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Influence of Condensed tannins Supplementation through *Ficus infectoria* and *Psidium guajava* Leaf Meal Mixture on Nutrient Intake and Clinical Chemistry in Lambs

A. K. Pathak[1,2]*, Narayan Dutta[1], A. K. Pattanaik[1] and K. Sharma[1]

[1]*Centre of Advanced Faculty Training in Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, INDIA*
Division of Animal Nutrition, F.V.Sc. & A.H., SKUAST-J, R.S. Pura, Jammu, INDIA

**Corresponding Author: Ak Pathak; Email:* dranand_pathak@Yahoo.com

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ABSTRACT

This study examined the effect of condensed tannins (CT) through *Ficus infectoria* and *Psidium guajava* leaf meal mixture (LMM) on nutrient intake, calcium and phosphorus retention and clinical chemistry in lambs. Twenty four non-descript lambs 6 months of age with average body weight 10.07±0.59 kg were randomly divided into 4 dietary treatments (CT-0, CT-1, CT-1.5 and CT-2) consisting of six lambs in each in a completely randomized block design. Blood-biochemical profile was monitored at 45 days intervals. The intake (g kg^{-1} $W^{0.75}$) of dry matter and organic matter were significantly ($P<0.05$) lower in control (CT-0) as compared to CT supplemented groups. LMM was given to lambs to supply CT @ 1, 1.5 and 2% in CT-1, CT-1.5 and CT-2, respectively. The Hb and PCV levels were highest ($P<0.05$) in CT-1.5 followed by CT-1, CT-2 and CT-0, respectively. CT Supplementation significantly ($P<0.01$) reduced serum urea level in CT-1, CT-1.5 and CT-2 groups as compared to CT-0. Serum proteins differed significantly ($P<0.05$) among various dietary treatments. Serum glucose, calcium, phosphorus, cholesterol, triglycerides and creatinine levels were comparable among all treatment groups. Similarly, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase did not differ significantly among dietary treatments. Alkaline phosphates was significantly ($P<0.05$) higher in CT-1.5 as compared to CT-2, however, CT-1 and CT-2 have intermediate values between CT-1.5 and CT-0. It may be concluded that dietary supplementation of CT at moderate level (1-2 %) did not exert any adverse effect on blood chemistry; however, some blood parameters and nutrient intake was improved considerably.

Keywords: Clinical chemistry, condensed tannins, lambs, leaf meal mixture, nutrient intake, retention

Ruminant animals play a significant role in conversion of low quality plant materials into high quality protein rich food besides playing greater role in conserving fertility of soil, through organic manure. However, ruminant production in mixed farming has lagged behind in productivity because of factors like poor genetic potential, poor health management and imbalance feeding (Singh *et al.* 1997). Poor nutrition results in low rates of production, reproduction as well as increased susceptibility of livestock to diseases. In this regard, tropical tanniferous tree leaves or leaf meal mixture (LMM) may be used as functional feed in the ration of small ruminants. Tree leaves are a component of most natural pastures for small ruminant diets because they are rich in protein, soluble carbohydrates, minerals, vitamins and natural antioxidants (Dubey *et al*. 2012; Pathak *et al*. 2015). Most species of plants seem to be competent of synthesizing plant secondary metabolites. Plants with potent bioactive compounds are often characterized as both poisonous and medicinal, and a beneficial or an adverse effect may depend on the quantity eaten by the animal. Some tropical tree leaves with bioactive compounds (tannins, saponins, essential oils, or other aromatic compounds) are usually considered as advantageous (Scalbert, 1991; Chung *et al*. 1998; Pathak, 2013).

Condensed tannins (CT) are a diverse group of polymeric flavanoids that readily complex with carbohydrates and proteins (Hagerman, 1992). Consequently, the tannin-protein reaction has been widely used to improve protein metabolism in ruminants (Aerts *et al*. 1999). The CT have been associated with decreased palatability and with reduced gut wall permeability (Kumar and Baithiyanathan, 1990), however, the palatability of browse species is closely related to the concentration of CT. There appears to be a threshold of CT contents (about 5%) below which no adverse effect is observed (Wang *et al*. 1996a, b; Terrill *et al*. 1992). Some positive effects have been reported in growing animals fed CT containing diets (Dey *et al*. 2008). Moderate levels (1-4%) of CT markedly reduce rumen degradation of soluble proteins and increase absorption of methionine and a range of essential amino acids from the small intestine, thereby improve the performance of animals (Waghorn *et al*. 1987; McNabb *et al*. 1993).

