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Enteric methane mitigation in sheep through leaves of selected tanniniferous tropical tree species

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ABSTRACT

This study was carried out to investigate the effect of supplementation of three selected tanniniferous tropical tree leaves of *Ficus benghalensis*, *Artocarpus heterophyllus* and *Azadirachta indica* on enteric methane emission, rumen fermentation characteristics and protozoal population in 20 native Indian adult male sheep (*Mandya*). The experiment was conducted in randomized block design with four treatments of five animals each. The sheep in control group (CON) were fed on complete feed block (CFB) comprising finger millet straw and concentrate in the ratio of 60:40 (DM basis) without any inclusion of tropical tree leaves. Sheep in three test groups *i.e.* FIC, ART and AZA were also fed complete feed block prepared from finger millet straw and concentrate in the same ratio (60:40) as control, but wheat bran in concentrate mixture of test groups was partially replaced (10 parts w/w) with selected tanniniferous tropical tree leaves of *Ficus benghalensis*, *Artocarpus heterophyllus* and *Azadirachta indica,* respectively. The condensed tannin intake in sheep between 7.15 and 10.8 g/kg dry matter intake *via* selected tanniniferous tropical tree leaves did not influence dry matter and digestibility. About 20–26% reduction ($p < 0.05$) in enteric methane emission is achievable simply through the supplementation of selected tree leaves in straw based diet. The reduction in protozoal numbers ($p < 0.05$) due to selected tropical leaves supplementation appeared as primary cause for the reduction in enteric methane emission. The study confirmed that entodinimorphs protozoa are more vulnerable to the condensed tannin supplementation. The study established that significant reduction in methane emission is feasible without affecting feed fermentation characteristics. However, studies in large productive (growing, lactating) ruminants are warranted in order to explore the persistency of ameliorative effect in long term and improvement in productive performance thereof.

1. Introduction

Increasing demand for livestock products in recent years has put livestock sector under tremendous pressure for productivity intensification. The efforts of increasing products availability would lead to larger methane emissions into surrounding atmosphere, if suitable and sustainable mitigation strategies are not being devised and followed correctly. Methane, a greenhouse gas is 25 times more potent in heat trapping and accelerating the warming of earth than CO_2 on equal mass basis. Globally, ruminants account for 65–85 Tg annual enteric methane emission which is not only one of the major causative factors for global warming, but also obligated for a substantial loss of biological energy which otherwise could be used by host animal for productive purpose. One litre of enteric methane emission is equated to a loss of 39.5 KJ feed energy. In developing countries like India where feed shortage particularly quality feeds, prevail and animals are forced to primarily thrive on crop residue based fibrous diet, the absolute and per unit of product methane emission is quite large. Therefore, reduction in enteric methane emission in developing world may be a major avenue to stabilize the global warming and vigorous improvement in livestock productivity.

Worldwide researchers are trying to reduce enteric methane emission with different approaches that do not interfere with beneficial rumen microbioata and functions. Some compounds may be effective in decreasing methane emission, but this could be associated with a concurrent decrease in feed intake and digestibility. In addition, rumen archaea may also adapt and therefore, loose anti-methanogenic action in long term. Thus, there is a need to identify phyto-sources which could potentially mitigate the emission and thereby conserve biological energy within the animal system.

Tannins are polyphenolic compounds with a vast structural diversity which affects methanogenesis and rumen functions depending on the source, level and type. The tannins exert their anti-methanogenic activity through direct inhibition of methanogen archaea or indirectly by interfering with protozoa and thereby restricting the interspecies H_2 transfer (Bhatta et al., 2009; Gemeda and Hassen, 2015). Apart from

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methane mitigation, the strategic supplementation of tropical browses optimizes the utilization of poor quality feeds. In our laboratory, more than 100 phyto-sources have been screened and evaluated (*in vitro)* in order to determine their methane mitigation potential and optimizing their level of inclusion in the animal diet (Bhatta et al., 2013a, 2013b). It is established that *in vitro* evaluation only simulates the ruminal fermentation and does not consider the factors such as long term ruminal microbe adaptation, continuous media flow, direct absorption, palatability of the phyto-sources under investigation, their toxic impact on habitant rumen microbes. Thus, *in vitro* studies only provide the clue and cannot predict the exact extent of methane mitigation and impact of phyto-sources inclusion.

Based on our previous *in vitro* results (Bhatta et al., 2012, 2013a, 2014) on methane reduction potential, three tanniniferous tropical tree leaves *viz. Ficus benghalensis* (Banyan); *Artocarpus heterophyllus* (Jackfruit) and *Azadirachta indica* (Neem) not yet been investigated *in vivo* were selected for the supplementation in sheep to ascertain effect of inclusion on enteric methane emission.

2. Materials and methods

The experiment was conducted at the Experimental Livestock Farm of National Institute of Animal Nutrition and Physiology, Bangalore (12.97°N 77.56°E), India after obtaining the necessary approval from the Institute Animal Ethics Committee (approval number 25/16/2015-CPCSEA dated 5th August 2015). The experiment was conducted in 20 male adult native Indian sheep (*Mandya*) following randomized block design.

