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The effect of condensed tannins supplementation through *Ficus infectoria* and *Psidium guajava* leaf meal mixture on erythrocytic antioxidant status, immune response and gastrointestinal nematodes in lambs (*Ovis aries*)

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ABSTRACT

This experimental study was carried out to assess the effect of condensed tannins (CT) through a leaf meal mixture of *Ficus infectoria* and *Psidium guajava,* on erythrocytic antioxidant status, immune response and gastrointestinal nematodes in lambs. Twenty-four non-descript lambs were randomly divided into four groups, consisting of six lambs in each, in a completely randomized block design, and randomly allocated to 4 dietary treatments: CT-0, CT-1, CT-1.5 and CT-2 containing 0, 1, 1.5 and 2.0 percent CT, respectively. The erythrocytic antioxidant status was monitored in all lambs at 0, 45, 90, 135, 180 days of feeding, however, humoral and cell mediated immune responses were determined at the end of the feeding trial. Hemoglobin was found to be highest ($P<0.05$) in CT-1.5 followed by CT-1, CT-2 and CT-0, respectively. CT supplementation significantly ($P<0.05$) improved the antioxidant status, as indicated by increased levels of glutathione peroxidase, catalase, reduced glutathione, glutathione-S-transferase, superoxide dismutase, total thiol and protein bound thiol group and decreased lipid peroxidase in the lambs. Supplementation of CT significantly ($P<0.05$) improved the cell mediated immune response in lambs. The fecal egg counts (FEC) in lambs were significantly ($P<0.01$) higher in the control group (CT-0), followed by CT-1, CT-1.5 and CT-2. The pooled fecal cultures of the lambs revealed that the majority of the infective larvae were from *Haemonchus contortus*. The FEC in the control

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was significantly higher ($P<0.05$) throughout the study period compared to the CT-1.5 and CT-2 groups. It may be concluded that dietary supplementation of CT (1-2%) through LMM improved the erythrocytic antioxidant status and immune response, and reduced FEC in lambs.

Key words: antioxidants status, condensed tannins, *Haemonchus contortus*, immune response, lambs

Introduction

Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit oxidation and reduce oxidative stress caused by increased levels of reactive oxygen species and free radicals that can initiate chain reactions in the cell, resulting in death or damage to the cell. Balanced proportions of nutrients in the diet play an important role in maintaining health and production performance of animals, and protecting them from various infectious and non-infectious diseases. Among all dietary factors, natural antioxidant rich diets have become especially important for growth, survival, maintenance and health status of animals (SURAI, 2002). In the Tropics nematode infection, particularly *Haemonchus contortus,* causes extensive protein losses in animals and is a significant economic burden to the animal husbandry (PATHAK and TIWARI, 2013). However, the development of parasitic resistance against anthelmintics in sheep and goats is of greater importance.

Alternative gastrointestinal nematode (GIN) control strategies using locally available condensed tannins (CT) containing a tree leaves/ leaf meal mixture (LMM), have been suggested (PATHAK, 2013). The CT are also known as proanthocyanidins, which are of great interest in nutrition and medicine because of their potent anthelmintic efficacy, and their protein protecting, immune stimulating (PATHAK et al., 2013a; PATHAK et al., 2013b; PATHAK et al., 2014; PATRA et al., 2006) and antioxidant capacity (ZHANG and LIN, 2008). The CT bind with dietary protein by making a CT-protein complex, which is not dissociated at neutral pH in the rumen. By making the protein unavailable for digestion and absorption until it reaches the more acidic abomasum, CT also enhances nutrition, by providing high-quality protein to the small intestines (BARRY et al., 2001). This high-quality protein bypass effect has the potential to enhance the immune response and increase resistance to GINs (PATHAK et al., 2014). By-passing amino acids, such as arginine, glutamine and cysteine, can enhance immune responses as these amino acids regulate activation of T and B lymphocytes, lymphocyte proliferation, and the production of antibodies (LI et al., 2007). The CT has antioxidant properties related to their radical scavenging capacity, and these properties have been used against heart disease through reducing lipid peroxidation (ROSENBLAT et al., 2006). Free radicals and reactive oxygen species, generated due to aerobic metabolism, can be extremely damaging to biological systems.

Current research is now directed towards finding naturally occurring antioxidants of plant origin. They provide a potentially important, socio-economical, eco-friendly and

sustainable alternative, to reduce anthelmintic resistance, oxidative stress and improve the health status of animals. Keeping this in view, the present study was conducted to assess the effect of dietary supplementation of CT through LMM on erythrocytic antioxidant status, immune response and GI nematodes in lambs.

