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Influence of Condensed Tannins Supplementation through Leaf Meal Mixture on Urinary excretion of Purine Derivatives, Microbial Protein Synthesis and Performance of *Haemonchus contortus* Infected Sheep

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ABSTRACT

Study was carried out to assess the effect of condensed tannins (CT) containing leaf meal mixture (LMM) on feed intake, body weight changes, parasitic load, urinary excretion of purine derivatives (PD) and microbial protein synthesis (MP) in *Haemonchus contortus* infected sheep. Eighteen adult male sheep of similar age and body weight (25.03±1.52) were randomly divided into three groups (negative control; NC, infected control; C and treatment; T) of six each in a completely randomized block design (CRD) for a period of 90 days. Twelve *H. contortus* infected sheep were allocated into C and T groups, containing 0 and 1.5% of CT, respectively. Six non-infected sheep was taken in NC group to compare their performance with C group. Concentrate intake was significantly (P<0.000) lower in T group as compared to C group, while roughage intake did not differ significantly irrespective of groups. Final body weights were comparable (P<0.063) among all three groups. Faecal egg counts (FECs) were significantly (P<0.001) higher in C group as compared to T group. MP synthesis was calculated by estimating urinary excretion of PD through High performance liquid chromatography (HPLC). Total PD excretion, absorption of PD and Microbial nitrogen (MN) supply (g d^{-1}) were comparatively (P<0.05) higher in T group than in the C group. It may be concluded that CT supplementation (1.5%) decreased *H. contortus* load in sheep by reducing FECs and has a potential benefits on protein nutrition by altering partitioning of nutrients towards higher microbial yield and absence of any depressing effect on rumen MN synthesis.

Keywords: Condensed tannins, *haemonchus contortus*, leaf meal mixture, microbial protein, purine derivatives, sheep

Sheep play a significant role in maintaining family stability by providing meat, milk, skin and wool; earn cash income and play traditional social and religious roles. Gastrointestinal nematodes (GIN) represent a major economic obstacle in small ruminant production, and chemical anthelmintics are estimated to account for about 53% of the total costs of chemical anthelmintics worldwide (Diaz Lira *et al*. 2008). *Haemonchus contortus,* is one of the most detrimental abomasal GINs of small ruminants, and the blood feeding activity of the parasite can lead to animal losses. Chemical anthelmintics have been used to combat this problem, but due to increasing anthelmintic resistant worldwide, incidence of *H. contortus* infection is becoming more

and more common. The moderate level of condensed tannins (CT) from leaf meal mixture (LMM) can work as natural anthelmintics, directly by affecting every developmental stage of *H. contortus* (Pathak, 2013; Pathak *et al*. 2013 a, b) or indirectly by increasing protein bioavailability post ruminally either by protecting the dietary protein or by increasing the microbial protein (MP) synthesis in the rumen (Patra and Saxena, 2010; Patra *et al*. 2009) to improve nutritional and health status of sheep.

Protein requirements for ruminants are the results of MP synthesis from protein degradation in the rumen, endogenous nitrogen recycled via saliva, dietary protein non-degraded in the rumen and animal protein (Boer *et al*. 1987). Thus, the quantitative estimation of bypass protein and MP synthesis is important for proper diet formulation for animals to get rid off from GIN infection and nullify their harmful effects. It should be possible to estimate the MP synthesis in the rumen of sheep with high performance liquid chromatography (HPLC) by estimating urinary excretion of purine derivatives (PD) during *in-vivo* experimental study (Chen and Gomes, 1992; Dey *et al*. 2008). The quantity of dietary rumen un-degradable protein supply to the gut depends on rumen degradation. The amount of MP synthesized in the rumen, as a result of microbial fermentation, is interesting because there are evidences that MP can be influenced by diet characteristics (Dove and Milne, 1994), potential source and type of CT present.

A similar amount of urinary PD excretion and thereby MP synthesis was reported in sheep fed undegradable protein (Webster *et al*. 2003). Several studies reported an increase in MP flow (up to 28% in sheep) when moderate levels of tannins were fed. Makkar *et al*. (1995; 1997) reported that this beneficial effect of tannins *in vivo* could also be due to higher efficiency of MP synthesis in rumen by decreasing the rate of digestion of feeds, which could help synchronizing the release of various nutrients. So the LMM of *Ficus infectoria* and *Psidium guajava* being rich in nitrogen, energy, minerals, CTs and their supplementation could increase the efficiency of utilization of wheat straw either by increasing the efficiency of MP synthesis in the rumen or by protecting the dietary protein at neutral pH in the rumen by making CT-protein complex, leading to higher amino acid availability to the intestine. Thus the recent concept of diet formulation is based on the manipulation of the diets in order to achieve high efficiency of protein utilization and high production of MP in the rumen by creating an efficient rumen ecosystem.