2.1. Test sources

Three tropical tree leaves from *Ficus benghalensis*, *Artocarpus heterophyllus* var. *varikka* and *Azadirachta indica var minor valeton* were evaluated for their effect on enteric methane emission when supplemented in sheep. The leaves from in and around Bangalore were harvested during summer, rainy and winter seasons from the nine trees of each (three trees in each season) *Ficus benghalensis* (15–18 yrs), *Artocarpus heterophyllus* (6–8 yrs) and *Azadirachta indica* (6–8 yrs). Both old and newly emerge type of leaves from trees were harvested and brought fresh to the institute immediately. The leaves from each season were air dried under shade having good provision for air ventilation. The air dried leaves for each tree species collected during different seasons were pooled and ground (2 mm size) prior to the determination of tannin and subsequent use in complete feed block preparation.

2.2. Sheep and diets

Twenty adult male *Mandya* sheep (BW 32 ± 0.17 kg) were randomly blocked into four groups of five animals each and allocated to one of the following treatments: CON (Control; without leaves), FIC (*Ficus benghalensis* leaves incorporated CFB), ART (*Artocarpus heterophyllus* leaves incorporated CFB) and AZA (*Azadirachta indica* leaves incorporated CFB). The complete feed blocks (CFB) were prepared by incorporating finger millet (*Eleusine coracana*) straw and concentrate in the ratio of 60:40 (DM basis). The nutritional value of wheat bran was reported at par with tropical tree leaves (Bhatta et al., 2014); hence, wheat bran in concentrate was partially replaced (10 parts, w/w basis, 4% of diet) with tropical tree leaves *Ficus benghalensis, Artocarpus heterophyllus* and *Azadirachta indica* in FIC, ART and AZA groups (Table 1), respectively. The finger millet straw was chopped to 2 mm size; while all the ingredients of concentrate were ground and mixed homogenously. The roughage and concentrate were properly mixed in 60:40 (DM basis) and complete feed blocks (2 kg) were prepared using complete feed blocking machine at 4000 psi. The animals were fed for maintenance to satisfy their energy and protein requirement following ICAR (2013) standard. The diets in the form of complete feed block were offered to the animals once per day in the morning (09.00 AM). The sheep were housed in individual pens with a concrete floor and fed with separate feed troughs. Animals had free access to clean water throughout the experimental period. Faeces were collected in faecal bags attached to the sheep. Faeces from each sheep were weighed every morning before feed offer (09.00 AM), and representative samples of faeces were dried daily at 80 °C overnight to determine the dry matter (DM) content. Experimental periods consisted of a 30-d diet adaptation period followed by 8 d of methane collection wherein minimum five successful gas collections (24 h) were ensure from each sheep. Total 30 observations (minimum five and maximum seven) from each group were taken into consideration to arrive on the average enteric methane emission value.

Table 1
Different diets (DM basis) offered to sheep under different groups.

Ingredients	% of diet (DM basis)			
	CON	FIC	ART	AZA
Finger millet straw	60	60	60	60
Maize grain	16	16	16	16
Soybean meal	14	14	14	14
Wheat bran	8.8	4.8	4.8	4.8
Tropical leaves*	0	4.0 (Banyan)	4.0 (Jackfruit)	4.0 (Neem)
Mineral mixture	0.8	0.8	0.8	0.8
Salt	0.4	0.4	0.4	0.4
Total	100	100	100	100

All ingredients were mixed homogenously on DM basis. Tropical tree leaves constituted 10% (DM basis) of the concentrate or 4% of total diet.

2.3. Chemical analysis

To record the dry matter intake and dry matter digestibility; feed, refusals and faecal samples from each sheep were collected during gas collection period (SF_6 experiment) of eight days. Feed, refusals and faecal samples were dried in hot air oven at 80 °C until no further weight loss to determine the dry matter content in samples. The dried feed samples from each animal in triplicate were subject to the following analysis: ash (method 942.05, AOAC, 1990), Kjeldhal N (method 984.13, AOAC, 1990); while aNDFom corrected for neutral detergent fibre (NDF)-ash was determined following standard procedure of Van Soest et al. (1991) using heat stable α-amylase (Sigma Aldirch A3306) at 1 ml per 100 ml of NDF solution. Feed samples were also analyzed for acid detergent fibre (ADF) and acid detergent lignin (ADL) following the procedure of Van Soest et al. (1991). The condensed tannin (CT) in air dried leaves samples was analyzed by butanol-HCl-iron method (Makkar, 2003) and expressed as leucocyanidin equivalents.

2.4. In vivo enteric methane measurement

The sulphur hexafluoride (SF_6) tracer technique (Johnson et al., 1994) was used to measure *in vivo* enteric methane emissions from individual sheep. The preliminary feeding of sheep with control and test diets in different groups was done for one month before conducting SF_6 trial. About 32 mm long brass permeation tubes were used for filling of SF_6 gas (99.9% purity; Chemix specialities gases, Bangalore), and placed at 39 °C for calibration. The tubes were monitored and weighed at weekly intervals to determine the SF_6 release rate. Once the SF_6 release became steady (3.07–4.82 mg/d), the permeation tubes were placed in the rumen.

The halters comprising teflon tube (1/8″ OD), air filter (15 μm; Swagelok USA), capillary tube (0.005″ ID, 1/16″ OD, 100 cm long; Supelco USA) and QC4 quick connects (QC4-B-200 & QC4-D-200, Swagelok USA) were assembled following standard guidelines. The exhaled air (breath) samples were collected for a complete feeding