Materials and methods

Animals, feeding, management and experimental protocol. Twenty-four non-descript lambs of about 6 months ofage,with average body mass 10.07 ± 0.59 kg, were randomly divided into four groups consisting of six lambs in each, in a completely randomized block design. The animals were randomly allocated to 4 dietary treatments: CT-0, CT-1, CT-1.5 and CT-2, containing 0, 1, 1.5 and 2.0 percent CT, respectively. All the experimental lambs were offered a basal diet of wheat straw *ad libitum,* along with the required amount of concentrate mixture (Table 1), 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 grams of oat hay (*Avena sativa*) were given to each lamb per day to take care of their vitamin-A requirement. A dried and ground leaf meal mixture of *Ficus infectoria* and *Psidium guajava* (70:30) was incorporated in different proportions to the concentrate mixtures of the three treatment groups by replacement of the concentrate so as to bring the CT content to 1.0, 1.5 and 2.0 percent of the diets. All experimental lambs were kept under uniform management conditions by housing them in a well-ventilated shed with facilities for individual feeding and watering. Samples of concentrate mixture, leaf meal mixture, oat hay and wheat straw were analyzed for proximate principles as per AOAC (2000), and fiber fractions were analyzed as per VAN SOEST et al. (1991). The CT content was estimated as per the method of MAKKAR (2000).

Antioxidant indices. Blood samples from all experimental lambs were collected early in the morning before feeding at 0, 45, 90, 135 and 180 days, post feeding, aseptically by jugular vein puncture. About 2 mL of whole blood was collected from every animal in sterilized vials containing acid citrate dextrose (ACD; 1.5 mL/10 mL of blood) as anticoagulant, for determination of hemoglobin by the cyanomethemoglobin method (DACIE and LEWIS, 1975), and for determination of antioxidant status as per the standard protocol. The samples were centrifuged at 2000 rpm for 15 min at 4 °C, and the resulting erythrocyte pellet (PRBC) was washed three times with 250 mOsm/litre (pH 7.4) phosphate buffered saline (PBS), as per YAGI et al. (1989). The PRBC obtained was mixed with PBS to form a RBC suspension, and it was mixed with a stabilizing solution to prepare a hemolysate.

The activity of superoxide dismutase (SOD) in erythrocytes was assessed by the ability of SOD to inhibit auto-oxidation of pyrogallol under specific conditions, as described by

MARKLUND and MARKLUND (1974). Reduced glutathione (GSH) was estimated by the dithio-bis-2-nitro benjoic acid (DTNB) method of PRINS and LOOS (1969). Glutathione peroxidase (GSH-Px) activity was determined by the method of PAGLIA and VALENTINE (1967). Glutathione-S-transferase (GST) activity was estimated by the rate of increase in optical density at 340 nm at 25 °C due to the formation of 1-chloro-2, 4-dinitrobenzene (CDNB) conjugate of glutathione (HEBIG et al., 1974). Catalase (CAT) activity was assayed in the erythrocytic hemolysate after appropriate dilution, as described by BERGMEYER (1983). The lipid peroxidation (LPO) level was determined by estimating the concentration of malonaldialdehyde (MDA) in RBC hemolysate, as per the method of PLACER et al. (1966). Total thiol (T-SH), non-protein thiol (NP-SH) and protein thiol (P-SH) groups in the RBC hemolysate were determined following the method of SEDLAK and LINDSAY (1968).

Immune response. Immunological responses (CMI and IgG) were tested towards the end of the experimental feeding trial. The CMI response was assessed by measuring the increase in skin thickness as a delayed-type hypersensitivity (DTH) reaction to intra-dermal inoculation of phytohaemagglutinin-p (PHA-p), as described by BLECHA et al. (1983) and KORNEGAY et al. (1993). Individual lambs were injected intra-dermally with 125 µL of PHA-p solution (0.16 µg/µL). Skin thickness was measured at 0, 12 h and then 24 h intervals up to 96 h post-inoculation. For assessing IgG response, *H. contortus* antigen was extracted and prepared as per the method described by AMARANTE et al. (2005). The protein concentration in the somatic extract of *H. contortus* antigen was determined by the Biuret method. The prepared antigen was stored at -20 °C until used for IgG assay. Specific IgG response was determined by ELISA. The ELISA conditions were similar to those described previously (CUQUERELLA et al., 1991). The optical density was measured at 492 nm, using an automated ELISA plate reader (Biored I Mark, India). The optimum dilutions of antigen and anti-sera were determined using checkerboard assay and sera from worm free sheep.

Fecal egg counts (FEC). The FEC was made at fortnightly intervals in lambs throughout the experimental period. Fresh fecal samples were collected (per rectum) from all experimental lambs. Each sample was put in a plastic bag bearing the number of the corresponding tag number of the animal. After collection, the samples were taken to the laboratory and FECs undertaken using a modified McMaster technique (ANONYM., 1984).