Supplementation of CT from LMM may be a possible alternative approach to protect the dietary protein from microbial degradation in the rumen, improved MP synthesis and reduce *H. contortus* load; however information regarding effect of CT from LMM on MP synthesis in *H. contortus* infected sheep is scare. This raises the possibility that feeding locally available LMM containing CT may be an alternative sustainable approach for controlling GIN infections by improving amino acids availability post ruminally. The CTs are currently the most studied natural class of compounds for their nutritional value (increase in protein uptake) and for reducing harmful effects, and *H. contortus* load in sheep. Therefore, potential source and optimum level of CT to be used in the diets to improve animal performance and reduce *H. contortus* load in sheep warrants investigation. Keeping this in view, it is proposed to investigate the effect of CT through LMM on feed intake, body weight changes, *H. contortus* load, and urinary excretion of PD and MP synthesis in sheep.

MATERIALS AND METHODS

The experimental study was conducted at the Animal Nutrition Research Sheds of the Indian Veterinary Research Institute (Deemed University), Izatnagar (Uttar Pradesh), India. A feeding trial of 90 days duration was undertaken to ascertain the effect of condensed tannins (CT) containing leaf meal mixture (LMM) on feed intake, body weight changes, faecal egg counts (FECs), urinary excretion of PD and MP synthesis in *H. contortus* infected sheep.

EXPERIMENTAL ANIMALS AND DESIGN

Experimental animals

Eighteen non-descript adult sheep of similar age and body weight (25.03±1.52) were selected from the well maintained stall fed herd and randomly allocated to three groups in a completely randomized design (CRD) for experimental study. Before the start of experiment all sheep were treated with broad spectrum drugs. During the adaptation period, all the sheep were vaccinated against prevalent contagious diseases to ensure that the sheep were in apparently healthy condition, free from any disease. Out of 18 sheep, 12 sheep were infected with infective 3rd stage larvae of *H. contortus* @ 2000 larvae per sheep. All sheep were allocated in three different groups (6 sheep in each group) i.e. negative control (NC; no infection), control (C; *H. contortus* infection) and treatment (T; *H. contortus* infection + CT

@ 1.5 % of the diet through LMM). A negative control group was taken to compare their health status and microbial nitrogen synthesis with infected control and treatment groups.

HOUSING, FEEDING AND MANAGEMENT

Housing and Management

All the sheep were kept under uniform managemental conditions by housing them in a well-ventilated shed with facilities for individual feeding and watering. Sheep were allowed to exercise daily, out-doors in an adjacent dry paddock for an hour in the morning (8:30 to 9:30 AM). All experimental sheep were offered a basal diet of wheat straw *ad libitum* along with required amount of concentrate mixtures to meet their nutrient requirements for maintenance as per Kearl (1982) for a period of 90 days. One hundred gram oat hay was given to each sheep per day to meet their vitamin-A requirement. The LMM was prepared by mixing of *Ficus infectoria* and *Psidium guajava* in the ratio of 70:30. The same LMM was used for whole experimental study. The LMM was incorporated in the concentrate mixtures of T group by replacement of concentrate so as to bring CT content to 1.5% of diet. The sheep 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 sheep consumed the concentrate mixtures completely. Offered and refusals of roughage from all the sheep were weighed daily and sampled at fortnightly intervals for subsequent analysis of dry matter to assess the feed intake (% DM) during the experimental period. The ration schedule was changed every fortnight after recording the body weights of each sheep.

Sample analysis

Sample of concentrate mixture, LMM, oat hay and wheat straw were milled to pass through a 1 mm sieve and analyzed for their proximate principles (AOAC, 1995) and fibre fractions (neutral detergent fibre: NDF and acid detergent fibre: ADF) as per the methods of Van Soest *et al.* (1991). The extraction and estimation of CT content of LMM were done by Butanol-Hcl method (Makkar, 2000). Faecal samples from all experimental sheep were collected at fortnightly interval directly from the rectum for FECs. After collection, FECs were done by modified McMaster technique as described by Anonymous (1984). At the beginning of the experiment, all experimental sheep were free from any GIN infections as indicated by nil FECs. However, after 21 days of *H. contortus* (L_3) administration in C and T groups showed passing parasitic eggs in their faeces. Though, FECs were zero in sheep of NC group throughout the experimental period, so they were not included in the statistical analysis.

