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Effect of condensed tannins from *Ficus infectoria* and *Psidium guajava* leaf meal mixture on nutrient metabolism, methane emission and performance of lambs

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Objective: The study examined the effect of condensed tannins (CT) containing *Ficus infectoria* and *Psidium guajava* leaf meal mixture (LMM) supplementation on nutrient metabolism, methane emission and performance of lambs.
Methods: Twenty four lambs of ~6 months age (average body weight 10.1±0.60 kg) were randomly divided into 4 dietary treatments (CT-0, CT-1, CT-1.5, and CT-2 containing 0, 1.0, 1.5, and 2.0 percent CT through LMM, respectively) consisting of 6 lambs each in a completely randomized design. All the lambs were offered a basal diet of wheat straw *ad libitum*, oat hay (100 g/d) along with required amount of concentrate mixture to meet their nutrient requirements for a period of 6 months. After 3 months of experimental feeding, a metabolism trial of 6 days duration was conducted on all 24 lambs to determine nutrient digestibility and nitrogen balance. Urinary excretion of purine derivatives and microbial protein synthesis were determined using high performance liquid chromatography. Respiration chamber study was started at the mid of 5th month of experimental feeding trial. Whole energy balance trials were conducted on individual lamb one after the other, in an open circuit respiration calorimeter.
Results: Intake of dry matter and organic matter (g/d) was significantly ($p<0.05$) higher in CT-1.5 than control. Digestibility of various nutrients did not differ irrespective of treatments. Nitrogen retention and microbial nitrogen synthesis (g/d) was significantly ($p<0.01$) higher in CT-1.5 and CT-2 groups relative to CT-0.Total body weight gain (kg) and average daily gain (g) were significantly (linear, $p<0.01$) higher in CT-1.5 followed by CT-1 and CT-0, respectively. Feed conversion ratio (FCR) by lambs was significantly (linear, $p<0.01$) better in CT-1.5 followed by CT-2 and CT-0, respectively. Total wool yield (g; g/d) was linearly ($p<0.05$) higher for CT-1.5 than CT-0. Methane emission was linearly decreased ($p<0.05$) in CT groups and reduction was highest ($p<0.01$) in CT-2 followed by CT-1.5 and CT-1. Methane energy (kcal/d) was linearly decreased ($p<0.05$) in CT groups.
Conclusion: The CT supplementation at 1% to 2% of the diet through *Ficus infectoria* and *Psidium guajava* LMM significantly improved nitrogen metabolism, growth performance, wool yield, FCR and reduced methane emission by lambs.

Keywords: Condensed Tannins; Lambs; Leaf Meal Mixture; Methane Emission

INTRODUCTION

Role of fodder tree leaves in the diet of ruminants is considered predominantly important in countries like India where small holdings and large ruminant densities result in severe problem of feed availability from conventional feed sources [1]. A wide variety of multi-purpose tropical trees grown at the farmers' field, therefore, can be used as supplementary feeds. These tree leaves not only provide a cheap source of nitrogen, energy and micro-nutrients but have also many other advantages like their wide spread on-farm availability and easy accessibility to farmers, their lax-

ative influence on the alimentary system, low degradability of nitrogen in the rumen, and above all the scope of adding variety to the diet [2,3]. The tree leaves can be harvested, sun-dried and used in compounded protein supplements. The concentration of phenolic compounds (particularly tannin) in multipurpose tree leaves is generally high; they may bind to protein thus rendering the protein un-degradable by rumen microbes [2]. Therefore, low levels of condensed tannins (CT) (15 to 40 g/kg dry matter [DM]) are now believed to be beneficial in ruminant diets [4-6]. Farmers usually minimize and overcome the toxic effects by feeding different leaves in mixtures in smaller quantities which not only dilutes and reduces the problem of palatability and toxic effects but also extends feed base for animals [7].