In this regard, current research is now directed towards finding naturally occurring CT from tropical tree leaves, which provide a potentially important, socio-economical, eco-friendly functional feed to improve the performance of lambs. Keeping this in view, the present study was planned to assess the effect of dietary supplementation of CT through *Ficus infectoria* and *Psidium guajava* LMM on nutrient intake, Calcium and Phosphorus retention and blood chemistry of lambs.

MATERIALS AND METHODS

Experimental animals, feeding and management

Twenty four non-descript lambs of about 6 months age were randomly divided into four groups consisting six lambs in each in a completely randomized block design (CRD). The animals were randomly allocated into 4 dietary treatments CT-0, CT-1, CT-1.5 and CT-2 containing 0, 1, 1.5 and 2% CT, respectively. All the experimental lambs were offered a basal diet of wheat straw *ad libitum* along with required amount of concentrate mixture (crushed maize: 28.0, wheat bran: 37.0, deoiled soybean meal: 32.0, mineral mixture: 2.0 and common salt: 1.0%) and the ration schedule was changed every fortnight after recording the body weights of each lamb to meet their nutrient requirements for maintenance and growth as per Kearl (1982) for a period of 180 days. One hundred gram oat hay was given to each lamb per day to meet their vitamin-A requirement. Dried and ground LMM of *F. infectoria* and *P. guajava* (70: 30) was incorporated in different proportion to the concentrate mixtures of three treatment groups by replacement of concentrate so as to bring CT content to 1.0, 1.5 and 2.0 percent of diet. All experimental lambs were kept under uniform managemental conditions by housing them in a well-ventilated shed with facilities for individual feeding and watering. At the start of the experiment, the lambs were weighed for two consecutive days to get their average initial body weight. For growth study, the weight of the individual lamb was recorded at fortnightly intervals in the morning before feeding and watering for six months in order to assess the change in body weight. A metabolism trial of 6 days duration was conducted in the middle of feeding trial on 24 lambs in specially designed cages to determine plane of nutrition and nutrient balances.

Nutritional analysis

Sample of concentrate mixture, LMM, oat hay and wheat straw were analyzed for proximate principles as per the methods described of AOAC (2000), and fibre fractions were analyzed as per Van Soest *et al*. (1991). The calcium (Ca) content in the feed, residues, faeces and urine samples was determined by the precipitation method as per Talpatra *et al*. (1940), however, phosphorus (P) content of the feeds, residue, faeces and urine samples was determined spectrophotometrically adopting the metavandate method (AOAC, 2000). The CT content was estimated as per the method of Makkar (2000).

Clinical chemistry

Blood samples from all experimental lambs were collected early in the morning before feeding and watering at 0, 45, 90, 135 and 180 days of post feeding aseptically by jugular vein puncture. About 5 ml of whole blood was collected from every animal, from that 1 ml was added with ethylene diamine tetra acetic acid (EDTA) for determination of haemoglobin (Hb) by cyanomethemoglobin method (Dacie and Lewis, 1975) and packed cell volume (PCV) by capillary microhaematocrit method, while 4 ml was collected in centrifuge tubes without EDTA for collection of serum to perform various biochemical tests.

Serum glucose (mg dl^{-1}) was estimated as per method described by Barham and Trinder (1972) and serum urea (mg dl^{-1}) level as per the method described by Hallett and Cook, 1971). Total protein and albumin concentrations (g dl^{-1}) were determined by method of Vatzidis (1977) and Doumas *et al* (1971), respectively. Globulin was determined by the difference between total protein and albumin concentration in the serum. Serum creatinine, cholesterol and triglycerides (mgdl^{-1}) were estimated as per method described by Newman and Price (1999), Richmond (1973) and Cole *et al.* (1997), respectively. Serum enzyme like alanine amino transferase (ALT) and aspartate amino transferase (AST) activities (IUl^{-1}) were determined as per the modified method described by Thefeld *et al* (1974), whereas, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated as per the methods of Bretaudiere *et al*. (1977) and Wroblewski and Duean (1955), respectively. The serum Ca and inorganic - P levels (mg dl^{-1}) were determined by Morgan *et al*. (1993) and Thomas (1998), respectively.