COLLECTION AND PREPARATION OF URINE SAMPLES

Preparation of urine samples

A metabolism trial of 6 days duration was conducted at the end of feeding trial. Urine samples were collected during metabolism and they were pooled in individual sample bottles and after 6 days of collection they were centrifuged and filtered through a Millipore filter. The urine samples were diluted tenfold with distilled water after adjusting the pH 4. A 20µl volume of the filtrate was injected into the HPLC column. Urine samples were stable for several weeks when stored at –20°C.

ANALYSIS OF URINE SAMPLES FOR PURINE DERIVATIVES AND MICROBIAL NITROGEN SUPPLY

Analysis of urine samples for purine derivatives and microbial nitrogen supply to animals

Purine derivatives and creatinine content of urine was analyzed using HPLC (Shimadzu 10 A; Kyoto, Japan with UV detector). The procedure is adapted from the method of Resines *et al.* (1992). Allantoin (A-7878), uric acid (U-0881), Xanthine (X-4002), Hypoxanthine (H-9377) and Creatinine (C-4255) from Sigma Aldrich Ltd. were used as the standards. Stock solutions (1mg ml^{-1}) of all compounds were prepared by dissolving pure standards in water and the pH 4 was adjusted with the help of 0.01 N NaOH and 0.01 N H_2SO_4 solutions except uric acid. However, uric acid standard (1 mg ml^{-1}) was dissolved in distilled water and adjusted the pH 7 using 0.01 N NaOH solutions. As the uric acid completely dissolved on pH 4, so immediately pH 4 was adjusted by using 0.01 N H_2SO_4. A series of working standards were prepared by dilution of each of the stock solutions with water. Quantification was achieved by regression analysis of the peak areas of each compound against concentration. Triplicate injections of each concentration were made.

Chromatographic conditions

A Phenomenex C18 reversed-phase column (250 × 4.60 mm I. D., 5mm particle size) was used. The mobile phase

was 10 mM potassium di-hydrogen phosphate buffer (pH 4.0). Before use, the mobile phase was filtered through a HA 0.45 mm pore size filter (Millipore) and further degassed by sonication. The flow rate was 0.5 ml minute^{-1}, the column was maintained at 25^{o}C and the absorbance detector was set at 218 nm. Compound peaks were identified by their retention times with authentic standards and quantified by comparison of the peak areas of the samples with those of authentic standards.

ESTIMATION OF PURINE DERIVATIVES AND PREDICTION OF MICROBIAL NITROGEN SUPPLY

Estimation of purine derivatives and prediction of microbial nitrogen supply

The amount (mmole d^{-1}) of each purine derivative (PD) and creatinine excreted by sheep were estimated by comparing peak area of each standard and sample. The rate of absorption of exogenous (microbial) purines (mmole d^{-1}) was calculated from total purine excretion (mmole d^{-1}) using the following equations from Chen and Gomes (1995). To correct for endogenous purine excretion:

$$\text{Sheep } Y = 0.84X + (0.150\ W0.75\ e\text{-}0.25X)$$

Where X is absorption of exogenous purines, Y is the excretion of purine derivatives, W is the body weight (kg).

Microbial nitrogen yield (g d^{-1}) was also calculated according to Chen and Gomes (1995).

$$MN\ (g\ d^{-1}) = 70X / (0.83 \times 0.116 \times 1000) = 0.727X$$

Where 0.83 is the true digestibility of purines, 0.116 is the ratio of purines N: total N in microorganisms and 70 is the N concentration in purines (mg mmol^{-1}).