Fecal culture technique. The fecal culture provides a suitable media and environment for hatching and development of GI-nematode eggs into infective 3rd stage larvae (L_3). The culture media was prepared by mixing powdered cow dung and its ash in a ratio of 70:30. The cow dung provides proper aeration to the culture media and the ash creates water-holding capacity in the media to maintain the adequate moisture necessary for the

cultivation of parasitic larvae. The fecal samples of the infected lambs, pooled group wise, were broken up finely using a stirring device and mixed thoroughly with culture media after moistening the culture media with distilled water. The infective L_3 were produced by the Petri dish method of fecal culture technique, as described by URQUHART et al. (1996). The culture was left at 27 °C in the incubator for 7-10 days. It was checked and distilled water added if required to the culture regularly (every 1-2 days) to maintain adequate moisture. Infective L_3 were recovered using Baermann's technique.

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 antioxidant enzymes and CMI response 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

Chemical composition of feeds and intake. The chemical composition (% dry matter: DM) of the concentrate mixture, LMM, oat hay and wheat straw offered to lambs is presented in Table 1. The crude protein (%) of concentrate mixture, LMM, oat hay and wheat straw were found to be 21.21. 10.03 and 7.03 and 3.69, respectively. The CT content of LMM was 10.39 percent. The intake of DM (gd^{-1}) was significantly higher in CT-1.5 as compared CT-0, while CT-1 and CT-2 had an intermediate position between CT-0 and CT-1.5 treatments (Fig. 1). However, the mean intake (gd^{-1}) of concentrate mixture, and roughage did not differ significantly ($P<0.05$), irrespective of dietary treatments. LMM intake was significantly ($P<0.01$) higher in the CT groups.

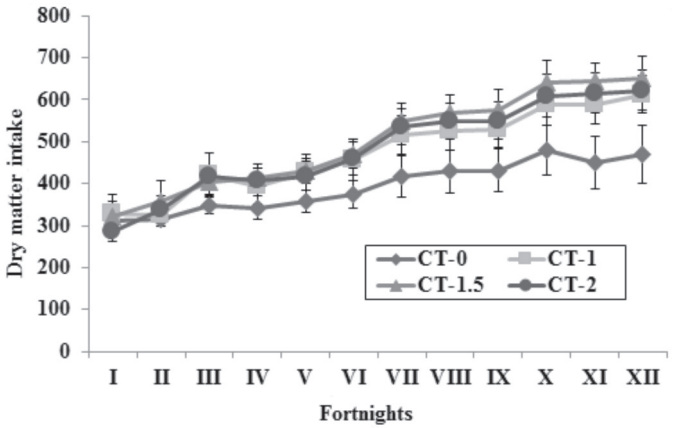

Fig.1. Effect of CT supplementation on fortnightly DM intake by lambs**.** CT-0: 0% CT; CT-1: 1% CT; CT-1.5: 1.5% CT; CT-2: 2% CT; CT - Condensed tannins

Table 1. Ingredients 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	-
Deoiled soybean meal	-	-	32.00	-
Mineral mixture	-	-	2.00	-
Common salt	-	-	1.00	-
Chemical composition (% DM basis)				
Organic matter	93.15	91.55	90.66	90.13
Crude protein	3.69	7.03	21.21	10.03
Ether extract	0.54	1.65	1.77	3.96
Total ash	6.85	8.45	9.34	9.87
Neutral detergent fiber	81.52	62.31	33.60	53.57
Acid detergent fiber	51.62	40.74	9.55	37.75
Condensed tannins	-	-	-	10.39

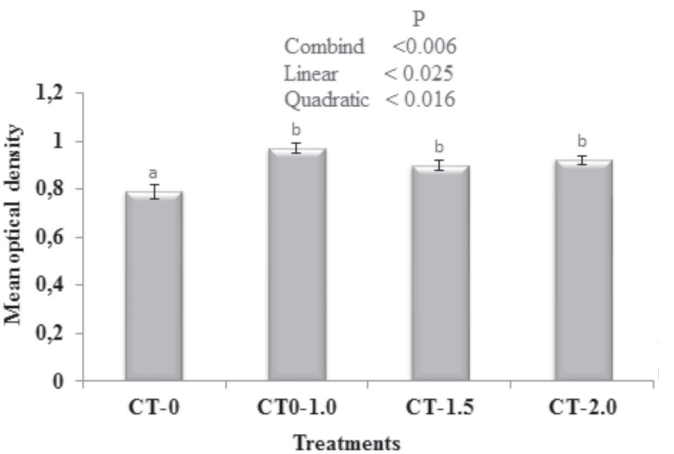

Fig. 2. Mean OD as detected by ELISA due to the effect of CT on IgG response in lambs. CT-0: 0% CT; CT-1: 1% CT; CT-1.5: 1.5% CT; CT-2: 2% CT; CT - Condensed tannins. Mean values with different letters (a, b) differ significantly

