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Research Article

EFFECT OF FEEDING OAK (*Quercus leucotrichophora*) LEAVES ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF PARASITIC INFECTED GOAT IN KUMAON HILLS

CHAURASIYA A.*[1], TAMBOLI P.[2], SAHOO B.[3], KURIYAL A.[4], NEHRA A.[5] AND SANKAR M.[6]
[1,4]Animal Nutrition, ICAR- Indian Veterinary Research Institute, Mukteswar, Nainital, Uttarakhand 263138, India
[2]ICAR-National Dairy Research Institute, Karnal, Haryana 132001, India
[3]Pr.Scientist, Animal Nutrition, ICAR- Central Institute for Women in Agriculture, Bhubaneswar, Odisha, 751003, India
[5,6]Veterinary Parasitology, ICAR- Indian Veterinary Research Institute, Mukteswar, Nainital, Uttarakhand 263138, India
*Corresponding Author: Email-amitspchaurasiya@gmail.com

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Abstract: The present study was undertaken to explore the haematological and biochemical changes due to feeding oak leaves (*Quercus leucotricophora*) having 3.35% condensed tannin and the comparative effect of oak (*Quercus leucotricophora*) leaves with or without polyethylene glycol (PEG) in goats infected with *Haemonchous contortus* for its anthelmintic property. Twenty four local male goats of about 6-7 months of age were randomly divided into three homogenous groups (T_1, T_2 and T_3) of eight animals each. Further, each group was subdivided in to 2 sub groups of 4 animals each and one sub group in each group was treated with synthetic anthelmintic (Ivermectin @ 200μg/kg body wt.) (TA+). Experimental feeding was similar in the three groups except for the roughage source, which was local green grass (*Pennisetum clandestinum*) in T_{1A-} and T_{1A+}, oak leaves (*Q. leucotricophora*) in T_{2A-} and T_{2A+} and oak leaves supplemented with PEG in group T_{3A-} and T_{3A+}, respectively. Concentrate and roughage ratio was maintained 30:70. DM intake (g d^{-1}) through roughage, total DM intake and organic matter intake in oak leaves fed groups (T_{2A-}, T_{2A+}, T_{3A-} and T_{3A+}) were higher ($P<0.05$) than the grass fed groups (T_{1A-}, T_{1A+}). Average daily gain (ADG; g d^{-1}) was lower ($P<0.01$) in grass fed control (T_{1A-}) than oak leaves fed groups with or without PEG supplementation and anthelmintic treatment. Mean Hb (g dl^{-1}) and PCV (%) levels were higher ($P<0.05$) in oak fed goats than in grass leaves fed groups. The BUN (mg dl^{-1}) was lower ($P<0.05$) in oak leaves fed groups while increased with PEG supplementation and highest in grass fed group (T_{1A-}). It was concluded that feeding of oak leaves improved the haematological parameters, blood glucose, serum proteins and maintains the BUN as well as liver enzymes level in the body. Their by it enhances the growth performance of parasitic infected goats showing anthelmintic property without any adverse effect in the animal health and performance.

Keywords: *BUN (Blood Urea Nitrogen), Condensed tannin, Oak leaves, Serum*

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Introduction

The Kumaon hills at an altitude of 2286 meters (7500 feet) occupy largest land area within the middle or lesser Himalayan region. Goat rearing is an integral part of hill farmer's nutrition and economy. It is universally accepted as a profitable animal without any threat to ecology. However, animal productivity is quite low mainly due to nutritional inadequacy pertaining to one of the principle cause, *i.e.* scarcity of forage. The availability of pasture in hilly region is limited to a very short period of the year in rainy season (July to October) only. Other than fodder scarcity, goats also suffer from heavy gastrointestinal parasitic infection in temperate hills of Himalaya, a major problem which adversely affects their health and performance. Worldwide, gastrointestinal nematode, mostly *Haemonchus contortus* infection (95-97%) remain a major threat for the economic viability of small ruminants [1]. *H. contortus*, an adult parasite can ingest 0.05 ml of blood /helminth/day [2] which leads to marked anaemia in the animal and decrease their growth and production [3]. The Indian government expends $103 millions every year to control gastrointestinal parasite with application of chemotherapeutic agents like benzimidazoles, levamisole and ivermectin but anthelmintic drug resistance become a major problem leading to failure of parasites control programme. Thus, alternative environment friendly sustainable novel strategies are required to reduce the exclusive reliance on anthelmintic and to control gastrointestinal nematodes without causing the drug resistance. Several studies in the small ruminant species have shown that the consumption of a condensed tannin (CT) rich feed was associated with improved haemato-biochemical parameters in animal. Oak leave (*Quercus leucotrichophora*) is the dominant, climax tree species and most abundantly available throughout the year in the moist temperate forests of the Indian Himalayan region [4]. Condensed tannin binding agent polyethylene glycol (PEG) has ability to neutralize CT by displacing protein-tannin complexes, as a consequence of CT interact more strongly with PEG than they do with protein. PEG has been used to reduce the negative effect of tannins. Keeping in view, the present study was designed to study the effect of feeding *Q. leucotrichophora* leaves on haematological and biochemical parameters, as an alternative to control *H. contortus* and performance of goats in Kumaon hills and to study the comparative effect of *Q. leucotrichophora* leaves with or without polyethylene glycol (PEG) in goats infected with *H. contortus* for its anthelmintic property.