The CT, are a diverse group of poly-phenolics that readily complex with proteins and carbohydrates [8]. Consequently, the tannin-protein reaction has been widely used to improve protein metabolism in ruminants. The CT reduces CH_4 emission by ruminants both *in vitro* and *in vivo*. The mechanism of action of CT on methanogenesis is not completely understood. It has been suggested that depending on type and dose, CT may directly inhibit the growth of methanogens in the rumen [9]. Indirect inhibition of methanogens could occur by decreasing the availability of hydrogen to microorganisms in the rumen [10]. Inhibition of methanogenesis by CT may also result in decreasing the acetate to propionate ratio [11], resulting from an increased transfer of hydrogen to propionate [12]. Another possibility is that CT is hydrogen (H_2) acceptors and reduces the amount of H_2 available in the rumen to form CH_4. The CT from different plant sources may affect CH_4 production in different ways. Varying the concentrations of CT will also affect the amount of CH_4 produced [13], but the greatest concentrations of CT will not always result in the greatest reductions of CH_4 [14]. Dietary supplementation of CT through leaf meal mixture (LMM) may be a possible alternative approach to improve protein bioavailability and reduce CH_4 emission. However, information regarding the effect of CT from LMM on CH_4 emission is scarce. Therefore, potential source and optimum level of CT to be used in the diets to improve protein metabolism and reduce CH_4 emission in growing lambs warrants investigation. Keeping this in view, the present study designed to study the effect of CT from a LMM of *Ficus infectoria* and *Psidium guajava* on nutrient metabolism, CH_4 emission, wool yield and performance of lambs.

MATERIALS AND METHODS

The experiment was carried out at Animal Nutrition Shed of ICAR-Indian Veterinary Research Institute, Izatnagar in Uttar Pradesh province of India. It was planned and conducted as per the prescribed guidelines of Institute Animal Ethics Committee. Before the start of experimental study we got ethical clearance from the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Animals, treatments and management

Twenty four local non descript lambs of ~6 months age, average body weight (BW) 10.1±0.60 kg were randomly divided into four dietary treatments consisting of 6 lambs each in a completely randomized design. The lambs were randomly allocated into four dietary treatments CT-0, CT-1, CT-1.5, and CT-2 containing 0, 1.0, 1.5, and 2.0 percent CT through LMM, respectively. All the lambs were kept under uniform management conditions by housing them in a well ventilated shed with facilities for individual feeding and watering. At the onset of experiment the lambs were treated with broad spectrum anthelmintic (Albomar suspension, Virbac Animal Health Pvt. Limited, Magha Thane, Maharashtra, India) at the rate of 2 mL/10 kg BW before the start of study. After shearing the lambs were treated with butox (Deltamethrin, Hoechst India Limited, Mumbai, India) at the rate of 2 mL/L of water for the control of ectoparasites. During adaptation period, all the lambs were vaccinated against prevalent contagious diseases (peste des petits ruminants, hemorrhagic septicemis, and Sheep pox) to ensure that the animals were in apparently healthy condition, free from any disease.

All the lambs were offered a basal diet of wheat straw *ad libitum*, oat hay (100 g/d) along with required amount of concentrate mixture to meet their nutrient requirements for maintenance and growth as per Kearl [15] for a period of six months. The feeding cum growth trial was conducted for 195 days duration including the first 15 days for adaptation and subsequent 180 days for measurement. Dried and ground LMM of *Ficu sinfectoria* and *Psidium guajava* (70:30) was incorporated in different proportion to the concentrate mixtures of three treatment groups (CT-1, CT-1.5, and CT-2) by replacing concentrate so as to bring CT content to 1.0, 1.5, and 2.0 percent, respectively of the diet. The leaves were harvested in one lot in the month of June and were dried and ground before mixing in the concentrate mixture. The ration schedule was adjusted fortnightly for each lamb as per the body weight changes to meet the nutrient requirements for growth [15].

Measurements

The lambs were individually offered measured quantities of respective concentrate mixtures in the morning (9.00 AM). Wheat straw was offered *ad libitum* along with hundred gram oat hay when all the lambs consumed the concentrate mixtures completely. At the start of experiment, the lambs were weighed for two consecutive days to get their average initial BW. For the growth study, the weight of the individual lambs was recorded at fortnightly intervals in the morning before feeding and watering for 6 months in order to assess the change in body weight and average daily gain (ADG).

After 3 months of experimental feeding, a metabolism trial of 6 days duration was conducted on all 24 lambs in specially designed cages to determine nutrient digestibility, nitrogen balance and yield of microbial-N. In the morning, the residue left,

faeces voided and urine excreted of each animal were collected and weighed and then a uniform representative sample of the residue, faeces and urine were kept in polythene bags and in container, respectively.

