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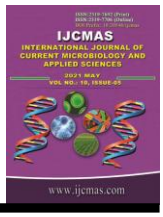

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Effect of Condensed Tannin Feeding on Serum Biochemical Profile and Faecal Egg Count in Nellore Brown Ewes

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A B S T R A C T

Present study was conducted to assess the effect of feeding condensed tannins supplemented through leaf meal mixture on haematology, serum biochemical profile and faecal egg count in Nellore brown ewes. 18 Nellore brown ewes of similar age and body weight (3-3.5 yrs, 25.03±1.52 kg) were selected and randomly divided into three groups Negative Control (dewormed), Control (without deworming), Treatment group (T) of six animals in each group. Both control groups were fed with complete feed, while treatment group ewes were fed with complete feed in which part of roughage was replaced with tanniferous leaf meal mixture to get 3% condensed tannin level in the diet and the experiment was conducted for the period of 120 days. Blood and serum samples were collected periodically (30 days interval) and analysed for haematology and serum biochemical profile. The mean Hb (g/dl) levels were significantly ($P<0.05$) low in C group compared to other groups and significantly ($P<0.05$) high in T, NC groups. The mean PCV (%) values were significantly ($P<0.05$) low in C group compared to other groups. The mean serum urea (mg/dl) levels in the treatment group of lambs were found to be significantly ($P<0.05$) lower compared to both control groups. The fecal egg count was significantly ($P<0.05$) low in treatment group. The results of the present study revealed that inclusion of Condensed tannin at 3% level in the diets of sheep does not cause any effect on health of the animal and also decreases the fecal egg count.

Introduction

Infestation of parasites particularly that of gastrointestinal (GI) tract like helminthes has been regarded as one of the major constraints for animal production as they interfere with nutrient bioavailability for production purposes and may create clinical conditions

leading to productive and economic losses (Githigia *et al.,* 2001). Control strategy for helminthic infection relies on the repeated use of dewormers which results in anti helminthic resistance. In this regard, tannins predominantly condensed tannin (CT) has emerged as a potential contender as natural anthelmintics (Iqbal *et al.,* 2007; Pathak *et al.,* 2013). It has proved its worth for the purpose as evident by several studies claiming reduction in faecal egg count (FEC) and worm load in host animal species (Gujja *et al.,* 2013). Many of Indian tree leaves are rich source of plant secondary metabolites (PSMs) including tannin and also constitute the natural component of small ruminants diets (Bhatta *et al.,* 2005). Due to high level of CT in their leaves, they have displayed their anthelmintic effect with elevation in nutritional and antioxidant status of farm animal models (Dutta *et al.,* 2012; Dey and De, 2014).

Keeping this in view, present study was planned to investigate the effect of feeding CT through LMM (leaf meal mixture) on haematology, serum biochemical profile and fecal egg count in Nellore brown ewes.

Materials and Methods

Present study was carried out at Livestock Farm Complex, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh during the period from 2019 to 2020.

Experimental Animals and Feeding

A total of 18 Nellore brown ewes aged 3-3.5 years with a mean live weight of 25.03±1.52 kg, tested positive for *Haemonchus* infestation were selected and randomly divided into three groups of 6 animals each in a completely randomized design.

Group I ewes (Negative control-NC) were fed with conventional complete feed containing concentrate and roughage (ground nut straw) in the ratio of 50:50 and dewormed using Albendazole suspension @ 10mg/kg body weight. Group II ewes were positive control (C) fed with conventional feed and were not dewormed throughout the experiment Group III animals were served as treatment group (T) they were fed with complete feed in which part of groundnut straw was replaced with dried and grounded tree leaves mixture of *L. leucocephala, F. Benghalensis* and *P. guajava* at 40:40:20 proportions so as to bring the condensed tannin (CT) levels 3% per cent of diet.

All the animals were kept under uniform managemental conditions by housing them in a well-ventilated shed with facilities for uniform feeding and watering.

Collection of Blood

Blood from all experimental animals were collected early in the morning before feeding, by jugular vein puncture.

About 4 ml of whole blood was collected from every animal in EDTA vaccutainer for analysing haematotological profile and another 4ml of blood was collected in another vaccutainer in which clot activator is there to hasten the clotting and allowed for clotting. After clotting, the tubes were centrifuged to collect sera. The collected sera samples were stored in deep freezer for further analysis.

Haematological Parameters

Haemoglobin (Hb) and PCV were analyzed using automatic MIND RAY haemoanalyser

Biochemical parameters

All the biochemical parameters were estimated by using Erba diagnostic kits (TRANSASIA BIO –MEDICALS LTD).

Fecal egg count

Faecal samples were collected (per rectum) from experimental animals for every 15 days. Each sample was put in a plastic bag bearing a number of corresponding tag number of the animal. After collection, the samples were taken to the laboratory and egg counts were made using the modified McMaster technique (Roepstorff and Nansen, 1998).