Antioxidant status. The mean Hb levels were significantly higher (P<0.05) in CT-1.5 followed by CT-1, CT-2 and CT-0, respectively. The level of Hb decreased significantly (P<0.05) with the advancement of time (0 to 180 days). The dietary supplementation of CT through LMM significantly (P<0.05) increased the mean values of GSH-Px, CAT, GSH, GST, SOD, T-SH and P-SH, however, LPO did not differ significantly (P<0.05), irrespective of dietary treatments (Tables 2 and 3). The highest activity of these enzymes was evident in CT-1.5 followed by CT-2, CT-1 and CT-0. The levels of various enzymes (GSH-Px, CAT, GSH, GST, SOD, T-SH, and P-SH) increased significantly (P<0.05) with the advancement of the feeding trial (0 to 180 days), while LPO and NP-SH level decreased significantly (P<0.05) from 0 to 180 days in the lambs.

Table 2. Effect of CT on hemaglobin and antioxidant enzymes (GSH-Px, CAT, GSH and GST) activity in lambs

Attributes	Period (Days)					Mean
	0	45	90	135	180	
Hemoglobin (g dL^{-1})						
CT-0	10.50	9.02	8.56	7.63	7.26	$8.59^{a} \pm 0.24$
CT-1	10.70	9.60	9.14	8.39	8.12	$9.19^{b} \pm 0.21$
CT-1.5	10.34	9.75	9.43	9.20	8.97	$9.54^{c} \pm 0.13$
CT-2	10.44	9.83	9.47	9.17	8.88	$9.56^{c} \pm 0.16$
PM ± SE	10.50^{d} ± 0.12	9.55^{c} ± 0.18	9.15^{b} ± 0.18	8.60^{a} ± 0.16	8.31^{a} ± 0.16	
GSH-Px (µmol NADPH mg^{-1} Hb min^{-1})						
CT-0	9.06	8.36	7.68	7.14	6.91	$7.83^{a} \pm 0.25$
CT-1	8.54	11.11	11.93	14.08	14.41	$12.01^{b} \pm 0.55$
CT-1.5	8.85	12.69	13.18	16.31	16.73	$13.55^{c} \pm 0.69$
CT-2	9.23	12.17	12.11	15.41	15.63	$12.91^{bc} \pm 0.55$
PM ± SE	8.92^{a} ± 0.43	11.08^{b} ± 0.56	11.22^{b} ± 0.59	13.23^{c} ± 0.85	13.42^{c} ± 0.86	
CAT (U mg^{-1} Hb)						
CT-0	0.87	0.72	0.69	0.58	0.53	$0.68^{a} \pm 0.05$
CT-1	0.85	0.94	1.19	1.96	2.20	$1.43^{b} \pm 0.14$
CT-1.5	0.84	1.11	1.50	2.22	2.38	$1.61^{b} \pm 0.15$
CT-2	0.89	1.04	1.72	2.12	1.95	$1.54^{b} \pm 0.17$
PM ± SE	0.86^{a} ± 0.10	0.95^{ab} ± 0.11	1.27^{c} ± 0.12	1.72^{d} ± 0.20	1.77^{d} ± 0.20	

Table 2. Effect of CT on hemaglobin and antioxidant enzymes (GSH-Px, CAT, GSH and GST) activity in lambs (continued)

Attributes	Period (Days)					Mean
	0	45	90	135	180	
GSH (µmol mg^{-1} Hb)						
CT-0	1.13	0.78	0.71	0.68	0.64	$0.79^{a} \pm 0.06$
CT-1	0.98	1.51	1.98	2.21	2.50	$1.83^{b} \pm 0.12$
CT-1.5	1.25	2.01	2.82	3.18	3.32	$2.52^{d} \pm 0.17$
CT-2	1.33	1.92	2.35	2.18	2.68	$2.09^{c} \pm 0.15$
PM ± SE	1.17^{a} ± 0.09	1.55^{b} ± 0.13	1.96^{c} ± 0.20	2.06^{cd} ± 0.21	2.28^{d} ± 0.22	
GST (µmol mg^{-1} Hb)						
CT-0	3.13	2.68	2.18	2.02	1.69	$2.34^{a} \pm 0.15$
CT-1	3.20	3.16	4.19	6.77	6.74	$4.81^{b} \pm 0.32$
CT-1.5	3.16	4.27	5.51	8.82	9.15	$6.18^{c} \pm 0.50$
CT-2	3.07	3.35	4.47	6.87	7.37	$5.03^{b} \pm 0.37$
PM ± SE	3.14^{a} ± 0.17	3.36^{a} ± 0.18	4.09^{b} ± 0.30	6.12^{c} ± 0.57	6.24^{c} ± 0.61	

^{abcd} Mean values with different superscripts with in a row and column differ significantly; CT-0: 0% CT of diet; CT-1: 1% CT of diet; CT-2: 2% CT of diet. PM ± SE: Period mean ± standard error