A well-mixed representative sample from supplement, LMM, wheat straw and oat hay was kept in previously labelled polythene bags at the time of feeding. Representative sample each from feed and residue was taken in aluminium tray and kept in an oven at 80°C±2°C, for 24 hrs for the estimation of DM content. The total quantity of faeces voided by individual lamb was collected and weighed quantitatively after every 24 h at 9:00 AM. Following thorough and uniform mixing in a clean plastic trough, representative samples were taken to the laboratory for further aliquoting. A suitable aliquot (20%) of daily faecal excretion was taken in previously weighed tray and dried in hot air oven at 80°C±2°C for determining the DM content.

The daily urinary output by individual lamb was collected quantitatively in plastic bottles containing known quantity of 10% sulphuric acid to bring pH around 3.0 and measured with a measuring cylinder. The representative samples were brought to the laboratory in sample bottles. A suitable aliquots (1% v/v), in duplicate, were measured daily into Kjeldahl flasks containing known quantity (40 mL) of laboratory grade sulphuric acid for nitrogen estimation. Another aliquot was taken and kept at –20°C for estimation of purine derivatives (PD).

Urine samples were centrifuged and filtered through a Millipore filter and diluted tenfold with distilled water after adjusting the pH 4.0. A 20 μL volume of the filtrate was injected into the high performance liquid chromatography (HPLC) column. Urine samples were stable for several weeks when stored at –20°C. A fixed aliquot (2%) of fresh faeces was preserved in pre-weighed air-tight glass bottles after adding 10.0 mL sulphuric acid (25%) in each bottle. At the end of collection period, the contents were weighed; mixed thoroughly and 5.0 g sample was taken in a Kjeldahl flask for digestion and estimation of nitrogen in faecal samples.

Respiration chamber study

The respiration chamber study was started at the mid of 5th month of experimental feeding trial. Whole energy balance trials were conducted on individual lamb one after the other (4 lambs from each group), in an open circuit respiration calorimeter developed for small animals and described by Khan and Joshi [16] for sheep and goat which consisted of a wooden chamber with internal dimensions (in meters) 1.5×0.9×1.75 (height). The chamber was maintained at 25°C with relative humidity about 65%. The selected lamb was weighed in the morning prior to feeding and watering and kept in a pre-respiration chamber.

Observations were recorded on two consecutive days for each lamb after proper suitable adaptation period in the respiration chamber. After keeping the feeds inside, the chamber was made air tight by closing the door. Blower was started along with fan and ventilation system of the chamber. The equipment was run for an hr in order to stabilize the recorder. Recording the temperature of dry and wet bulb, flow rate, volume and atmospheric pressure was done manually at one hr interval.

The samples of out-going and in-coming air were collected in Douglas bags separately with continuous sampling device oxygen, methane and carbon-dioxide contents of the samples of the out-going and incoming air from the respiration chamber. The flow rate, dry and wet bulb temperature and atmospheric pressure oxygen, carbon dioxide and methane were also recorded by an infra red gas analyzer (Fuji Electric System Co. Ltd, Tokyo, Japan) with the help of a flow meter having totaliser (Teledyne, Lincoln, NE, USA). The chamber was opened after 24 h the residue of feeds, faeces voided and urine excreted were collected and representative samples were processed for further analysis in the laboratory. The open circuit respiration calorimetry involves the quantification of concentrations of oxygen, carbon dioxide and methane of the respiration chamber.

Wool yield

Wool shearing of all lambs was done at the prior and completion of experiment by hand scissors. The total wool yield was weighed for each lamb and average daily wool yield was calculated (g/d). The wool samples were taken from the second-to-last rib, middle of the lambs. Staple length was measured by metric scale and fibre diameter and total modulation by lanometer. An average of 10 wool fibre taken at random was used as the representative measurement.

Laboratory analyses

Representative samples of feed offered, residues left and faeces were milled to pass through 1 mm sieve and analyzed as per AOAC [17] to determine of DM by oven drying method, organic matter (OM) by muffle furnace incineration, crude protein (CP) by kjeldahl method, ether extract (EE), and ash. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated by the method of VanSoest et al [18]. NDF was assayed with sodium sulphite, in the NDF reagent without alpha-amylase and the results were expressed with residual ash.

Urinary PD viz. Allantoin, uric acid, xanthine, hypoxanthine and creatinine were estimated using HPLC as described Resines et al [19] using pure standards from Sigma Aldrich Limited, New Delhi, India. Microbial nitrogen (MN) production (g/d) was calculated using the equations of Chen and Gomes [20]. The CT content of LMM was estimated by Butanol-HCl method [21]. The estimation of gross energy (cal/g) of feeds, residue, faeces and urine samples was determined by Adiabatic Bomb Calorimeter along with computer operated soft ware (Toshniwal Brothers Pvt. Ltd, New Delhi, India).