Statistical Analysis

The results obtained were subjected to analysis of variance using SPSS 25.0 software and treatment means were ranked using Duncan's multiple range tests. The degree of freedom of the treatments was portioned into orthogonal polynomial, depicting linear and quadratic trends associated with increasing levels of CT supplementation. 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

Effect of CT feeding on hematological parameters

Blood samples were collected from sheep on day one of the experiment and thereafter at 30 days intervals for a period of 120 days. The blood was used for estimation of hematological parameters *viz.*, Hemoglobin (Hb) level and packed cell volume (PCV).

Effect on hemoglobin (g/dl) and PCV (%)

The mean Hb levels (g/dl) and PCV (%) of ewes under different experimental groups were presented in Table 1. The Hb (g/dl) values as well as PCV (%) values were found to decrease gradually from beginning to the end of experiment in the control group and this decrease could be attributed to increased parasitic burden. In other groups, there was no significant change in the Hb (g/dl) and PCV (%) values with the time. The mean PCV (%) values were significantly ($P<0.05$) low in C group compared to other groups, while the difference was not significant ($P<0.05$) among other groups.

Therefore, feeding of condensed tannin (CT) reduced the parasitic burden which results no significant change in the Hb (g/dl) and PCV (%) values in the sheep. Similar findings with respect to Hb (g/dl) and PCV (%) were reported by Pathak *et al.,* (2013) in sheep fed CT through leaf meal mixture.

Parallel to the present findings, Moore *et al.,* (2008) observed that feeding of CT up to 4% in the diet has no adverse effect on hematological parameters during the 120 days of feeding experiment. However, it was reported that supplementation of CT at 6% level in goats decreased ($P<0.05$) PCV (%) and Hb (g/dl) level due to binding of the tannin especially with Fe (Olafadehan, 2011).

Based on the results obtained in the present study, the anthelminthic effect of CT may be responsible (Hoste *et al.,* 2012) for optimum PCV (%) and Hb (g/dl) levels in treatment group compared to the control sheep.

Effect of CT feeding on serum biochemical parameters

Different biochemical parameters studied during the experiment were serum glucose, serum proteins (Total protein, Albumin, Globulin & AG Ratio), calcium, phosphorous, copper, total cholesterol, serum creatinine, serum urea, SGOT and SGPT.

Effect on liver function tests

The mean serum total protein, albumin and globulin (g/dl) levels and AG ratio values for

different experimental groups at different periods of the experiment were presented in Table 2. The mean serum total protein, serum albumin and globulin (g/dl) values at the end of the experiment were significantly (P<0.05) lower in control group compared to other groups, while the difference was not significant among other groups. A/G ratio in control groups was significantly (P<0.05) higher when compared to treatment groups. Among the treatment groups, the A/G ratio of T group was found to be significantly (P<0.05) lower. Ahmed *et al.,* (1990) also reported a decrease in total protein, albumin and an increase in Y-globulin (g/dl) levels in *H. contortus* infected sheep. It is possible to link the decreased level of total protein and albumin (g/dl) in infected control group which may occurs as a result of abomasal haemorrhage caused by *H. contortus*. It is attributable to the loss of serum proteins into the gut and the subsequent impaired synthesis of albumin (Sharma *et al.,* 2001) as a result of acute phase response associated with elevated level of globulin (Murata *et al.,* 2004).

However, the elevated globulin (g/dl) level in CT fed group may be due to indirect effect of CT to synthesize more immunoglobulins against *H. contortus.* Similar findings were reported by Dubey *et al.,* (2007) in goats fed with tanniferous tree leaves.

Effect on liver enzymes

No significant difference was observed among different groups with respect to SGOT and SGPT (Table 3). Present results were in agreement with the findings of Pathak *et al.,* (2013) in sheep and Olafadehan (2011) in goats fed with tanniferous forages.

The non significant difference in serum enzyme levels between treatment and control groups in the present study indicated that up to 3 % level of CT feeding does not cause any damage to the internal organs like kidney and liver.

Effect on serum biochemical profile

The difference in mean serum glucose (mg/dl) values among the experimental groups was not statistically significant (P<0.05) and were found to be within the normal physiological range suggested for sheep (Boyd, 1984). The present results were consistent with the findings of Pathalk *et al.,* (2013) and Cenci *et al.,* (2007) who reported no significant difference in serum glucose (mg/dl) values in treatment and control groups.

Contrary to the present findings Wang *et al.,* (1996b) reported lower plasma glucose (mg/dl) level in lactating ewes grazed on tanniferous *Lotus corniculatus* pasture, probably due to increased uptake of glucose from blood for milk lactose synthesis.