Table 3. Effect of CT supplementation on antioxidant enzymes (LPO, SOD, T-SH, NPSH and P-SH) activity in lambs

Attributes	Period (Days)					Mean
	0	45	90	135	180	
LPO (µmol mg^{-1} Hb)						
CT-0	3.46	2.73	2.35	2.49	2.82	2.77 ± 0.11
CT-1	3.22	2.82	2.58	2.46	2.12	2.64 ± 0.10
CT-1.5	3.17	2.96	2.64	2.62	2.02	2.68 ± 0.12
CT-2	3.30	2.80	2.96	2.31	1.98	2.67 ± 0.11
PM ± SE	3.29^{c} ± 0.12	2.83^{b} ± 0.06	2.67^{ab} ± 0.09	2.47^{a} ± 0.08	2.24^{a} ± 0.13	
SOD (SOD U mg^{-1} Hb)						
CT-0	3.77	3.65	3.08	2.85	2.44	$3.16^{a} \pm 0.16$
CT-1	3.58	5.60	5.93	9.22	9.39	$6.74^{b} \pm 0.51$

Table 3. Effect of CT supplementation on antioxidant enzymes (LPO, SOD, T-SH, NPSH and P-SH) activity in lambs (continued)

Attributes	Period (Days)					Mean
	0	45	90	135	180	
CT-1.5	3.66	6.64	8.02	11.16	11.32	$8.16^{c} \pm 0.59$
CT-2	3.74	5.73	6.54	9.77	10.26	$7.21^{b} \pm 0.52$
PM ± SE	3.69^{a} ± 0.23	5.41^{b} ± 0.30	5.89^{b} ± 0.45	8.25^{c} ± 0.72	8.35^{c} ± 0.77	
T-SH (µmol mg^{-1} Hb)						
CT-0	2.46	1.89	1.83	1.58	1.32	$1.82^{a} \pm 0.11$
CT-1	2.38	2.23	2.43	3.45	3.18	$2.73^{b} \pm 0.13$
CT-1.5	2.60	2.37	2.56	3.72	3.48	$2.95^{b} \pm 0.14$
CT-2	2.41	2.99	2.64	3.32	3.02	$2.87^{b} \pm 0.11$
PM ± SE	2.46^{ab} ± 0.13	2.37^{a} ± 0.12	2.37^{a} ± 0.12	3.02^{c} ± 0.20	2.75^{bc} ± 0.20	
NPSH (µmol mL^{-1})						
CT-0	0.87	0.59	0.53	0.49	0.43	$0.58^{ab} \pm 0.03$
CT-1	0.88	0.60	0.40	0.50	0.42	$0.56^{ab} \pm 0.04$
CT-1.5	0.87	0.58	0.50	0.61	0.54	$0.62^{b} \pm 0.03$
CT-2	0.85	0.62	0.34	0.46	0.41	$0.54^{a} \pm 0.04$
PM ± SE	0.87^{d} ± 0.03	0.60^{c} ± 0.01	0.44^{a} ± 0.03	0.51^{b} ± 0.03	0.45^{ab} ± 0.02	
P-SH (µmol mL^{-1})						
CT-0	1.59	1.31	1.30	1.09	0.90	$1.24^{a} \pm 0.10$
CT-1	1.50	1.63	2.03	2.95	2.75	$2.17^{b} \pm 0.13$
CT-1.5	1.73	1.79	2.07	3.11	2.95	$2.33^{b} \pm 0.15$
CT-2	1.56	2.36	2.30	2.86	2.61	$2.34^{b} \pm 0.13$
PM ± SE	1.59^{a} ± 0.12	1.77^{ab} ± 0.12	1.92^{b} ± 0.13	2.50^{c} ± 0.20	2.30^{c} ± 0.20	

[abcd] Mean values with different superscripts with in a row and column differ significantly CT-0: 0% CT of diet; CT-1: 1% CT of diet; CT-2: 2% CT of diet. PM ± SE: Period mean ± standard error

Immune response. The CMI response assessed by the *in vivo* DTH reaction to PHA-p differed significantly ($P<0.05$) between all dietary treatments and periods (Table 4). The mean values of skin indurations were significantly ($P<0.05$) higher in CT-1.5 as compared to the control (CT-0) and CT-1, however, CT-2 exhibited an intermediate value between

CT-1.5 and CT-0. The maximum thickness of skin was observed after 12 hours, followed by 24 hours and then it gradually declined up to 96 hours.

The serum anti-*Haemonchus* antibody (IgG) response of lambs fed graded levels of CT was assessed using antigenic crude extracts of *H. contortus*. The optical density (OD) values after 180 days' feeding were significantly (linearly and quadratic $P<0.01$) higher in the CT supplemented groups compared to the control (CT-0), however, OD values were comparable irrespective of CT levels (Fig. 2).

