control)</th>
<th>Group 2 (Horse gram inclusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Rice bran</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>Soya meal</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Horse gram meal</td>
<td>00</td>
<td>28</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>02</td>
<td>02</td>
</tr>
<tr>
<td>Common Salt</td>
<td>01</td>
<td>01</td>
</tr>
</tbody>
</table>

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 m² 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.

2.3 Statistical analysis
The results obtained were subjected 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

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

*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.

3.3 Statistical analysis
The results obtained were subjected 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].

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

Fig 2: Gas chromatography

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 soybean 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 Gemade 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.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group I</th>
<th>Group II</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI kg/day</td>
<td>1.049 ± 0.01</td>
<td>0.996 ± 0.03</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>DMI % LW</td>
<td>4.80 ± 0.32</td>
<td>4.63 ± 0.36</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>OMI kg/day</td>
<td>0.961 ± 0.01</td>
<td>0.908 ± 0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Apparent digestibilities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM %</td>
<td>64.32 ± 0.08</td>
<td>70.48 ± 1.19</td>
<td>0.93</td>
<td>0.01</td>
</tr>
<tr>
<td>OM %</td>
<td>67.95 ± 0.67</td>
<td>73.2 ± 1.07</td>
<td>0.81</td>
<td>0.02</td>
</tr>
<tr>
<td>CP %</td>
<td>66.94 ± 0.68</td>
<td>73.25 ± 0.98</td>
<td>0.93</td>
<td>0.05</td>
</tr>
<tr>
<td>NDF %</td>
<td>68.19 ± 0.71</td>
<td>73.4 ± 1.16</td>
<td>0.84</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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. Rajee Sharifabadi et al. (2014) [30] reported no effect on DM, OM digestibility 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.

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 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 (CH₄) emissions as affected by feeding experimental rations in Deccani ram lambs

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group I</th>
<th>Group II</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.Wt</td>
<td>22.73 ± 1.32</td>
<td>24.29 ± 0.92</td>
<td>0.82</td>
<td>0.83</td>
</tr>
<tr>
<td>M.bwt</td>
<td>10.39 ± 0.45</td>
<td>10.94 ± 0.31</td>
<td>0.28</td>
<td>0.81</td>
</tr>
<tr>
<td>DMI/day</td>
<td>1.05 ± 0.01</td>
<td>1.00 ± 0.01</td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>DM Digest %</td>
<td>64.32 ± 0.08</td>
<td>70.48 ± 1.19</td>
<td>0.93</td>
<td>0.01</td>
</tr>
<tr>
<td>OM Digest %</td>
<td>67.95 ± 0.67</td>
<td>73.2 ± 1.07</td>
<td>0.81</td>
<td>0.02</td>
</tr>
<tr>
<td>CH₄ gm/L-Day</td>
<td>11.59 ± 0.70</td>
<td>10.50 ± 0.39</td>
<td>0.38</td>
<td>0.01</td>
</tr>
<tr>
<td>CH₄ gm/L WT</td>
<td>11.07 ± 0.72</td>
<td>10.57 ± 0.46</td>
<td>0.38</td>
<td>0.02</td>
</tr>
<tr>
<td>CH₄ gm/M.BWT</td>
<td>0.51 ± 0.00</td>
<td>0.43 ± 0.00</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>CH₄ gm/Kg DMI</td>
<td>1.11 ± 0.02</td>
<td>0.96 ± 0.01</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>CH₄ L/Day</td>
<td>8.28 ± 0.50</td>
<td>7.50 ± 0.28</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>CH₄ L/L WT</td>
<td>7.91 ± 0.51</td>
<td>7.54 ± 0.33</td>
<td>0.27</td>
<td>0.02</td>
</tr>
<tr>
<td>CH₄ L/M.BWT</td>
<td>0.35 ± 0.00</td>
<td>0.31 ± 0.01</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>CH₄ L/Kg DMI</td>
<td>0.79 ± 0.01</td>
<td>0.68 ± 0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

DMI: Dry matter intake; CH₄: 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 CH₄ emissions and Similarly Liu et al., (2011) [24] reported sheep fed with chestnut tannins (CT) significantly decreased methane emissions. Similar reports were reported by Archimède (2016) [15] and Malik et al (2017) [18] Whereas Wischer et al (2014) [34] 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

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potential to reduce CH₄ 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.

5. References