Statistical Analysis

The results obtained were subjected to analysis of variance and treatment means were ranked using Duncan's multiple range test. The periodic alterations in body weight changes and FECs were analyzed using repeated measures design (General linear model; GLM, Multivariate). Significance 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 ingredients and chemical composition (g/kg DM)

Table 1: Ingredients and chemical composition of experimental feeds

Attributes	CM	LMM	OH	WS
Ingredients (g/kg)				
Crushed maize	290	—	—	—
Wheat bran	370	—	—	—
Soybean meal	320	—	—	—
Mineral mixture[a]	10	—	—	—
Common salt	10	—	—	—
Chemical composition (g/kg DM)				
Organic matter	917.9	908.2	909.5	927.2
Crude protein	221.0	108.0	91.0	34.3
Ether extract	29.8	3.21	16.3	15.5
Total Ash	82.1	91.8	90.5	72.8
Calcium	12.4	16.1	03.4	02.0
Phosphorus	07.3	02.0	03.3	0.4
NDF	351.5	572.9	701.7	847.5
ADF	110.3	449.7	462.0	556.8
Condensed tannins[b]	—	104.4	—	—

[a]Mineral mixture contained (gkg^{-1}) : calcium 215, phosphorus 95, sodium chloride 285, potassium iodide 2.5, iron 5.0, copper 0.8, cobalt 1.0, manganese 1.0 and sulphur 1.0; [b]It was assumed that only LMM contains condensed tannins (CT).

Table 2: Fortnightly feed intake (gd^{-1}) in *H. contortus* infected sheep (on % DM bases)

Group*	Period							GM±SE
	0	1	2	3	4	5	6	
Concentrate intake								
NC	0.00	183.06	182.62	182.56	184.10	182.54	183.12	156.86^{b} ±10.12
C	0.00	183.06	182.62	182.56	184.10	182.54	183.12	156.86^{b} ±10.41
T	0.00	146.45	146.10	146.05	147.28	146.04	146.49	125.49^{a} ±8.11
PM±SE	0.00	170.85 ±5.40	170.44 ±5.39	170.39 ±5.39	171.83 ±5.43	170.37 ±5.38	170.91 ±5.40	
Leaf meal mixture intake								
NC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T	0.00	72.02 ±3.69	71.97 ±3.69	71.49 ±3.66	71.76 ±3.68	71.59 ±3.67	70.52 ±3.61	61.33 ±4.09
PM±SE	0.00	24.01 ±8.32	23.99 ±8.31	23.83 ±8.25	23.92 ±8.29	23.86 ±8.26	23.51 ±8.14	
Total roughage intake (Oat hay + wheat straw)								
NC	0.00	499.44	503.77	460.50	476.02	478.68	491.88	415.76 ±38.31
C	0.00	498.61	500.16	411.61	414.83	419.46	363.55	372.60 ±38.93
T	0.00	474.87	476.94	420.24	413.16	420.04	404.66	372.84 ±34.65
PM±SE	0.00	490.97 ±47.74	493.62 ±46.60	430.78 ±42.45	434.67 ±44.71	439.40 ±48.50	420.03 ±50.21	
Total feed intake								
NC	0.00	682.50	686.39	643.06	660.12	661.23	675.00	572.61 ±45.95
C	0.00	681.67	682.78	594.17	598.93	602.00	546.67	529.46 ±47.21
T	0.00	693.33	695.00	637.78	632.20	637.67	621.67	559.66 ±44.88
PM±SE	0.00	685.83 ±50.47	688.06 ±49.26	625.00 ±44.65	630.42 ±47.02	633.63 ±50.90	614.44 ±52.53	

^{ab}Means with different superscripts within a row and column differ significantly,

*NC: Negative control; C: Control; T: Treatment;**G x P: group and period interaction,

PM±SE: Period mean ± standard error; GM±SE: Group mean ± standard error.

of feeds offered to sheep for a period of 90 days of feeding trial is presented in the table 1. The chemical composition of concentrate, LMM, oat hay and wheat straw used in the experiment was comparable with the values reported by many workers (Dutta and Sharma, 2004; Patra *et al*. 2006; Dey *et al*. 2008). The total feed intake was numerically lower in C group than that of NC and T groups (Table 2) but it was statistically similar (P<0.05) among three groups (NC, C and T). The roughage (oat hay (@ 100g d^{-1}) and wheat straw) intake (g) was also found to be statistically non significant (P<0.05) under three dietary treatments. However, intake (g d^{-1}) of concentrate mixture was reduced significantly (P<0.01) in the T group as compared to NC and C group. The LMM as CT source was given to sheep in T group only. The slight depression in feed intake was probably due to the panic reaction by the sheep of C group. *H. contortus* while sucking the blood from deeper mucosa of abomasum resulted into profuse loss of N in the form of desquamated epithelial cells of damaged tissues, which consequently caused aggravated pain. This caused reduction in the overall nutrient intake. The findings were in accordance to the earlier report (Rowe *et al*. 1988) in sheep and kids (Pathak and Tiwari, 2012) infected