Statistical analyses

The results obtained were subjected to analysis of variance and treatment means were ranked using Duncan's multiple range test. The periodic alterations in clinical chemistry were analyzed using repeated measures design. Significance of treatments was declared at P<0.05 unless otherwise stated. All the statistical procedures were done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Chemical composition of feed

The chemical composition of concentrate mixture, LMM, oat hay and wheat straw offered to lambs during the feeding trial of 180 days is presented in table 1. 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 (Patra *et al*. 2006; Dey *et al*. 2008; Pathak *et al*. 2013). The concentration of NDF and ADF was higher in LMM as compared to concentrate this could be attributed to the high cell-wall constituents usually present in LMM.

Nutrient intake and growth

Daily nutrient intake by lambs under different dietary treatments is given in the table 2. The intakes of DM and OM (g kg^{-1} W$^{0.75}$) were significantly higher (P<0.05) in CT supplemented groups as compared to control, while concentrate intake (g kg^{-1} W$^{0.75}$) was significantly higher in control group than CT supplemented groups. The roughage intake (g kg^{-1} W$^{0.75}$) was significantly (quadratic, P<0.05) higher in CT-1 as compared to CT-0, while CT-1.5 and CT-2 have an intermediate position between CT-0 and CT-1 groups. The LMM was given to lambs only in CT groups. The intake of DM (58.6-72.7 g DM d^{-1} kg W$^{0.75}$) by lambs was within the normal range (Kearl, 1982) and this clearly indicates that all the experimental diets were palatable.

Table 1. Chemical composition of feeds

Attributes	WS	OH	CM	LMM
Chemical composition (g kg^{-1}DM)				
Organic matter	931.50	915.50	906.60	901.30
Crude protein	36.90	70.30	212.10	100.30
Ether extract	5.40	16.50	17.70	39.60
Total ash	68.50	84.50	93.40	98.70
Calcium	2.60	4.00	10.70	19.60
Phosphorus	1.20	3.40	7.60	2.20
Neutral detergent fibre	815.20	623.10	336.00	535.70
Acid detergent fibre	516.20	407.40	95.50	377.50
Condensed tannins	-	-	-	103.90

WS: Wheat straw; OH: Oat hay; CM: Concentrate mixture; LMM: Leaf meal mixture.

Similar to the present study, higher voluntary intake at moderate (1-4%) CT containing diets was reported by many workers (Terrill *et al*., 1992; Ramirez-Restrepo *et al*. 2004; Dey *et al*., 2008; Pathak *et al*., 2013). Daily intake of DOM and TDN (kg^{-1} W$^{0.75}$) was significantly lower (P= 0.13, P=0.012) in CT-0 as compared to CT-1 and CT-1.5. DCP intake did not differ significantly (P<0.05) irrespective of dietary treatments. The findings suggest that plane of nutrition was not affected with CT supplementation in conformity with the earlier reports (Terrill *et al*. 1992; Waghorn *et al*. 1994 a, b; Dey *et al*. 2008). The palatability of browse species was closely related to the level of CT. There appears to be a threshold of CT levels (around 5%) below which no adverse effect was noticeable on nutrient metabolism.

The fortnightly body weight changes by lambs under different dietary treatments are depicted in Fig 1. Initial body weights of lambs did not differ significantly (P<0.05) irrespective of dietary treatments, however,

Table 2: Effect of CT supplementation on nutrient intake by lambs

Attributes	Treatments				SEM	P values*		
	CT-0	CT-1	CT-1.5	CT-2		C	L	Q
Metabolic body size (kgW$^{0.75}$)	7.56	8.02	8.52	8.30	0.23	0.53	0.22	0.48
Feed intake (g d^{-1}kg^{-1} W$^{0.75}$)								
Concentrate	27.46 ^{b}	25.37^{a}	25.11 ^{a}	23.43 ^{a}	0.42	0.003	0.000	0.754
LMM	0.00	7.24	11.12	14.70	1.16	0.000	0.000	0.000
Oat hay	13.05	12.10	11.24	11.55	0.42	0.467	0.172	0.465
Wheat straw	18.11	28.03	24.41	20.45	1.60	0.128	0.801	0.031
Roughage	31.15 ^{a}	40.13 ^{b}	35.66^{ab}	32.01^{ab}	1.44	0.101	0.874	0.028
Nutrient intake (gkg^{-1} W$^{0.75}$)								
Dry matter	58.61^{a}	72.74 ^{b}	71.89 ^{b}	70.14 ^{b}	1.90	0.017	0.027	0.021
Organic matter	54.48^{a}	68.35^{b}	66.67 ^{b}	64.60 ^{b}	1.75	0.013	0.038	0.012
Crude protein	7.51 ^{a}	8.06 ^{b}	8.22 ^{b}	8.24 ^{b}	0.10	0.028	0.008	0.148
DCP	5.16	5.48	5.57	5.56	0.13	0.685	0.298	0.559
DOM	30.55 ^{a}	37.29 ^{b}	36.34 ^{b}	34.24 ^{ab}	0.84	0.013	0.120	0.005
TDN	32.08^{a}	39.16 ^{b}	38.16 ^{b}	35.95 ^{ab}	0.89	0.013	0.121	0.005