Table 4. Effect of CT supplementation on DTH response of lambs to PHA-p

	Period (Hrs post inoculation)							P values		
Treatment	0	12	24	48	72	96	GM ± SE	T	P	T x P
CT-0	3.98	7.70	7.30	6.03	4.28	4.10	5.56^{a} ± 0.36	0.030	0.000	0.390
CT-1	2.73	7.28	7.60	6.80	5.33	3.43	5.53^{a} ± 0.43			
CT-1.5	3.33	8.90	8.30	7.43	5.70	3.75	6.23^{b} ± 0.48			
CT-2	3.08	8.73	8.00	6.78	5.38	3.95	5.98^{ab} ± 0.46			
PM ± SE	3.28^{a} ± 0.19	8.15^{d} ± 0.30	7.80^{d} ± 0.25	6.76^{c} ± 0.25	5.17^{b} ± 0.27	3.8^{a} ± 0.17				

[abcd] Means with different superscripts within a row and column differ significantly. CT-0: 0% CT; CT-1: 1% CT; CT-1.5: 1.5% CT; CT-2: 2% CT; T: treatment; P: period. GM ± SE: Group mean ± Standard error; PM ± SE: Period mean ± Standard error

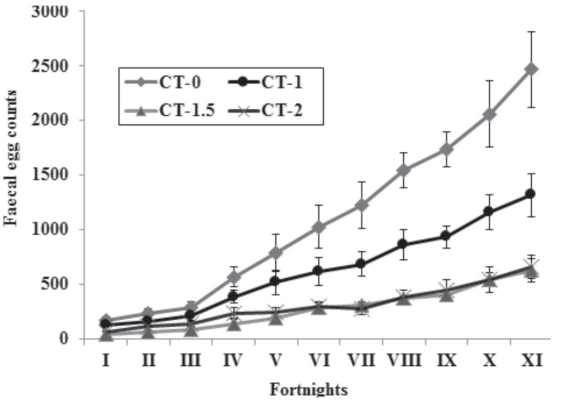

Fig. 3. Fortnightly fecal egg counts in lambs fed on condensed tannins supplemented diets. CT-0: 0% CT; CT-1: 1% CT; CT-1.5: 1.5% CT; CT-2: 2% CT; CT - Condensed tannins

Fecal egg counts. CT supplementation at various levels (1-2%) significantly ($P<0.01$) reduced the FEC in the lambs. The FEC were significantly ($P<0.01$) higher in the control group (CT-0) followed by CT-1, CT-1.5 and CT-2. The FEC per gram in lambs at fortnightly intervals is presented in Fig. 3. The FEC differed significantly ($P<0.05$) between different periods and the interaction between periods and treatment was also significant. FECs in the control (CT-0) increased significantly ($P<0.05$) throughout the study period relative to their counterparts in CT-1.5 and CT-2, however, CT-1 exhibited comparatively higher FECs than their counterparts in CT-1.5 and CT-2.

Morphological identification of nematodes larvae. In the present study the pooled fecal culture of the four groups of lambs revealed that about 90% of L_3 were of *Haemonchus* and the remaining 10% were of *Oesophagostomum* and *Trichostrongylus*.

Discussion

The chemical composition of the experimental diets in the study was comparable with the values reported by DEY et al. (2008) and PATHAK et al. (2013c). The intake of DM by lambs was within the normal range (KEARL, 1982) and this clearly indicates that all the diets were palatable. Similarly, higher DM intake in moderate (1-4%) CT containing diets has been reported by many workers (DEY et al., 2008; RAMIREZ-RESTREPO et al., 2004; TERRILL et al., 1992). The present results are in agreement with the findings of KABASA et al. (2000), who reported that CT played a significant role in reducing the negative effects of GINs, and suggested that goats prefer a tanniferous diet to resolve GIN load up to some extent.

The Hb levels were significantly higher ($P<0.05$) in CT-1.5 followed by CT-1 and CT-2 and CT-0, respectively. This observation indicates that CT as an additive of up to 2% in the diet induced no adverse effect on Hb in lambs (DEY et al., 2008) during the six month feeding trial, contrary to the findings of BHATTA et al. (2002) who reported lower Hb (9.16 vs. 11.02 g dL^{-1}) in kids fed on tanniferous leaves of *Prosopis cineraria*.