Statistical analysis

The experimental data obtained were subjected to analysis of vari-

ance using SPSS 11.0 software and treatment means were ranked using Duncan's multiple range tests. The degree of freedom of the treatments was partitioned into orthogonal polynomial, depicting linear and quadratic trends associated with increasing levels of CT in diets. Significance of treatments with respect to different characters was declared at p<0.05 unless otherwise stated. All the statistical procedure was done as per Snedecor and Cochran [22].

RESULTS

Chemical composition of feeds

The chemical composition (% DM basis) of concentrate mixture, LMM, oat hay and wheat straw offered to lambs for a period of 6 months and during metabolism trial is presented in the Table 1. The CT content of LMM was 10.39 percent.

Nutrients intake, digestibility, and nitrogen retention

The intake of DM and OM (g/d) during the metabolism trial was significantly (p<0.05) higher in CT-1.5 as compared to CT-0, while CT-1 and CT-2 have intermediate position between CT-0 and CT-1.5 groups. However, concentrate, wheat straw, oat hay and total roughage intake (g/d) did not differ significantly (p>0.05) irrespective of dietary treatments. The LMM intake was significantly (p<0.01) higher in CT-2 followed by CT-1.5 and CT-1, respectively. The digestibility coefficients of DM, OM, CP, EE, NDF, and ADF did not differ significantly (p>0.05) irrespective of dietary treatments (Table 2). Total N intake (g/d) did not differ significantly (p>0.05) among dietary treatment by lambs. Faecal excretion of N g/d was significantly (p<0.05) higher by lambs in CT-1.5 as compared to CT-0, however, CT-1 and CT-2 have intermediate position between CT-0 and CT-1.5. The urinary N excretion was linearly (p<0.01) reduced in CT-1.5 and CT-2 groups as compared to control (CT-0) (Table 2). Similarly, urinary N excretion (% of intake) was linearly (p<0.01) reduced in CT supplemented groups, while it was analogous between CT-1.5 and CT-2 groups. N-retention (g/d, % of intake or abs. N) was significantly (linear, p<0.01) higher in CT-1.5 and CT-2 relative to CT-0, however, CT-1 has an intermediary position. The nutrient density (%) of composite diets and intake (g/d) in terms of digestible crude protein (DCP) and total digestible nutrient (TDN) was comparable among dietary treatments.

Urinary excretion of purine derivatives and microbial nitrogen synthesis

The excretion of allantoin, hypoxanthine (linear, p<0.01), total PD (linear, p<0.01) in urine by lambs increased significantly in CT groups as compared to control (CT-0). However, dietary supplementation of CT did not exert any effect on uric acid excretion. The PD:creatinine (C) ratio was significantly higher (p<0.01) in CT-1.5 and CT-2 relative to their counter parts in control group (CT-0) and CT-1 (Table 3). The PD absorption (mmol/d) and MN synthesis in terms of kg^{-1} DOMI and/or DOMR/kg OM digested in the rumen did not differ significantly (p>0.05) irrespective of dietary treatments.

Body weights and lamb performance

The initial body weights of lambs were comparable irrespective of treatments, however, final body weights, were significantly (p<0.05) higher in CT supplemented groups as compared to control. Total weight gain (kg), ADG (g) for a period of 6 months were significantly (linear, p<0.01) higher in CT-1.5 followed by CT-1 and CT-0, respectively, while CT-2 has an intermediate position between CT-1 and CT-1.5 (Table 4). The overall intake (g/d) of concentrate, roughage and DM did not differ significantly (p>0.05) irrespective of dietary treatments. The feed conversion ratio (FCR) by lambs (kg DM/kg gain) was significantly (linear, p<0.01) better in CT-1.5 followed by CT-2 and CT-0, respectively,