^{abc} Mean values with different superscripts with in a row differ significantly; DOM: Digestible organic matter; DCP: Digestible crude protein; TDN: Total digestible nutrients

*C: combined; L: linear; Q: quadratic

Table 3. Effect of condensed tannins supplementation on nutrient retention by lambs

Attributes	Treatments				SEM	P values*		
	CT-0	CT-1	CT-1.5	CT-2		C	L	Q
Calcium retention (g d^{-1})								
Intake	3.29^{a}	4.15^{ab}	4.90^{b}	5.02^{b}	0.22	0.009	0.001	0.315
F excretion	1.85^{a}	2.31^{ab}	2.60^{bc}	2.93^{c}	0.11	0.001	0.000	0.708
U excretion	0.06	0.06	0.06	0.04	0.01	0.176	0.191	0.079
T excretion	1.91^{a}	2.37^{ab}	2.66^{bc}	2.97^{c}	0.11	0.001	0.000	0.623
Balance	1.38^{a}	1.77^{ab}	2.24^{b}	2.05^{ab}	0.13	0.089	0.029	0.235
Phosphorus retention (g d^{-1})								
Intake	2.34	2.31	2.30	2.31	0.08	0.950	0.989	0.787
F excretion	1.32	1.32	1.38	1.32	0.05	0.969	0.937	0.764
U excretion	0.08^{b}	0.02^{a}	0.06^{b}	0.06^{b}	0.008	0.005	0.553	0.011
T excretion	1.40	1.35	1.43	1.37	0.05	0.943	1.000	0.937
Balance	0.94	0.96	0.99	0.93	0.04	0.957	1.000	0.629

^{abc} Mean values with different superscripts with in a row differ significantly

*C: combined; L: linear; Q: quadratic; F: Faecal; U: Urinary; T: Total

Table 4. Effect of condensed tannins supplementation on serum proteins in lambs

Attributes	Periods					TM±PSE	P values**		
	I	II	III	IV	V		T	P	T× P
Total protein (g dl^{-1})									
CT-0	5.67	5.22	4.91	4.84	4.54	5.04^{a}±0.11	0.000	0.027	0.003
CT-1	5.64	6.17	5.91	6.31	5.96	6.00^{b}±0.09			
CT-1.5	5.89	6.31	6.34	6.57	6.32	6.28^{c}±0.08			
CT-2	5.77	6.59	6.28	6.39	6.03	6.21^{ab}±0.10			
PM ± SE	5.74^{a} ±0.13	6.07^{b} ±0.14	5.86^{ab} ±0.13	6.03^{b} ±0.17	5.71^{a} ±0.1				
Albumin (gdl^{-1})									
CT-0	2.72	3.01	2.57	2.45	2.20	2.59^{a}±0.08	0.000	0.038	0.000
CT-1	2.55	2.77	3.27	3.56	3.31	3.09^{c}±0.09			
CT-1.5	2.84	2.75	2.77	2.86	2.94	2.83^{b}±0.07			
CT-2	2.48	2.75	2.97	3.15	2.92	2.85^{b}±0.08			
PM ± SE	2.65^{a} ±0.09	2.82^{ab} ±0.06	2.89^{b} ±0.09	3.00^{b} ±0.11	2.84^{ab} ±0.12				
Globulin (g dl^{-1})									
CT-0	2.95	2.21	2.35	2.40	2.34	2.45^{a}±0.10	0.000	0.169	0.079
CT-1	3.09	3.40	2.64	2.76	2.66	2.91^{b}±0.10			
CT-1.5	3.05	3.56	3.58	3.71	3.37	3.45^{c}±0.09			
CT-2	3.28	3.85	3.31	3.25	3.11	3.36^{c}±0.12			
PM ± SE	3.09^{ab} ±0.12	3.25^{b} ±0.17	3.09^{ab} ±0.19	3.03^{ab} ±0.15	2.87^{a} ±0.14				
A:G ratio									
CT-0	0.93	1.51	1.23	1.06	0.99	1.14^{b}±0.09	0.000	0.285	0.011
CT-1	0.88	0.83	1.25	1.34	1.36	1.13^{b}±0.07			
CT-1.5	0.95	0.82	0.79	0.77	0.89	0.84^{a}±0.04			
CT-2	0.79	0.72	0.93	1.06	1.00	0.90^{a}±0.05			
PM ± SE	0.89 ±0.05	0.97 ±0.09	1.05 ±0.07	1.06 ±0.07	1.06 ±0.08				