Materials and methods

The present study was undertaken to explore the haematological and biochemical changes due to feeding oak leaves (*Quercus leucotricophora*) having 3.35% condensed tannin. Twenty four local adult male goats of about 6-7 months of age were selected and randomly divided into three homogenous groups (T1, T2 and T3) of eight animals each.

Table-1 Chemical composition (% DM basis) of different feeds and fodders

Nutrients	Concentrate mixture	*Pennisetum clandestinum*	*Quercus leucotrichophora*
Organic matter	92.23	91.62	96.45
Crude protein	21.74	9.84	10.61
Ether extract	4.41	1.50	5.01
Total carbohydrates	66.08	80.28	80.82
Neutral detergent fibre	36.80	76.70	62.30
Acid detergent fibre	14.92	47.80	52.67
Ash	7.77	8.38	3.55
Calcium	1.22	0.67	1.21
Phosphorus	0.72	0.42	0.19
Total tannin	-	-	6.45
Condensed tannin	-	-	3.35
Hydrolysable tannin	-	-	3.10

Table-2 Intake of feed, DM and OM in kids during metabolism trial

Attributes	T_{1A-}	T_{1A+}	T_{2A-}	T_{2A+}	T_{3A-}	T_{3A+}	SEM	P value
Concentrate								
g d^{-1}	144	162	177	172	208	169	12.17	0.83
g $d^{-1}kg^{-1}W^{0.75}$	21.67	22.00	22.76	22.54	23.77	22.34	0.89	0.82
% body weight	1.16	1.15	1.16	1.16	1.15	1.16	0.02	0.20
Roughage								
g d^{-1}*	243^{a}	284^{a}	400^{b}	383^{b}	454^{c}	391^{b}	47.40	0.02
g $d^{-1}kg^{-1}W^{0.75}$**	37.00^{a}	38.83^{a}	51.15^{b}	49.83^{b}	51.90^{b}	51.83^{b}	3.92	0.01
% body weight**	2.19^{a}	2.16^{a}	2.39^{b}	2.46^{b}	2.58^{c}	2.69^{b}	0.05	0.01
Dry matter								
g d^{-1} *	387^{a}	446^{a}	577^{b}	555^{b}	662^{c}	560^{b}	69.73	0.02
g $d^{-1}kg^{-1}W^{0.75}$**	58.67^{a}	60.83^{a}	73.91^{b}	72.37^{b}	75.63^{c}	74.17^{b}	2.69	0.01
% body weight**	3.35^{a}	3.31^{a}	3.55^{b}	3.62^{b}	3.73^{c}	3.85^{b}	0.07	0.01
Organic matter								
g d^{-1}*	352^{a}	406^{a}	549^{b}	528^{b}	630^{c}	533^{b}	39.56	0.03
g $d^{-1}kg^{-1}W^{0.75}$**	51.31^{a}	55.33^{ab}	70.28^{b}	68.80^{b}	71.92^{b}	70.65^{b}	2.23	0.01
% body weight**	2.85^{a}	2.91^{ab}	3.56^{b}	3.52^{b}	3.50^{b}	3.56^{b}	0.08	0.01
Concentrate: Roughage	37:63	36:64	31:69	31:69	31:69	30:70		

*a,b,c Means bearing different superscripts in a row differ significantly *P<0.05,**<0.01*