Table 1. Ingredient and chemical composition of feeds fed to lambs

Attributes	Wheat straw	Oat hay	Concentrate mixture	Leaf meal mixture
Ingredient composition (%)	-	-		-
Maize	-	-	28.00	-
Wheat bran	-	-	37.00	-
De-oiled soybean meal	-	-	32.00	-
Mineral mixture	-	-	2.00	-
Common salt	-	-	1.00	-
Chemical composition (% DM basis)				
OM	93.15	91.55	90.66	90.13
CP	3.69	7.03	21.21	10.03
EE	0.54	1.65	1.77	3.96
Total ash	6.85	8.45	9.34	9.87
NDF	81.52	62.31	33.60	53.57
ADF	51.62	40.74	9.55	37.75
Gross energy (kcal/kg DM)	3,905.15	4,063.60	4,198.32	4,025.04
Condensed tannins	-	-	-	10.39

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Table 2. Effect of CT supplementation on nutrient intake, utilization and nitrogen balance by lambs during metabolism trial

Attributes	Treatments[1)]				SEM	p values[2)]		
	CT-0	CT-1	CT-1.5	CT-2		C	L	Q
Body weight (kg)	14.95	16.13	17.45	16.83	0.62	0.55	0.23	0.48
Intake (g/d)								
DM	445.33[a]	585.04[ab]	617.95[b]	588.02[ab]	26.84	0.091	0.049	0.100
Concentrate	210.55	205.05	214.48	194.84	7.90	0.851	0.617	0.675
LMM	0.00	58.34[a]	95.40[b]	122.99[b]	10.09	0.851	0.617	0.675
Roughage[3)]	234.78	321.65	308.08	270.20	15.78	0.203	0.501	0.052
Nutrient digestibility (%)								
DM	52.84	51.39	52.02	51.09	0.91	0.923	0.601	0.893
OM	56.17	54.62	54.78	53.35	0.85	0.741	0.310	0.972
CP	68.29	67.87	67.76	67.53	1.31	0.99	0.85	0.97
EE	67.14	67.14	73.17	73.85	1.56	0.25	0.07	0.91
NDF	43.87	44.61	44.06	42.77	0.85	0.90	0.64	0.58
ADF	29.03	30.86	30.53	31.82	1.14	0.87	0.46	0.91
Nitrogen balance (g/d)								
Intake	9.16	10.38	11.25	10.98	0.40	0.26	0.08	0.35
Faecal excretion	2.74[a]	3.30[ab]	3.57[b]	3.41[ab]	0.13	0.11	0.04	0.14
Urinary excretion	3.92[b]	3.73[b]	3.12[a]	3.05[a]	0.14	0.05	0.01	0.814
Balance (g/d)	2.51[a]	3.36[ab]	4.57[b]	4.53[b]	0.29	0.02	0.00	0.37
Nitrogen excretion (% intake)								
Faecal	37.71	32.13	32.76	31.15	1.30	0.99	0.87	0.85
Urine	43.22[c]	36.66[b]	27.80[a]	27.78[a]	1.62	0.00	0.00	0.10
Retention (%)								
N intake	25.07[a]	31.21[ab]	40.44[b]	41.07[b]	2.21	0.02	0.00	0.47
N absorbed	33.70[a]	45.71[ab]	59.25[b]	59.65[b]	3.20	0.00	0.00	0.25

CT, condensed tannins; SEM, standard error of the mean; DM, dry matter; LMM, leaf meal mixture; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre.
[1)] CT-0, 0% CT; CT-1, 1% CT; CT-1.5, 1.5% CT; CT-2, 2% CT of diet. [2)] C, combined; L, linear; Q, quadratic. [3)] Roughage: Oat hay at 94.89 g/d DM+wheat straw.
[ab] Mean values with different superscripts with in a row differ significantly.

while CT-1 has an intermediate position between CT-0 and CT-2.

Methane emission

The intake of DM and digestible OM (DOMI; g/d) during respiration study was comparable irrespective dietary treatments. The emission of CH_4 (L/d, L/kg DMI or DOMI) was linearly

Table 3. Urinary excretion of purine derivatives and microbial nitrogen supply to lambs