Table 3: Comparative plane of nutrition with Kearl (1982) feeding standards of sheep supplemented with CT

Attributes	Groups		
	NC	C	T
Digestible crude protein			
Requirement (g $kg^{-1}W^{0.75}$)	3.26	3.26	3.25
Intake (g $kg^{-1}W^{0.75}$)	3.36	3.19	3.34
Deviation (%)	+3.15	-2.33	+2.58
Total digestible nutrient			
Requirement (g $kg^{-1}W^{0.75}$)	37.59	37.69	37.57
Intake (g $kg^{-1}W^{0.75}$)	31.36	33.36	34.26
Deviation (%)	-16.52	-11.50	-8.84

*NC: Negative control; C: Control; T: Treatment; $gkg^{-1}W^{0.75}$: Metabolic body weight.

Table 4: Effect of condensed tannins supplementation on urinary excretion of PD and microbial nitrogen supply to sheep

Attributes	Groups*			SEM	P value
	NC	C	T		
Urinary excretion of PD (m mol d^{-1})					
Allantoin	4.47^{ab}	4.07^{a}	5.05^{b}	0.17	0.040
Uric acid	0.13	0.19	0.16	0.02	0.347
Xanthine	0.18	0.14	0.13	0.02	0.582
Hypoxanthine	0.13	0.20	0.11	0.02	0.083
Total	4.91	4.60	5.45	0.16	0.093
Creatinine	2.65	2.43	2.55	0.12	0.797
PD:C	1.90	2.01	2.22	0.12	0.567
PD absorption (m mol d^{-1})	5.28	4.85	6.02	0.22	0.086
Microbial-N supply (g Nd^{-1})	3.84	3.52	4.38	0.16	0.085
Efficiency of microbial N synthesis					
gN kg^{-1}DOMI	11.34	11.00	12.71	1.00	0.783
gN kg^{-1} DOMR	11.34	11.00	12.71	1.00	0.783

*NC: Negative control; C: Control; T: Treatment; m $mold^{-1}$: Mili mole per day;
PD:C : Purine derivatives and creatinine ratio; DOMI: Digestible organic matter intake;
DOMR: Organic matter digested in the rumen; gNd^{-1}: Gram nitrogen per day.

with *H. contortus*. Significant relationship was established (Poppi *et. al.* 1985, 1986 and Pathak and Tiwari, 2012) between the severity of infection and the feed intake by the animals. The plane of nutrition of sheep compared with Kearl (1982) feeding standard is presented in table 3. The comparative study revealed that experimental sheep of NC and T groups had higher DCP intake (+ 3.15 and +2.58 %) as compared to infected control (-2.33) and TDN intake (-8.84 to -16.50 %) lower than the maintenance requirement which clearly indicated that CT supplementation at lower level increased the bioavailability of protein even in *H. contortus* infected sheep either by protecting the dietary protein or by increasing and maintaining the MP in T group of sheep and nullify the deleterious effects of *H. contortus* infection.

Body weight changes

The periodical body weight changes in experimental sheep of all three groups (NC, C and T) for experimental feeding of 90 days are depicted in the Figure 1. The initial body weights (kg) of sheep did not differ significantly ($P<0.05$) irrespective of all three groups, however, final

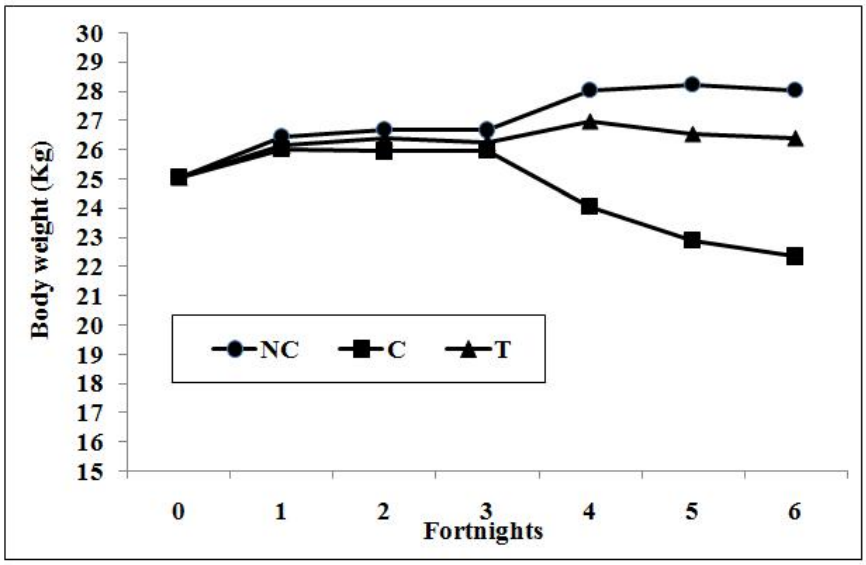

Fig. 1. Effect of condensed tannins on body weight changes of *H. contortus* infected sheep