Table-3 Effect of feeding tanniferous oak leaves on TLC and DLC

TLC											
Fortnight	T_{1A-}	T_{1A+}	T_{2A-}	T_{2A+}	T_{3A-}	T_{3A+}	Mean	SEM	T	P	T*P
Initial	19825	20125	13875	17625	16025	15075	17091	583.94	0.09	0.08	0.67
2 wk	15550	13400	14550	14625	16875	15725	15120				
4 wk	17325	15225	17225	12025	11325	10950	14012				
6 wk	13450	13025	15525	12800	10525	9350	12445				
8 wk	14500	12050	14450	11825	9275	8650	11791				
10 wk	15325	13325	14550	14150	11075	9400	12970				
12 wk	15275	13525	14175	12225	10900	9550	12608				
14 wk	13700	13400	13300	12000	10500	8875	11962				
16 wk	13200	11550	12675	10650	10000	8950	11170				
17 wk	13100	10950	11950	10100	9425	8875	10733				
Mean	15125	13657	14227	12802	11592	10540					
SEM	452.21										
Eosinophil											
Initial	1.50	1.00	2.75	0.50	0.75	1.50	1.33^{a}	0.22	0.02	0.05	0.31
2 wk	2.00	0.00	1.00	2.50	1.00	1.50	1.33^{a}				
4 wk	5.50	5.00	2.75	2.50	5.00	3.00	3.96^{c}				
6 wk	4.75	1.25	1.00	1.00	4.00	1.75	2.29^{b}				
8 wk	2.75	1.50	1.50	1.00	2.00	1.00	1.63^{a}				
10 wk	3.00	0.50	0.75	1.50	1.25	0.00	1.17^{a}				
12 wk	2.75	1.25	1.00	1.25	1.00	1.50	1.46^{a}				
14 wk	3.50	1.50	1.25	2.75	1.25	1.50	1.96^{a}				
16 wk	2.00	1.50	1.25	2.25	2.00	1.50	1.75^{a}				
17 wk	2.50	1.00	1.00	2.00	2.00	1.25	1.63^{a}				
Mean	3.03^{c}	1.45^{a}	1.43^{a}	1.73^{a}	2.03^{b}	1.45^{a}					
SEM	0.17										

*a,b,c Means bearing different superscripts in a row differ significantly*P<0.05,**P<0.01*

Table-4 Haematlological and biochemical indices in different groups of kids

Treatments	Days post experimental feeding			Mean	SEM	P values		
	0	60	120			T	P	T*P
			Haemoglobin (gdl^{-1})					
T_{1A-}	7.55	7.90	8.25	7.90^{a}	0.21	0.02	0.01	0.09
T_{1A+}	7.25	9.15	8.60	8.33^{a}				
T_{2A-}	8.38	9.35	9.65	9.13^{b}				
T_{2A+}	8.13	9.73	9.48	9.11^{b}				
T_{3A-}	8.95	9.13	9.00	9.03^{b}				
T_{3A-}	8.75	9.35	9.65	9.25^{b}				
Mean	8.17^{a}	9.10^{b}	9.10^{b}					
SEM	0.14							
			PCV (%)					
T_{1A-}	22.65	23.7	24.75	23.70^{a}	0.62	0.02	0.01	0.09
T_{1A+}	21.75	27.45	25.8	25.00^{a}				
T_{2A-}	25.13	28.05	28.95	27.38^{b}				
T_{2A+}	24.38	29.18	28.43	27.33^{b}				
T_{3A-}	26.85	27.38	27.00	27.08^{b}				
T_{3A-}	26.25	28.05	28.95	27.75^{b}				
Mean	24.50^{a}	27.30^{b}	27.31^{b}					
SEM	0.44							
			Glucose (mg dl^{-1})					
T_{1A-}	53.33	45.67	46.50	48.50	2.12	0.23	0.18	0.63
T_{1A+}	52.00	52.17	50.33	51.50				
T_{2A-}	54.33	51.50	51.00	52.28				
T_{2A+}	52.33	54.33	54.33	53.66				
T_{3A-}	51.02	52.33	52.33	51.89				
T_{3A-}	53.34	51.02	51.02	51.79				
Mean	52.73	51.17	50.92					
SEM	1.68							
			BUN (mg dl^{-1})					
T_{1A-}	18.21	19.61	19.61	19.15^{d}	0.52	0.07	0.04	0.33
T_{1A+}	18.91	18.21	18.21	18.4^{cd}				
T_{2A-}	21.02	15.41	13.31	16.58^{ab}				
T_{2A+}	19.61	14.01	12.61	15.41^{a}				
T_{3A-}	19.61	16.11	16.11	17.28^{bc}				
T_{3A-}	19.61	15.41	15.41	16.81^{ab}				
Mean	19.50^{b}	16.46^{a}	15.88^{a}					
SEM	1.62							