Antioxidant enzymes, viz. GSH-Px, CAT, GSH, GST, SOD, T-SH and P-SH, play a significant role in body defense mechanisms by scavenging reactive oxygen species (ROS). Antioxidants comprising of an array of endogenous and exogenous substances serve to stabilize these highly reactive free radicals, thereby preserving the structural and functional integrity of cells, including important immune cells (HAN et al., 2004). There was a trend of increased GSH-Px, CAT, GSH, GST, SOD, T-SH and P-SH levels in all the CT groups, which was statistically significant ($P<0.05$) from 0-180 days. However, levels of LPO and NP-SH decreased significantly ($P<0.05$) from 0-180 days. The primary physiological role of GSH-Px in RBCs appears to be the detoxification of organic peroxides. It catalyzes the conversion of H_2O_2 to H_2O. It also catalyzes the reduction of fatty acid hydroperoxides, and 1-monoacylglycerol hydro peroxides. The CAT appears to

be important in protecting RBCs against higher levels of exogenous H_2O_2 (JOHNSON et al., 2005). In addition to its benefits to RBCs, the presence of CAT in RBCs may help protect somatic cells exposed to high levels of H_2O_2, such as in sites of active inflammation. GSH present in high concentrations in mammalian cells is important in protecting erythrocytes from oxidative damage by serving as an important sink for free radicals and ROS. In the present study, the increased level of GSH-Px, CAT and GSH clearly indicates that CT had a positive effect on these enzymes, at up to 1-2% of the diets. The increased level of SOD in the present study is in conformity with the finding of HO et al. (1999), who reported that the proantheocyanidinsA-1 component of tannins from *Vaccinium vits-idaea* had strong SOD activity.

Increased T-SH and P-SH levels in CT groups, as compared to the control, indicates the better antioxidant status of the lambs. The thiol group acts as an intracellular antioxidant by scavenging free radicals and through enzymatic reactions. The water soluble thiol group protects biological membranes (DEY et al., 2015; DUBEY et al., 2012; MOSCIO et al., 1994). LPO is used as an indicator of oxidative stress in cells and tissues. The LPO level had decreased significantly ($P<0.05$) in CT groups at 180 days. LIN et al. (2001) reported that tannin extract of *Terminalia catappa* had the ability to prevent lipid peroxidation and modification of mitomycin-C induced clasto-genicity. Moreover, the increased concentrations of thiol groups could explain the reduced lipid peroxidation, as these are known to play a greater protective role in preventing the lipid peroxidation of membranes. The NP-SH level also decreased significantly ($P<0.05$) in the CT groups. Similar to the presentstudy, earlier reports also indicated that there is a direct relationship between antioxidant activity and total phenolic content in selected herbs, vegetables and fruits (OSZMIANSKI et al., 2007) and the free radical scavenging capacities and ferric reducing power of CT from leaves, twigs and stem bark of *Canarium album* (ZHANG and LIN, 2008).

The increased CMI response in all CT supplemented lambs may be attributed to the higher availability of essential amino acids (cysteine and methionine) in the small intestine. A moderate concentration of CT (20-35 g kg^{-1} DM) in forage given to sheep has been reported to increase non-ammonia nitrogen (NAN) flux to the small intestine, and to increase the absorption of essential amino acids (BARRY et al., 1986; WAGHORN et al., 1994a; WAGHORN et al., 1994b). ROSS et al. (2001) observed that the administration of *P. granatum* fruit rind powder (PGFRP, containing tannins) in rabbits, at the dose of 100 mg/kg, resulted in significant increases in skin thickness compared to the control group.

The specific IgG response is consistently associated with a reduction in the helminth size and parasitic fecundity (AMARANTE et al., 2005) by metabolic enzyme neutralization, interfering with the feeding and metabolism of *H. contortus* (STRAIN and STEAR, 2001). The results of the present study reveal that supplementation of CT enhanced the serum

IgG response against *H. contortus*. KUMAR et al. (2007) observed that birds given tannins containing raw red sorghum exhibited a higher humoral immune response assessed by HA titre. By making the protein unavailable for digestion and absorption until it reaches the more acidic abomasum, CT also enhances nutrition by providing high-quality protein to the small intestines (BARRY et al., 2001). By-passing amino acids such as arginine, glutamine and cysteine can enhance immune responses, as these amino acids regulate activation of T and B lymphocytes, lymphocyte proliferation, and the production of antibodies (LI et al., 2007). Parasitized Angora does, grazing forage containing CT (*Sericea lespedeza*), had enhanced immune responses (MIN et al., 2005), and lambs grazing CT containing sulla (*Hedysarum coronarium*) had higher antibody titers against secretory-excretory antigens to *Ostertagia circumcincta* and to *Trichostrongylus colubriformis* (NIEZEN et al., 2002).