^{abc}Mean values with different superscripts with in a row differ significantly

PM ± SE: Pooled means± Standard errors; TM±PSE: Treatment means ± Pooled standard errors

final body weights were significantly (P<0.05) higher in CT supplemented groups as compared to control. The encouraging results of higher body weight (kg) at 1-2% 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 (Dey *et al*. 2008). At appropriate 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 (McNabb *et al*. 1993; Wang *et al*. 1994) and thereby could improve the performance of lambs.

Table 5. Effect of condensed tannins supplementation on biochemical profile in lambs

Treatment	Periods					TM±PSE	P values		
	I	II	III	IV	V		T	P	T × P
Calcium (mg dl^{-1})									
CT-0	10.40	10.57	10.08	9.98	9.69	10.14±0.14	0.454	0.250	0.915
CT-1	9.95	10.54	10.33	10.41	10.02	10.25±0.16			
CT-1.5	10.02	10.67	10.70	10.62	10.30	10.46±0.13			
CT-2	10.28	10.28	10.20	10.43	10.14	10.26±0.13			
PM ± SE	10.16 ±0.15	10.51 ±0.16	10.33 ±0.22	10.36 ±0.12	10.04 ±0.08				
Phosphorus (mg dl^{-1})									
CT-0	5.96	5.98	5.89	5.49	5.18	5.70±0.12	0.897	0.130	0.995
CT-1	5.68	5.79	5.48	5.72	5.29	5.59±0.13			
CT-1.5	5.83	5.70	5.56	5.71	5.44	5.65±0.12			
CT-2	5.72	5.85	5.82	5.82	5.40	5.72±0.14			
PM ± SE	5.80 ±0.13	5.83 ±0.13	5.69 ±0.16	5.68 ±0.16	5.33 ±0.11				
Cholesterol (mg dl^{-1})									
CT-0	38.91	43.88	38.58	59.47	56.80	47.53±2.30	0.215	0.000	0.928
CT-1	38.82	42.23	46.71	62.47	65.13	51.07±2.43			
CT-1.5	39.24	42.06	48.62	64.00	65.16	51.82±2.38			
CT-2	38.74	42.82	46.09	61.45	63.11	50.44±2.32			
PM ± SE	38.93^{a} ±1.77	42.75^{ab} ±1.62	45.00^{b} ±1.85	61.84^{c} ±1.58	62.55^{c} ±1.50				
Triglycerides (mg dl^{-1})									
CT-0	9.62	10.11	10.68	10.65	9.50	10.11±0.51	0.576	0.117	0.980
CT-1	9.58	10.52	10.45	11.07	11.07	10.54±0.38			
CT-1.5	9.54	9.75	11.75	11.69	11.97	10.94±0.36			
CT-2	10.05	9.72	10.78	11.83	11.93	10.84±0.52			
PM ± SE	9.69^{a} ±0.37	10.02^{ab} ±0.42	10.9^{ab} ±0.58	11.31^{b} ±0.57	11.09^{ab} ±0.49				
Creatinine (mg dl^{-1})									
CT-0	0.63	0.79	0.92	1.07	1.32	0.95±0.08	0.952	0.001	0.999
CT-1	0.60	0.75	0.85	0.98	1.19	0.87±0.09			
CT-1.5	0.62	0.74	0.67	1.08	1.38	0.90±0.10			
CT-2	0.61	0.72	0.66	1.19	1.36	0.91±0.11			
PM ± SE	0.61^{a} ±0.08	0.75^{a} ±0.07	0.78^{a} ±0.12	1.08^{b} ±0.10	1.31^{b} ±0.09				