^{a,b,c,d,ab,bc,cd} *Means bearing different superscripts in a row differ significantly*P<0.05,**P<0.01*

Table-5 Serum biochemical profile in different groups of kids

Treatments	Days post experimental feeding			Mean	SEM	P values		
	0	60	120			T	P	T*P
			Total Protein (g dl^{-1})					
T_{1A-}	6.81	6.35	6.50	6.55	0.128	0.06	0.25	0.91
T_{1A+}	6.94	6.65	6.65	6.75				
T_{2A-}	6.80	6.8	6.80	6.80				
T_{2A+}	6.94	6.94	6.94	6.94				
T_{3A-}	6.80	6.65	6.65	6.71				
T_{3A-}	6.94	6.8	6.80	6.84				
Mean	6.87	6.7	6.72					
SEM	0.09							
			Albumin (g dl^{-1})					
T_{1A-}	3.21	3.24	3.29	3.25^{a}	0.69	0.04	0.09	0.63
T_{1A+}	3.36	3.36	3.36	3.36^{a}				
T_{2A-}	3.38	3.38	3.43	3.40^{a}				
T_{2A+}	3.5	3.43	3.5	3.48^{b}				
T_{3A-}	3.33	3.33	3.33	3.33^{a}				
T_{3A-}	3.48	3.48	3.38	3.41^{a}				
Mean	3.38	3.37	3.38					
SEM	0.04							
			Globulin (g dl^{-1})					
T_{1A-}	3.58	3.11	3.21	3.31	0.15	0.06	0.55	0.79
T_{1A+}	3.59	3.29	3.29	3.43				
T_{2A-}	3.41	3.41	3.37	3.40				
T_{2A+}	3.44	3.51	3.44	3.45				
T_{3A-}	3.46	3.31	3.31	3.36				
T_{3A-}	3.47	3.32	3.41	3.43				
Mean	3.49	3.33	3.34					
SEM	0.1							
			Albumin: Globulin (A: G) ratio					
T_{1A-}	0.94	1.08	1.04	1.02	0.6	0.29	0.09	0.76
T_{1A+}	0.96	1.06	1.06	1.03				
T_{2A-}	1.01	1.02	1.05	1.02				
T_{2A+}	1.02	0.98	1.02	1.01				
T_{3A-}	0.98	1.01	1.01	1.00				
T_{3A-}	1.05	1.09	1.01	1.05				
Mean	0.99	1.04	1.03					
SEM	0.04							

^{a,b,c} *Means bearing different superscripts in a row differ significantly*P<0.05*

Table-6 Serum enzyme profile in different groups of kids

Treatments	Days post experimental feeding			Mean	SEM	P values		
	0	60	120			T	P	T*P
ALT (IU L^{-1})								
T_{1A-}	13.13	15.00	16.88	15.00	2.29	0.36	0.05	0.65
T_{1A+}	15.00	15.00	16.88	15.63				
T_{2A-}	15.00	16.88	22.50	18.13				
T_{2A+}	13.13	16.88	22.50	17.50				
T_{3A-}	15.00	18.75	26.25	20.00				
T_{3A-}	15.00	18.75	26.25	20.00				
Mean	14.38^{a}	16.88^{a}	21.88^{b}					
SEM	1.62							
AST (IU L^{-1})								
T_{1A-}	72	96	102	90^{a}	7.93	0.03	0.01	0.11
T_{1A+}	69	81	102	84^{a}				
T_{2A-}	93	117	132	114^{bc}				
T_{2A+}	84	105	99	96^{ab}				
T_{3A-}	87	123	156	122^{c}				
T_{3A-}	93	111	138	114^{bc}				
Mean	83^{a}	105.6^{b}	121.5^{c}					
SEM	5.52							
ALP (IU L^{-1})								
T_{1A-}	99.02	120.1	195.1	138.07^{ab}	0.55	0.04	0.01	0.17
T_{1A+}	97.55	120.59	193.63	137.25^{a}				
T_{2A-}	96.73	160.13	192.65	149.84^{bc}				
T_{2A+}	96.41	159.64	191.5	149.18^{ab}				
T_{3A-}	97.06	171.9	194.93	154.63^{c}				
T_{3A-}	97.22	176.31	194.12	155.88^{bc}				
Mean	97.33^{a}	151.44^{b}	193.65^{c}					
SEM	0.38							