Attributes	Treatments[1)]				SEM	p values[2)]		
	CT-0	CT-1	CT-1.5	CT-2		C	L	Q
Urinary excretion of PD (mmol/d)								
Allantoin	3.36[a]	4.42[b]	4.54[b]	4.55[b]	0.15	0.005	0.003	0.039
Uric acid	0.07	0.10	0.10	0.19	0.02	0.186	0.055	0.447
Xanthine	0.12[ab]	0.08[a]	0.21[bc]	0.26[c]	0.02	0.021	0.008	0.277
Hypoxanthine	0.06[a]	0.08[a]	0.14[a]	0.28[b]	0.03	0.009	0.002	0.176
Total	3.62[a]	4.79[b]	4.99[b]	5.27[b]	0.16	0.000	0.000	0.044
Creatinine	2.36[b]	2.35[b]	1.40[a]	1.62[a]	0.13	0.003	0.002	0.559
PD:C	1.67[a]	2.09[a]	3.73[b]	3.55[b]	0.25	0.001	0.000	0.408
PDA (mmol/d)	3.78[a]	5.32[b]	5.56[b]	5.93[b]	0.21	0.000	0.000	0.045
MN (g N/d)	2.75[a]	3.87[b]	4.04[b]	4.31[b]	0.15	0.000	0.000	0.045
Efficiency of microbial N synthesis								
g N/kg DOMI	12.50	13.28	13.48	15.52	0.68	0.468	0.147	0.653
g N/kg DOMR	16.66	17.71	17.97	20.69	0.91	0.468	0.147	0.653

CT, condensed tannins; SEM, standard error of the mean; PD, purine derivatives; C, creatinine; PDA, absorbable purine derivatives; MN, microbial nitrogen; DOMI, digestible organic matter intake; DOMR, digestible organic matter in rumen.
[1)] CT-0, 0% CT; CT-1, 1% CT; CT-1.5, 1.5% CT; CT-2, 2% CT of diet. [2)] C, combined; L, linear; Q, quadratic.
[abc] Mean values with different superscripts with in a row differ significantly.

Table 4. Effect of CT on growth rate, feed conversion ratio, wool yield and quality and plane of nutrition during 180 days experimental feeding

Attributes	Treatments[1]				SEM	p values[2]		
	CT-0	CT-1	CT-1.5	CT-2		C	L	Q
Body weights (kg)								
Initial	10.10	10.08	10.09	10.01	0.28	1.00	0.93	0.96
Final	18.46[a]	20.08[b]	23.15[b]	20.91[b]	0.63	0.050	0.049	0.097
Total gain (kg)	8.36[a]	10.00[b]	13.05[c]	10.89[bc]	0.51	0.004	0.007	0.026
ADG (g)	46.43[a]	55.55[b]	72.55[c]	60.52[bc]	2.84	0.004	0.007	0.0026
Total DMI (g/d)	391.56	472.64	499.26	480.95	19.89	0.232	0.100	0.209
Feed conversion ratio	9.29[c]	8.88[bc]	6.90[a]	8.12[b]	0.24	0.001	0.001	0.020
Wool yield and quality								
Total yield (g)	507.00[a]	652.50[ab]	704.00[b]	679.00[ab]	31.61	0.111	0.042	0.161
Yield (g/d)	2.82[a]	3.63[ab]	3.91[b]	3.77[ab]	0.18	0.110	0.042	0.159
Staple length (cm)	7.27	7.82	9.17	8.47	0.31	0.160	0.077	0.304
Fibre diameter (μm)	38.84	38.09	38.41	38.66	1.30	0.998	0.986	0.859
Total medullation (%)	54.69	56.84	67.70	59.39	2.74	0.375	0.318	0.348
Nutrient density (%)								
DCP	8.81	7.53	7.83	8.03	0.24	0.289	0.344	0.133
TDN	54.80	53.92	53.37	51.61	0.85	0.628	0.211	0.803
Nutrient intake (g/d)								
DCP	40.13	44.31	47.70	46.31	1.97	0.579	0.237	0.497
TDN	245.64	313.50	328.09	300.85	13.41	0.139	0.125	0.073

CT, condensed tannins; SEM, standard error of the mean; ADG, average daily gain; DMI, dry matter intake; DCP, digestible crude protein; TDN, total digestible nutrient.
[1] CT-0, 0% CT; CT-1, 1% CT; CT-1.5, 1.5% CT; CT-2, 2% CT of diet. [2] C, combined; L, linear; Q, quadratic.
[abc] Mean values with different superscripts with in a row differ significantly.

reduced (p<0.07; p<0.05) in CT groups relative to control (CT-0). The reduction (%) in CH_4 emission by lambs due to CT supplementation was highest (linear and quadratic, p<0.01) in CT-2, followed by CT-1.5 and CT-1. The CH_4 energy (mE; kcal/d) was linearly reduced (p<0.05) in CT-1.5 and CT-2 groups than CT-0, however, CT-1 has an intermediary position (Table 5). The heat production (kcal/d) was comparable irrespective of dietary treatments.