The serum cholesterol (mg/dl) values in the present study (Table 4) did not show any significant difference (P<0.05) among all the study groups. Similar results were reported by pathak *et al.,* (2013) for serum cholesterol (mg/dl) in *H. Contortus* infected sheep fed with CT through leaf meal mixture. Similar to the present study Olafadehan (2011) reported no significant change in serum cholesterol (mg/dl) level in Red Sokoto goats fed tannin-rich *Pterocarpus erinaceus* forage diets corroborated with present findings.

The range of creatinine (mg/dl) values reported in the present study was parallel with the findings of Pathak *et al.,* (2013) who reported creatinine (mg/dl) values from 0.94±0.07 to 1.11±0.09 with no significant difference between treatment and control in sheep. Similar results were reported by Olafadehan *et al.,* (2014) in goats fed diets containing varying ratios of tanniferous forage (*Ficus polita).*

Table.1 Effect of condensed tannin feeding on haemoglobin concentration (g/dl) and packed cell volume (%) in ewes

Treatment	Day 1	120 Day	Mean
Haemoglobin concentration (g/dl)			
NC	9.78±0.32	9.18±0.30^{b}	9.39±0.14^{b}
C	8.6±0.24	6.82±0.13^{a}	7.64±0.14^{a}
T	9.87±0.33	9.58±0.51^{b}	9.55±0.19^{b}
Packed cell volume (%)			
NC	40.32±0.37	40.59±0.40^{bc}	39.89±0.23^{b}
C	41.09±0.75	29.00±0.55^{a}	35.07±0.86^{a}
T	40.37±0.39	40.90±0.23^{bc}	41.09±0.20^{b}

^{abc} means with different superscripts with in a column differ significantly (P<0.05)
*NC: Negative control, C: Control, T: Treatment

Table.2 Effect of condensed tannin feeding on serum total protein, albumin, serum globulin and AG ratio

Treatment	Day 1	120 Day	Mean
Serum total protein (g/dl)			
NC	5.8±0.10	6.19±0.08^{b}	6.03±0.04^{b}
C	5.52±0.15	4.98±0.04^{a}	5.20±0.05^{a}
T	5.8±0.18	6.37±0.15^{b}	6.06±0.08^{b}
Serum albumin (g/dl)			
NC	2.63±0.05	3.09±0.13^{b}	2.88±0.05^{b}
C	2.61±0.09	2.49±0.15^{a}	2.5±0.05^{a}
T	2.77±0.08	2.50±0.09^{a}	2.72±0.04^{b}
Serum globulin (g/dl)			
NC	3.17±0.14	3.11±0.16^{b}	3.16±0.05^{b}
C	2.92±0.09	2.50±0.13^{a}	2.70±0.05^{a}
T	3.03±0.12	3.87±0.13^{c}	3.34±0.08^{b}
AG ratio			
NC	0.84±0.05	1.02±0.08	0.92±0.03^{b}
C	0.9±0.04	1.03±0.12	0.94±0.04^{b}
T	0.92±0.04	0.84±0.04	0.83±0.02^{a}

^{abc} means with different superscripts with in a column differ significantly (P<0.05)

Table.3 Effect of condensed tannin feeding on liver enzymes

Treatment	Day 1	120 Day	Mean
SGOT (IU/L)			
NC	55.28±2.77	73.89±1.03	64.10±1.53
C	50.42±3.21	72.64±1.95	59.50±1.85
T	57.36±1.79	71.93±1.20	62.79±1.24
SGPT (IU/L)			
NC	23.17±0.86	28.54±0.41	26.98±0.46
C	25.15±1.42	28.74±0.54	27.75±0.46
T	24.66±0.75	29.63±0.31	28.16±0.41

Table.4 Effect of condensed tannin feeding on serum biochemical profile in ewes

Treatment	Day 1	120 Day	Mean
	Serum creatinine (mg/dl)		
NC	0.85±0.03	1.21±0.03	0.97±0.04
C	0.81±0.02	1.30±0.09	1.00±0.04
T	0.87±0.04	1.30±0.03	1.03±0.04
	Serum urea (mg/dl)		
NC	41.76±0.22	40.25±0.40^{b}	40.65±0.15^{b}
C	42.28±0.20	42.77±0.28c	42.24±0.11^{b}
T	42.02±0.29	28.30±0.31^{a}	34.49±1.00^{a}
	Serum glucose (mg/dl)		
NC	51.56±0.90	54.06±0.76	52.83±0.38
C	47.24±1.90	47.02±1.24	47.82±0.71
T	49.26±1.53	51.40±0.87	50.39±0.58
	Serum cholesterol (mg/dl)		
NC	56.73±1.75	63.64±0.98	60.34±1.09
C	63.25±1.11	60.77±0.95	60.85±1.17
T	59.54±4.04	65.88±1.56	61.12±1.04

^{abc} means with different superscripts with in a column differ significantly (P<0.05)