^{a,b,c,ab,bc} *Means bearing different superscripts in a column differ significantly*P<0.05,**P<0.01*

Further, each group was subdivided in to 2 sub groups of 4 animals each and one sub group in each group was provided with synthetic anthelmintics (Ivermectin @ 200μg/kg body wt.) (TA+). Experimental feeding was similar in the three groups except for the roughage source, which was local green grass (*Pennisetum clandestinum*) in groups T1A- and T1A+, tanniferous oak tree leaves (*Quercus leucotricophora*) (Banjh) in group T2A- and T2A+ and oak tree leaves (*Quercus leucotricophora*) (Banjh. The body weights of all the goats were recorded at fortnightly intervals before o) supplemented with PEG in group T3A- and T3A+, respectively. The oak leaves were procured daily and fed to the animals in T2A-, T2A+, T3A- and T3A+, local grass was fed to T1A- and T1A+ preferably in the afternoon offering feed and water throughout the experimental period. The ground samples of feed and feces were analyzed for different proximate constituents as per the methods described by [5]. The extraction and estimation of total phenolics and tannins were done as per the methods of [6]. Blood from all animals was collected at fortnightly intervals of experimental period to study the hematological, serum biochemical, enzymatic profile by puncturing the jugular vein with the help of a clean sterilized needle into two separate test tubes. The first test tube contained sodium EDTA (anticoagulant) and second one was without anticoagulant. The serum was separated carefully from the second test tube to check haemolysis and serum samples were stored at -200C for further analysis. Haemoglobin estimation was done by Sahli's acid haematin method [7]. Haemtocrit estimation was done by Macrohaematocrit (Wintrobe) method [7]. Total leucocyte count (TLC) estimation was done by haemocytometer while Differential leucocyte count (DLC) estimation was done by Giemsa stained blood smear under oil immersion. All the serum biochemical parameters were estimated by using diagnostic kits (Cogent) manufactured by Span Diagnostics Pvt. Ltd., Surat, India. All the data generated in the above experiments were statistically analyzed using [8] computer package. For comparison of groups, Generalized Linear Model ANOVA procedure and Duncan's multiple range tests were used [9].

Result and Discussion

Nutritional parameters: The chemical composition and fiber fraction of concentrate mixture, oak leaves (*Quercus leucotricophora*) and native grass (*Pennisetum clandestinum*) offered to goats (kids) was within the normal range [Table-1] [10]. The organic matter (OM) and ether extract (EE) content of oak leaves were comparatively more than the concentrate mixture and grass. The tannin content (CT) analyzed in oak leaves of Himalayan temperate hills were low-moderate (3.35%) and also similar to the findings of many workers [11]. [Table-2] showing higher DM intake (DMI) through roughage (oak leaves) raised the total DMI in anthelmintic treated and oak fed groups T1A+, T2A-, T2A+, T3A- and T3A+ than in grass fed group T1A-. The higher organic matter intake in oak leaves fed group of animals was attributed to higher level of OM along with higher DM intake through oak leaves. In consistent with the present findings, dry matter intake was reported to be higher in goats fed on *Q. leucotricophora* [12] and cattle fed on *Q. leucotricophora* based diet [13]. Higher growth rate of kids fed on oak leaves based diet in the present study could be due to less adverse effects of CT of oak leaves, variable nature of tannin, anti- nutritive activity on feed digestibility and its better nutrients utilization efficiency in vivo [14]. Haematological parameters: Haematological indices like haemoglobin (Hb) and packed cell volume (PCV) [Table-4], the indicators of erythrocytic normalcy and wellbeing of animals were significantly higher in oak fed animals and did not show any adverse effect feeding tanniferous oak leaves. The TLC was decreased linearly with advance of period, and DLC was comparable in all the groups except eosinophil which was increased at 4^{th} week due to peak parasitic infection [Table-3]. The values were lower in oak leaves fed group (T2A-, T2A+) which was attributed to anthelmintic property of oak leaves. Similar results were reported in small ruminants by several authors [15, 16] suggesting that CT as an additive up to 1.6 % of diet induced positive effect on target haematological parameters. Glucose: Serum glucose level, the indicator of normal physiology remained similar in all the experimental kids were within the normal range [Table-4][17]. Any alteration in glucose level is an indicator of stress to the animals. [15] reported no significant change in serum glucose with added CT. BUN: Serum urea nitrogen 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 urea pool [18]. In the present study, the urea nitrogen level in case of oak leaves fed group (T2A-, T2A+, T3A- and T3A+) was significantly lower (P<0.05) at 120 days of post feeding than the other groups [Fig-1]. This lower level of urea nitrogen may be due to reduced rumen protein breakdown and increased EAA absorption in the small intestine [18]. Low serum urea nitrogen level in oak leaves fed group indicates the effect of CT on serum urea nitrogen concentration [13]. Condensed tannins reduce rumen proteolysis, limiting the ruminal microbial activity [19, 21] resulting into lower rumen ammonia concentration which might have contributed to the low