The reduction inFEC could be attributed to the direct effect of CT on fecundity (reduced), and killing adult worms, and indirectly by improving immune function against GI parasites through enhanced tissue protein supply (BARRY et al., 2001; NIEZEN et al., 2002; PATHAK et al., 2013a; PATHAK et al., 2013b; PATHAK et al., 2014; SHAIK et al., 2006). MOORE et al. (2008) reported that feeding *Sericea lespedeza* (SL) hay can reduce FEC and increase performance as compared with bermuda grass hay fed goats. Interestingly, higher FEC was observed in the control group (above the threshold level: 600-2000; EFC) as compared to the CT fed groups, which warranted anthelmintics treatment (McKENNA, 1981). However, FEC in the CT groups remained lower than the threshold level and consequently the frequency of medication could be curtailed in the CT fed animals. The combined effects (i.e. antioxidant and availability of EAAs and anthelmintic property) might be a reason for the improved humoral immune response and lowered FEC observed in the present study. The role of tannins on immune response can be understood, since flavonoids, tannins and microelements have been suggested to act as antioxidants and exert their antioxidant activity by scavenging the lipid peroxidation (YUTING et al., 1990). It is apparent that CT supplementation at as little as 1-2% of the diet can noticeably reduce the effect of *H. contortus*. GI parasitism increases the amino acid requirement of GI tissues and consequently peripheral tissues are denied the nutrients required for optimal growth. Normal animal performance in the face of larval challenge may be possible if the protein supply is increased, and this is possible by CT supplementation, which improves the bio-availability of proteins in the rumen (DEY et al., 2008; DUBEY et al., 2012).

On the basis of the morphological characteristics of GI nematode larvae (VAN WYK et al., 2004) the present study showed about 90% of the infective 3rd stage larvae (L_3) were *Haemonchus*.

Conclusion

On the basis of the above results, it may be concluded that dietary supplementation of tanniferous LMM of *F. infectoria* and *P. guajava* (70:30) has the potential to improve the normal health of lambs by improving their erythrocytic antioxidant status, cell mediated and humoral immune response, and the noticeable negative impact on GI nematodes in lambs fed a diet containing 1-2% CT. Thus, LMM of *F. infectoria* and *P. guajava* may be used as a functional feed in the diet of sheep, as it provide a potentially important socio-economical, eco-friendly and sustainable alternative, to reduce anthelmintic resistance, oxidative stress and improve the health status of animals, as well as the standard of living of poor farmers in an organic environment.

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PATHAK, A. K., N. DUTTA, A. K. PATTANAIK, K. SHARMA, P. S. BANERJEE, T. K. GOSWAMI: Učinak dodatka kondenziranih tanina putem obroka od mješavine lišća *Ficus infectoria* i *Psidium guajava* na antioksidacijski status eritrocita, imunosni odgovor i želučano-crijevne obliće u janjadi (*Ovis aries*). Vet. arhiv 87, 139-156, 2017.

SAŽETAK

Istraživanje je provedeno u svrhu procjene učinka kondenziranih tanina (KT), dodanih putem obroka od mješavine lišća *Ficus infectoria* i *Psidium guajava*, na antioksidacijski status eritrocita, imunosni odgovor i želučano-crijevne obliće u janjadi. Dvadeset i četiri janjeta slučajnim su odabirom bila razvrstana u četiri skupine po šest janjadi u svakoj skupini. Randomiziranim kompletnim blok-dizajnom i nasumičnim pridjeljivanjem formirane su četiri skupine s različitim udjelom KT u obroku: skupina KT-0 (0% KT), skupina KT-1 (1%), skupina KT-1,5 (1,5%) i skupina KT-2 (2%). Kod sve janjadi praćen je antioksidacijski status eritrocita 0., 45., 90., 135. i 180. dan hranidbe, a na kraju istraživanja određen je humoralni i stanični imunosni odgovor. Najviša razina ($P<0,05$) haemoglobina utvrđena je u skupini KT-1,5 nakon koje su slijedile skupine KT-1, KT-2 i KT-0. Dodani je KT signifikantno ($P<0,05$) poboljšao antioksidacijski status janjadi što su pokazale povišene razine glutation-peroksidaze, katalaze, zatim redukcija razine glutationa, glutation-S-transferaze, superoksid-dismutaze, ukupnog tiola i protein vezane tiol skupine kao i sniženje razine lipidne peroksidaze. Dodavanje KT signifikantno je ($P<0,05$) poboljšalo stanični imunosni odgovor janjadi. Broj jaja u fecesu bio je signifikantno ($P<0,01$) viši u kontrolnoj skupini (KT-0) janjadi nakon koje su slijedile skupine KT-1, KT-1,5 i KT-2. Skupne kulture iz fecesa janjadi pokazale su da većina invazivnih larvi pripada vrsti *Haemonchus contortus*. Broj jaja u fecesu kontrolne skupine janjadi bio je tijekom cijeloga istraživanog razdoblja signifikantno povišen ($P<0,05$) u odnosu na skupine KT-1,5 i KT-2. Može se zaključiti da je dodavanjem mješavine lišća s 1 – 2% KT u obroku kod janjadi poboljšan antioksidacijski status eritrocita i imunosni odgovor te smanjen broj prazitskih jajašaca u fecesu.

Ključne riječi: antioksidacijski status, kondenzirani tanini, *Haemonchus contortus*, imunosni odgovor, janjad