body weight (kg) was significantly (P<0.020) lower in C group as compared to NC groups, however the T group did not show any marked variation in their final body weights. As the time period of *H. contortus* infection in sheep increased the body weight declined significantly (P<0.001) in C group as compared to NC and T groups. The decline in body weight was observed after 45 days of *H. contortus* post infection. Similar to present findings Swarnkar *et al.* (2007) also reported that there was no marked variation in body weight in sheep and goats infected with *H. contortus* having tanniferous plant (*Prosopis cineraria*) or CT supplemented with in their diet in comparison to control. At appropriate concentration, the CT reduced the degradation of sulphur containing 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 likely to maintain body weights in CT supplemented infected (T) group. Though, a marked reduction in body weights of C group, it might be due to heavy parasitic loads.

H. contortus load

Mean FEC were significantly (P<0.001) higher in C group (3400±523) as compared to T group (1106±256). The present findings are in agreement with the previous reports (Min *et al*. 2003; Sokerya and Preston, 2003), who reported that dietary supplementation of CT may be used as an alternative parasite management strategy. Dosing nematode infected sheep with quebracho tannins at concentrations found in plants (approximately 4.9 g/kg of $BW^{0.75}$) produces a rapid decrease in FEC (50%), worm burdens (30%), and parasite fecundity (Athanasiadou *et al*. 2000). Lambs consuming 4.5 g/kg of $BW^{0.75}$ quebracho tannins for 8 day showed a 47% reduction in FEC (Lisonbee *et al*. 2009). The mechanism of action of CT is mainly through a direct anthelmintic effect (Athanasiadou *et al*. 2000), but CT may also enhance resistance to GIN infection by increasing protein supply (Niezen *et al*. 2002) and inhibiting GIN metabolism by binding nutrients and decreasing their availability (Min and Hart, 2003). Thus, CT has the potential benefits through their negative impacts on GINs. Pathak *et al*. (2013a, b) have observed that different CT extracts from various tree leaves can disrupt the life cycle of *H. contortus* by preventing their eggs from hatching and by preventing larval development to the infective stage.

URINARY EXCRETION OF PD AND MN SYNTHESIS

Urinary excretion of PD, MN supply and efficiency of MP synthesis in sheep is presented in table 4. Hypoxanthine, total PD excretion, absorption of PD and MN synthesis (g d^{-1}) were comparatively higher in T group as compared to C group, though NC group has intermediate position between C and T groups. Efficiency of MN in terms of per kg digestible organic matter intake (DOMI) or per kg digestible organic matter in the rumen (DOMR) did not differ significantly (P<0.05) irrespective of dietary treatments. Allantoin was the main constituent of the PD excreted through urine and accounted for more than 90% of total PD excreted. Present results are in consistent with the findings of Chen and Gomes (1995). Efficiency of MN synthesis was comparable irrespective of groups. The present 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 (Baba *et al*. 2002). MP synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Firkins *et al*. 2007). Increased MP synthesis in T group agrees with the observations recorded earlier in sheep fed tannin containing *Acacia* pods and *Ficus infectoria* (Ngwa *et al*. 2002; Dey *et al*. 2008). The findings suggest the possibility of using CT source in practical diets by increasing MN supply.

CONCLUSION

On the basis of present findings, it may be concluded that dietary supplementation of CT through LMM of *F. infectoria* and *P. guajava* has the potential to improve the health status of sheep by maintaining body weights and feed intake and decreasing FECs in *H. contortus* infected sheep. The infected sheep given CT supplemented diet (T group) comparatively perform better than infected control which might be attributed to beneficial effect of CT as it improved bioavailability of protein and has a potentially beneficial effect to protein nutrition by altering partitioning of nutrients towards higher microbial yield and absence of any depressing effect on rumen MP synthesis.

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