^{abc}Mean values with different superscripts with in a row differ significantly

Table 6. Effect of condensed tannins supplementation on serum enzymes activities in lambs

Treatment	Periods					TM±PSE	P values**		
	I	II	III	IV	V		T	P	T × P
Alkaline phosphatase (IUL^{-1})									
CT-0	39.04	61.35	66.88	72.87	84.53	64.93^{a}±3.36	0.011	0.000	0.219
CT-1	38.01	70.77	71.52	76.70	98.37	71.07^{ab}±4.39			
CT-1.5	41.44	60.81	83.98	88.18	112.68	77.42^{b}±5.26			
CT-2	40.22	58.98	69.07	80.79	104.12	70.64^{ab}±4.67			
PM ± SE	39.67^{a} ±1.17	64.55^{b} ±2.81	71.29^{b} ±3.65	79.63^{c} ±2.98	99.93^{d} ±3.84				
Alanine amino transferase (IUL^{-1})									
CT-0	14.46	14.19	14.88	18.25	23.25	17.00±1.14	0.823	0.000	0.999
CT-1	13.10	15.04	15.11	18.26	24.92	17.29±1.28			
CT-1.5	13.25	16.14	17.61	18.04	26.87	18.38±1.40			
CT-2	13.29	14.20	16.43	17.71	26.05	17.54±1.24			
PM ± SE	13.52^{a} ±0.44	14.89^{ab} ±1.05	16.01^{ab} ±0.92	18.06^{b} ±1.40	25.27^{c} ±1.55				
Aspartate amino transferase (IUL^{-1})									
CT-0	34.33	50.31	55.14	104.08	114.42	71.66±6.26	0.965	0.000	0.984
CT-1	34.17	48.78	52.99	98.42	123.42	71.55±7.10			
CT-1.5	33.67	46.37	52.52	102.57	114.23	69.87±6.31			
CT-2	34.53	47.05	60.71	100.37	110.65	70.66±6.11			
PM ± SE	34.17^{a} ±1.79	48.13^{b} ±1.31	55.34^{b} ±2.57	101.36^{c} ±3.34	115.68^{d} ±4.57				
Lactate dehydrogenase (IUL^{-1})									
CT-0	227.68	231.01	253.98	243.88	250.55	241.42±5.95	0.875	0.037	1.000
CT-1	225.10	227.58	254.78	241.80	260.13	241.88±8.52			
CT-1.5	229.68	232.53	261.88	257.03	268.70	249.97±9.21			
CT-2	231.80	225.67	259.67	249.58	266.25	246.59±9.09			
PM ± SE	228.57^{a} ±9.91	229.20^{a} ±6.85	257.58^{b} ±10.32	248.08^{ab} ±10.11	261.41^{b} ±6.42				

^{abc}Mean values with different superscripts with in a row differ significantly

Retention of Ca and P

The nutrient retention of Ca and P are presented in the table 3. In the present study, improved Ca intake and balances observed in CT groups could be attributed to high Ca level in LMM as evidenced by the positive Ca and P balance in all experimental animals fed on tree leaves based diets (Khatta *et al*. 1998; Kantwa *et al*. 2006; Ganai *et al*. 2010). P intake and balance was comparable irrespective of dietary treatments. In the present study, analogous Ca and P balances in all lambs irrespective of dietary treatments clearly indicate that low to moderate level of CT (1-2%) in the diet did not exert any adverse effect on mineral balance by lambs. However, contrary to some reports describing that CT (3.64-7.7 %) tended to decrease intakes of Mg and Ca as well as body Fe retention (Scharenberg *et al*. 2008). However, Scharenberg *et al*. (2007) reported that supplementing PEG to sheep fed on sainfoin may lead to greater retention of P, Ca and Mg.

Clinical chemistry

The levels of Hb and PCV (Fig. 2 and 3) were within the normal physiological range (Hb 9-15 g dl^{-1} and PCV 27-45 %) suggested for sheep (Kaneko, 1997). The observation indicates that CT as an additive up to 2% of diet induced no adverse effect on target haematological parameters in lambs up to 6 months feeding (Dey *et al*. 2008).