Wool growth and quality

Total wool yield (g) for the period of 6 months and yield (g/d) were (p<0.05) significantly higher for the CT-1.5 as compared to CT-0, however, CT-1 and CT-2 have an intermediate values between CT-0 and CT-1.5. The wool quality in terms of staple length (cm), fibre diameter (μm) and total medullation (%) was found to be comparable in lambs irrespective of dietary treatments (Table 4).

DISCUSSION

Chemical composition of feeds

The chemical composition of concentrate mixture, LMM, oat hay and wheat straw used in the experiment was comparable with the values reported by many workers [2,3]. The concentration

Table 5. Effect of condensed tannins supplementation on methane emission of lambs during respiration chamber study

Attributes	Treatment[1]				SEM	p values[2]		
	CT-0	CT-2	CT-1	CT-1.5		C	L	Q
DMI (g/d)	561.13	608.15	686.83	577.32	22.59	0.208	0.512	0.088
DOMI (g/d)	288.61	304.93	339.50	290.30	10.35	0.308	0.672	0.135
CH_4 emission								
L/d	9.90	9.02	8.48	7.28	0.52	0.373	0.072	0.877
% reduction	0.00[a]	8.89[b]	14.35[c]	26.48[d]	2.48	0.000	0.000	0.000
L/kg DMI	17.65[b]	15.16[ab]	12.20[a]	12.66[a]	0.87	0.082	0.020	0.340
L/kg DOMI	34.29[b]	30.34[ab]	24.71[a]	25.15[a]	1.73	0.149	0.035	0.495
CH_4 energy (kcal/d)	93.57[b]	85.24[ab]	80.14[a]	68.80[a]	4.92	0.023	0.029	0.379
Heat production (kcal/d)	636.37	627.58	616.26	579.59	31.38	0.940	0.567	0.843

CT, condensed tannins; SEM, standard error of the mean; DMI, dry matter intake; DOMI, digestible organic matter intake.
[1] CT-0, 0% CT; CT-1: 1% CT; CT-1.5: 1.5% CT; CT-2: 2% CT of diet. [2] C, combined; L, linear; Q, quadratic.
[abcd] Mean values with different superscripts with in a row differ significantly.

of NDF and ADF was higher in LMM as compared to concentrate mixture this could be attributed to the high cell wall constituents usually present in leaf meal [2,3]. The LMM was found to be a good source of calcium.

Nutrient intake, digestibility and N retention

The intake of DM (58.60 to 72.70 g DM/d/kg $W^{0.75}$) by lambs was within the normal range [15] and this clearly indicates that all the experimental diets were palatable. Similar to the present results, higher voluntary feed intake at moderate (1% to 4%) CT containing diets was reported by many workers [2,3,23]. The digestibility coefficients of DM, OM, CP, EE, NDF, and ADF did not differ significantly ($p<0.05$) irrespective of dietary treatments. The results of present study are in consistency with earlier workers [2,24,25], who reported that tannins are beneficial to ruminants at low concentration because they protect plant proteins from degradation in the rumen. Contrary to above reports Ndluvo [26] observed that dietary effects of tannins are related with their ability to bind with proteins (dietary and enzymes), structural carbohydrate polymers found in plant cell walls and minerals with an overall effect of lowering the bioavailability of nutrients at specific sites in the gastrointestinal tract. In the present study the supplementation of CT at 1% to 2% of diets does not seems to interfere with the total tract digestibility of nutrients. The comparable nutrient density (%) of diets and intake (g/d) in terms of DCP and TDN in lambs irrespective of treatments suggests that plane of nutrition was not affected adversely with CT supplementation are in conformity with earlier reports [2,27,28]. The absence of any detectable adverse effect on the health of experimental animals suggests that lambs were on balanced diets with no apparent deleterious consequences.