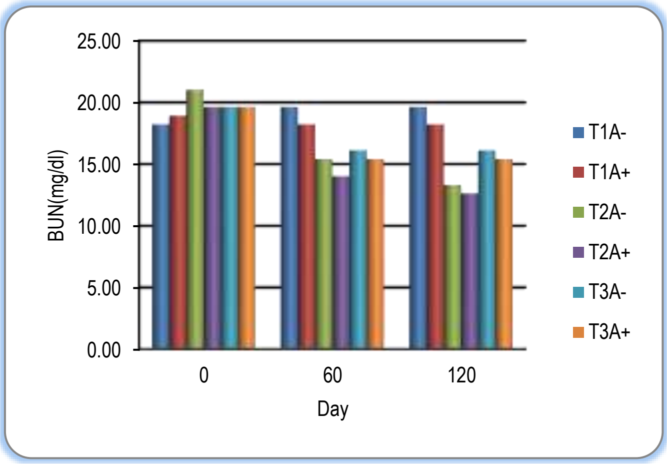

Fig-1 Serum urea nitrogen in different groups of kids

concentration of plasma urea nitrogen. This correlates very well with increased N utilisation efficiency in oak leaves-fed groups. Low level of urea nitrogen was observed in kids fed leaves of Prosopsis cineraria [21]. [16] reported significantly lower serum urea nitrogen level in lambs supplemented with graded levels (0-2%) of CT. Also [22] reported decrease serum urea nitrogen in goats fed with kermes oak foliage (CT-7.1%) supplemented with PEG. Serum Protein: In parasitic infection the serum protein level decreased due to the loss of protein in injured gut [23], but the mean values of protein, albumin, globulin and A:G ratio of kids under different treatments were within the normal physiological range [Table-5] indicating that tannin at low to moderate level induced no adverse effect on goats [15]. In the present study, total protein (TP) and globulin was comparable within the treatments and albumin was significantly higher ($P<0.01$) in anthelmintic treated oak leaves fed group (T2A+) compared to the grass fed groups which might be due to high intake of dietary protein and positive interaction of CT with protein [24]. Similar results were found by [16] with graded levels (0-2%) of CT and [25] with lambs fed diets having 1-2% CT. Serum enzymes: Serum enzyme levels of AST, ALT and ALP are conventionally used for diagnosing hepatic damage and to detect bile obstruction, *i.e.* mild and progressive damage to the liver in animals [26]. Release of some toxins by *Haemonchus contortus* causes dysfunction of liver cells resulting increased activities of AST, ALT and alkaline phosphatase [27]. In the present study, there was no significant difference on enzymatic activity of ALT while AST and ALP was higher ($P<0.05$) in oak leaves fed groups [Table-6]. The level of ALT, AST and ALP was also increased with the advancement of period, but the values were within the normal range. In contrary to the present findings, [16] reported non-significant effect on serum enzymes (ALT and AST) in lambs supplemented with graded levels (0-2%) of CT. Thus, feeding of *Q. leucotricophora* (3.35% CT) leaves may not have any adverse effect on the vital organs like liver, kidney and heart *etc.*

Conclusion

Feeding of oak leaves (3.35% CT) improved the haematological parameters, blood glucose, serum proteins and maintains the BUN as well as liver enzyme level in the body. There by it enhances the growth performance of parasitic infected goats showing anthelmintic property without any adverse effect in the animal health and performance.

Application of research: Study of anthelmintic property

Research Category: Animal Nutrition

Abbreviations

Hb: haemoglobin
PCV: packed cell volume
DLC: Differential leucocyte count
TLC: Total leucocyte count

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***Research Guide: Dr Amit Chaurasiya and Dr B. Sahoo**
***Research Co-Guide: Dr M Sankar**
Institute: ICAR- Indian Veterinary Research Institute, Mukteswar, Nainital, Uttarakhand 263138
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