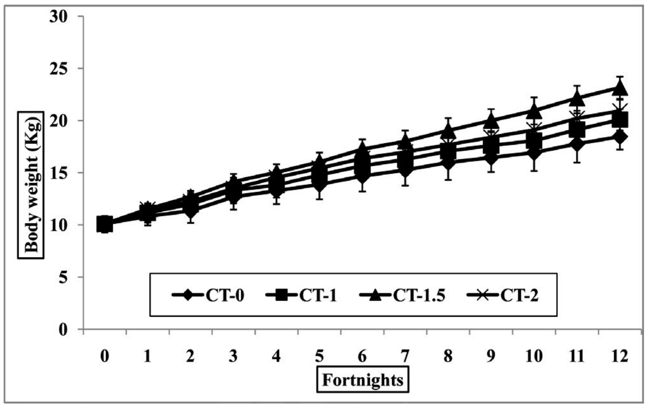

Fig. 1. Fortnightly body weight changes of lambs supplemented with condensed tannins

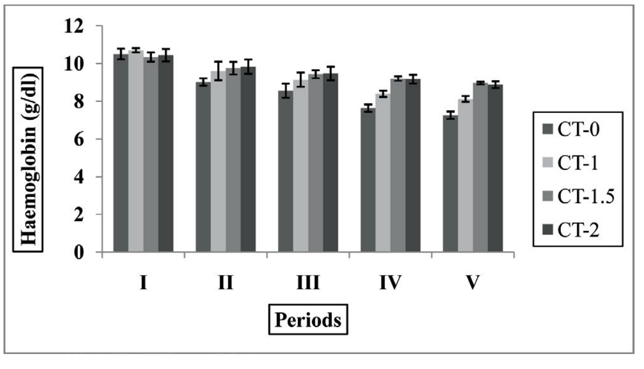

Fig. 2. Effect of condensed tannins supplementation on haemoglobin content in lambs

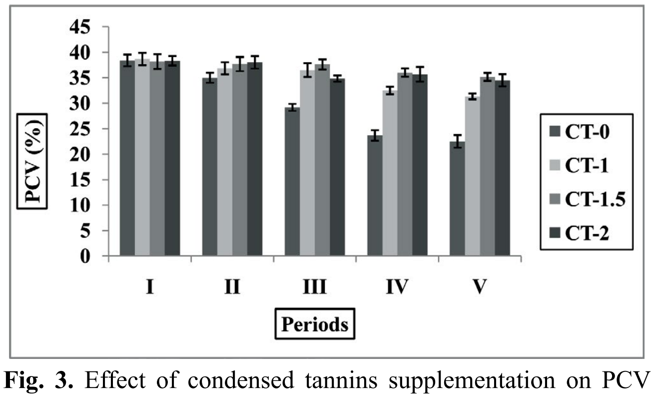

Fig. 3. Effect of condensed tannins supplementation on PCV values in lambs

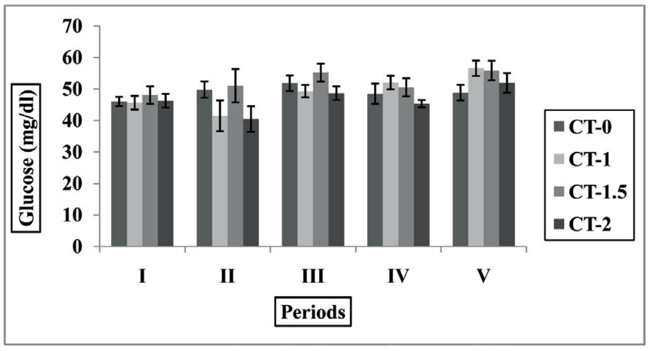

Fig. 4. Effect of condensed tannins supplementation on blood glucose in lambs

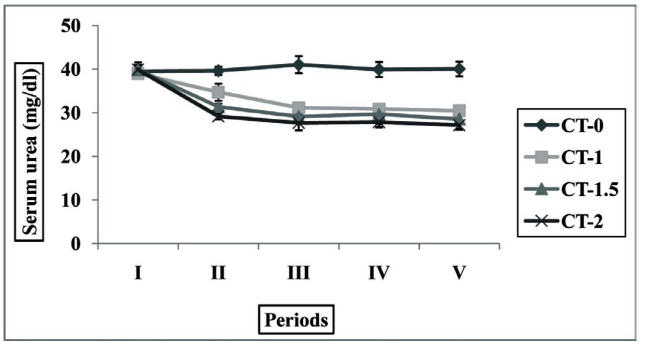

Fig. 5. Effect of condensed tannins supplementation on serum urea levels in lambs