Irrespective of dietary treatments all the lambs had positive N balance indicating satisfactory nutritional level of lambs. Though, CT supplemented diets significantly influenced N utilization and improved N retention. An additional feature of N utilization as evident by significantly higher N retention as percent absorbed N (an indicator of availability of amino acids-N at tissue level) in animals given CT protected diets was apparently due to better amino acid availability and apparent biological value of CT protected diets [29,30]. This examination is further authenticated by the fact that lambs given CT treated diets exerted significantly lower N in urine as percent intake relative to control lambs. A reasonable concentration of CT (20 to 35 g/kg DM) in forage given to sheep has been reported to increase non ammonia nitrogen flux to the small intestine, to increase the absorption of essential amino acids [27,28,31]. Similar to the present study, increased N retention in sheep and goats given tanniferous feeds at modest levels due to lowered N excretion through urine has been reported earlier by several workers [2,3,28]. The CT protein complex protects the dietary protein from microbial hydrolysis and deamination in the rumen and increases the proportion of dietary amino acids by-passed the rumen.

Urinary excretion of purine derivatives and microbial nitrogen synthesis

The urinary excretion of PD is directly related to microbial purine absorption and increasingly being used as a well accepted indicator for estimating microbial protein synthesis in the rumen [32]. In the present study, the major amount of allantoin and the minor amount of uric acid, xanthine and hypoxanthine excreted in the urine of growing lambs were consistent with the results of Chen and Gomes [20]. The results are in conformity with the earlier reports that the presence of CT has a potentially beneficial effect to protein nutrition of the host animal by altering partitioning of nutrients towards higher microbial yield rather than short chain fatty acids [33]. Absence of any depressing effect of CT supplementation on rumen microbial protein synthesis agrees with the observations recorded earlier in sheep fed tannin containing Acacia pods and *Ficus infectoria* [2,34]. The findings suggest the possibility of using LMM as CT source in practical diets by increasing MN supply.

Live weight changes and lamb performance

The voluntary feed intake (g/d) during 180 days growth trial did not differ significantly ($p<0.05$) irrespective of dietary treatments, indicating no adverse effect of CT from LMM on intake of DM by lambs during entire growth trial. The encouraging response on total weight gain (kg), ADG (g) and FCR at 1% to 2% of CT in the diets gives an indication that the binding effect of CT was pronounced at this level by supplying protein to the lower gut and subsequently its more efficient use for tissue growth [2,34,35]. At suitable concentration, the CT reduced the degradation of sulphur amino acids in the rumen, increases the irreversible loss of cystine from plasma and increased the flow of cystine to body synthetic reaction [25,36] and thereby could improve the performance of lambs.

Methane emission

The percentage reduction in methane emission by lambs due to dietary supplementation of CT was highest ($p<0.001$) in CT-2 (26.48), followed by CT-1.5 (14.35) and CT-1 (8.89). Plants rich in CT have been shown to reduce rumen methanogenesis in sheep and cattle [37,38] probably due to both directly through an inhibition of the growth of methanogens and indirectly through a reduction in fiber digestion, which decreases H_2 production [39].

The results are in agreement with the findings of Woodward et al [38], who reported 24% to 29% decrease in CH_4 emission in CT containing forages (*Lotus pedunculatus*) fed sheep when expressed as relative to digestible dry matter intake. A similar decrease (23%) in CH_4 emission relative to DMI was also observed by the same authors when cows were fed *L. corniculatus* silage as compared to ryegrass silage. Supplementation of forages containing CT in the diet of ruminants inhibited CH_4 emission by about 15% [40]. Similarly, CH_4 emission was reduced signifi-

cantly by including tanniferous legumes [41].

Wool growth

It has been suggested that positive response to wool growth and N retention in lambs fed CT containing diets may be the result of an increase in the irreversible loss rate of cystine from blood plasma, mainly due to reducing the loss of sulphur containing amino acids (SAA) in the rumen [25]. Part of the increase in fleece weight in the present experiment in CT supplemented group could be due to CT increasing the absorption of SAA and also that of all other essential amino acids [42].This is similar to earlier observations that CT in diets increased the efficiency of wool production [2,23]. The CT supplementation in the present study had no significant effect on wool quality in terms of staple length (cm), fibre diameter (μm) and total medullation (%) similar to the earlier reports [2], however, contrary to some reports describing beneficial effect of CT on these parameters in grazing sheep [23,42]. Further, in the present study local non-descript lambs were used and they are known for mediocre (carpet) quality wool production.

CONCLUSION

On the basis of the above results, it may be concluded that CT supplementation at 1% to 2% of the diet through tanniferous tree LMM (*Ficus infectoria* and *Psidium guajava*) significantly improved nitrogen metabolism, growth performance, wool yield, FCR and reduced enteric methane emission by lambs. Therefore, CT containing tanniferous tree LMM may be used as a possible alternative functional feed resource for lambs.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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