Table.5 Effect of condensed tannin feeding on serum mineral profile in ewes

Treatment	Day 1	120 Day	Mean
	Serum calcium (mg/dl)		
NC	10.18±0.23	10.23±0.22	10.16±0.13
C	10.16±0.19	10.63±0.25	10.47±0.15
T	10.09±0.23	9.80±0.47	10.20±0.15
	Serum phosphorous (mg/dl)		
NC	5.27±0.06	5.14±0.14	5.29±0.04
C	5.35±0.10	5.68±0.19	5.41±0.05
T	5.29±0.011	5.57±0.16	5.48±0.07
	Serum copper (mg/dl)		
NC	0.53 ±0.01	0.41 ±0.04	0.50 ±0.01
C	0.59 ±0.07	0.55 ±0.01	0.56 ±0.02
T	0.52 ±0.05	0.46 ±0.01	0.49 ±0.02

^{abc} means with different superscripts with in a column differ significantly (P<0.05)

Table.6 Effect of condensed tannin feeding on faecal egg count

		Fortnight Intervals							
Treatment	**Day 1**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
NC	425.00 ±58.81^{a}	350.00 ±51.64^{a}	425.00 ±38.19^{a}	575.00 ±58.81^{a}	758.33 ±66.35^{a}	200.00 ±22.36^{a}	366.67 ±24.72^{a}	583.33 ±44.10^{a}	783.33 ±62.80^{b}
C	2441.67±10 0.35^{b}	2716.67±14 8.16^{c}	2883.33±13 2.71^{c}	2850.00±3 4.16^{d}	3783.33 ±89.47^{d}	3916.67±11 9.49^{d}	3866.67±10 2.20^{d}	4316.67±8 4.58^{c}	4533.33±8 4.33^{c}
T	2500.00±14 3.76^{b}	2300.00±11 5.47^{b}	1983.33±10 7.75^{b}	1233.33±7 1.49^{b}	1050.00 ±42.82^{b}	866.67 ±76.01^{b}	600.00 ±68.31^{b}	466.67 ±80.28^{a}	350.00 ±34.16^{a}

^{abc} means with different superscripts with in a column differ significantly (P<0.05)

The insignificant effect of feeding of tannins on creatinine levels in the present study might be due to absence of wasting or catabolism of muscle tissues (Olafadehan 2011).

Serum urea (mg/dl) level is an indicator of protein degradation in rumen. The mean urea concentrations in condensed tannin fed groups were found to be significantly (P<0.05) lower compared to the control groups. Serum urea (mg/dl) levels in ewes were ranged from 42.24±0.11 in control group to 34.49±1.00 in T group. Pan and Maitra (1992) also observed lower (29.48 vs. 36.94 mg/dl) blood urea levels in sheep with and without CT supplemented diets.

Effect on serum mineral profile

The serum Ca, P and Cu (mg/dl) values (Table 5) did not differ significantly (P<0.05) among different dietary treatments and the values were found to be within the normal physiological range for sheep (Boyd, 1984). Similar findings of non significant difference in Ca and P (mg/dl) values were reported by Pathak *et al.,* (2013) in sheep supplemented with CT through leaf meal mixture.

Effect on fecal egg count

The faecal egg counts (Table 6) on day 120 were reduced by 86.0% in T group, when compared with the day one faecal egg counts of the experiment. Similar to the present findings, Cenci *et al.,* (2007) reported that faecal egg count (FEC), was significantly reduced in lambs fed with tanniferous herbage (*Acácia negra*) within eight weeks of the experiment. Similarly Moore *et al., (*2008) and Shaik *et al.,* (2006) also observed that feeding of *Sericea lespedeza* hay and *L. cuneata* hay, respectively significantly reduced the FEC in goats compared to control animals. Several earlier studies were also demonstrated the anthelminthic effect of tanniferous herbage in sheep and goat (Gregory *et al.,* 2017; Manolaraki *et al.,* 2010).

Several workers reported repressed female nematode reproductive activity as another direct effect of CT on GIN, which explained the decrease in faecal egg count (FEC) measured in several trials where feeding experiments were conducted with tannin-rich forages (Ahmed *et al.,* 2010; Heckendorn 2007). An indirect effect of tannins by a stimulation of the local immune response, due to their protective effect for proteins against ruminal degradation (Mangan, 1988) has also been evoked as one option to explain their action on nematodes.

CT supplementation from various forages and tree have been found to reduce the level of GI parasitic infestation in cattle (Novobilsky *et al.,* 2011), buffalo (Netpana *et al.,* 2001), goat (Joshi *et al.,* 2011) and sheep (Cresswell *et al.,* 2004).

The present study concluded that dietary supplementation of CT at 3% effectively reduce the faecal egg count in sheep, thereby reduce the use of chemical dewormers and provides an alternative path for organic sheep production.

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