Serum glucose level was significantly ($P<0.05$) higher in CT-1.5 as compared to CT-2, however, other groups did not differ with CT-1.5 (Fig.4). The normal glucose level in control and CT supplemented lambs indicates normal physiological condition of the lambs (Dey *et al*. 2008). In contrary, Wang *et al*. (1996 b) reported lower plasma glucose level in lactating ewes grazed on tanniferous *Lotus corniculatus* pasture, probably due to increased uptake of glucose from blood for milk lactose synthesis. Total protein, albumin, globulin and A:G ratio were significantly ($P<0.05$) lower in CT-0 as compared to CT groups (table 4). However, the mean values of TP, albumin, globulin and A: G ratio in lambs under different treatments were within the normal physiological range (Kaneko, 1997), which clearly indicates that low levels of CT did not have any adverse effect on all the three parameters

Mean serum urea level was significantly ($P<0.01$) higher in CT-0 (control) as compared to CT fed lambs (Fig. 5). Similarly, mean urea level was significantly ($P<0.01$)

higher at 0 day (I period) relative to comparable urea levels during subsequent periods (II, III, IV and V). Serum urea level is an indicator of protein degradation in rumen. The effects of CT were associated with low rumen ammonia concentration and rapid turnover of the plasma pool (Waghorn *et al*. 1994a). In the present study significantly ($P<0.001$) lower level of serum urea was observed in lambs fed 1.0, 1.5 and 2.0% CT in the diets relative to control (CT-0). Significantly lower level of serum urea in CT fed lambs may be attributed to the reduced rumen protein breakdown and increased EAA absorption (Waghorn *et al*. 1987; 1990). Similarly, lower serum urea level was reported in lambs given diets containing CT supplied through *Ficus infectoria* leaves at 1.5-2% (Dey *et al*. 2008) and in kids fed leaves of *Prosopis cineraria* (Bhatta *et al*. 2002).

Mean cholesterol and triglycerides levels did not differ significantly irrespective of dietary treatments and were within the normal physiological range (Kaneko 1997). However, cholesterol and triglycerides levels were increased gradually with the advancement of feeding trial. Mean values of serum creatinine varied from 0.90-0.95 mg dl^{-1} and were within the physiological range suggested for sheep (Merck Veterinary Manual 2005).

Serum ALP level was significantly ($P<0.05$) higher in CT-1.5 as compared to CT-2, however, CT-1 and CT-2 have intermediate values between CT-1.5 and CT-0 treatments. The mean ALP levels in all experimental lambs were within the physiological range for sheep (Merck Veterinary Manual 2005). CT fed lambs exhibited higher growth as compared to control. This could be attributed to higher deposition of protein in muscular tissues besides increased rate of deposition of Ca in bone tissues. The mechanism of action of alkaline phosphatase is little known. However, Guyton and Hall (1998) explained that osteoblasts cells secrete large quantity of ALP when they are actively depositing bone matrix. This enzyme is believed either to increase the local concentration of inorganic phosphatase or to activate the collagen fibres in such a way that they cause deposition of Ca salts. Since some alkaline phosphatase diffuses out during this process into blood stream, the blood level of ALP is increased. The mean values of ALT and AST were comparable and within the normal physiological range irrespective of dietary treatments (Boyd, 1984). LDH levels also did not differ significantly ($P<0.05$) among treatment groups.

Serum calcium and inorganic phosphorus levels under various dietary treatments did not differ significantly ($P<0.05$) and were within the normal physiological range as suggested for sheep (Boyd, 1984). The results clearly indicates that a moderate level of CT (1-2%) in the diet of lambs do not have any adverse effect on serum Ca and P levels.

CONCLUSION

On the basis of results, it may be concluded that dietary supplementation of CT through leaf meal mixture of *F. infectoria* and *P. guajava* at moderate level (1-2 %) did not exert any adverse effect on blood chemistry, however, blood parameters such as hemoglobin, PVC, serum glucose and nutrient intake were improved considerably. Serum urea level was reduced noticeably in CT fed lambs, which clearly indicates that low to moderate level of CT reduced protein degradation in rumen and increased bioavailability of amino acids at lower digestive tract. Therefore, CT containing leaf meal mixture may be used as an alternative functional feed resource for organic animal production.

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