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Effect of Feeding Tannin Rich Oak (*Quercus leucotrichophora*) Leaves on Immunological Parameters, Antioxidant Status and Microbial Nitrogen Supply of Parasitic Infected Goats in Kumaon Hills

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The present study was undertaken to explore the immunological, antioxidant effect as well as microbial protein synthesis potency of oak leaves (*Quercus leucotricophora*) having 3.35% condensed tannin with 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.) (T_{A+}). 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. Microbial nitrogen (gd^{-1}) was higher ($P<0.01$) in oak fed groups ($T_{2A-,}$ T_{2A+}) than PEG supplemented groups (T_{3A-}, T_{3A+}) and grass fed groups (T_{1A-}, T_{1A+}). The IgG and IgA were decreased at 28th day PI and again improved significantly in groups treated with anthelmintic and group fed with oak leaves ($T_{1A+,}$ $T_{2A-,}$ $T_{2A+,}$ $T_{3A+)}$ while it decreased with PEG supplementation (T_{3A-}) and lowest in the non-treated grass fed group (T_{1A-}). The antioxidant property from GSH, SOD and catalase was significantly increased in oak fed groups ($T_{1A+,}$ $T_{2A-,}$ $T_{2A+,}$ T_{3A+}) than grass fed groups (T_{1A-}, T_{1A+}). It was concluded that feeding of oak leaves having CT upto 1.6% in diet improved the microbial nitrogen supply, immunoglobulin status and antioxidant status of growing kids.

Introduction

Livestock rearing is an integral part of hill farmer's nutrition and economy. Goat is the popular species among marginal, sub-marginal and landless farmers in Himalayan region which 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 which witness the prevalence of highest parasitism in grazing animals (Gupta *et al.,* 1987). Other than fodder scarcity, goats also suffer from heavy gastrointestinal parasitic infection in temperate hills of Himalaya (Kumar *et al.,* 2015), 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 (Hostea *et al.,* 2012). Several studies in the small ruminant species have shown that the consumption of a condensed tannin (CT) rich feed was associated with a modulation of the biology of adult worm populations, affecting particularly the egg excretion (Shaik *et al.,* 2006) through direct and indirect effect. Tannin also has some antioxidant property (Andrade *et al.,* 2005) which hampers parasitic growth. 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 (Singh *et al.,* 1996) and forms the bulk of livestock feed during the critical forage scarcity period of winters. feeding of oak leaves (3.35% CT) improves the feed intake and growth performance of goats (Chaurasiya *et al.,* 2018b) with improved the haematological parameters, blood glucose, serum proteins and maintains the BUN as well as liver enzymes level in the body (Chaurasiya *et al.,* 2018a). According to Chaurasiya *et al.,* 2018c *Quercus leucotrichophora* (3.35% CT) leaves also having anthelmintic property without any adverse effect. It also improves the humoral-cell mediated immune response with antioxidant property in small ruminants (Pathak *et al.,* 2014) which provide potency to counteract with parasitic infection. 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 in livestock which facilitate microbial protein synthesis *in vivo* (Landau *et al.,* 2000). Keeping in view, the present study was designed to explore the immunological, antioxidant effect as well as microbial protein synthesis potency of feeding *Q. leucotrichophora* leaves as an alternative to control *H. contortus* with or without polyethylene glycol (PEG) in goats infected with *H. contortus* for its anthelmintic property.

Materials and Methods

The *in vivo* experiment was carried out at experimental goat farm of Indian Veterinary Research Institute, Mukteshwar in Uttarakhand (28^{o} 53' 24"-31^{o} 27' 50" N and 77^{o} 34' 27"-81^{o} 02' 22" E) of India. It is located at 2250-2350 m above sea level in the Kumaon regions of temperate Sub Himalayas. The ambient temperature ranges from -10.0°C to 26°C and the relative humidity ranges from 43 to 86% (150-200 cm precipitation per annum).

Animals: Selection and grouping

Twenty four local adult male goats of about 6-7 months of age were selected at goat farm, Surmane and randomly divided into three homogenous groups (T_1, T_2 and T_3) of eight animals each based on age and body weight. 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 (T_{A+}) to compare its effect with natural anthelmintic property of condensed tannin of oak leaves.

Feeds and feeding

Experimental feeding was similar in the three groups except for the roughage source, which was local green grass (*Pennisetum*

clandestinum) in groups T_{1A-} and T_{1A+}, tanniferous oak tree leaves (*Quercus leucotricophora*) (Banjh) in group T_{2A-} and T_{2A+} and oak tree leaves (*Quercus leucotricophora*) (Banjh) supplemented with PEG in group T_{3A-} and T_{3A+}, respectively. The concentrate mixture was fed twice a day, morning and evening. The oak leaves were procured daily and fed to the animals in T_{2A-}, T_{2A+}, T_{3A-} and T_{3A+}, local grass was fed to T_{1A-} and T_{1A+} preferably in the afternoon so as to allow sufficient time for the consumption of concentrate mixture. The PEG was given with the concentrate mixture after dissolving in clean water. Fresh water was offered *ad libitum* twice daily to all the animals.

Collection of blood

Blood from all animals was collected at fortnightly intervals of experimental period to study the hematological, serum biochemical, enzymatic profile, immunological parameter and antioxidant property 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 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 -20^0C for further analysis.

Collection and aliquoting of urine

Urine excreted daily by individual animal was collected separately in urine bottle for 24h and were measured with the help of measuring cylinder, then representative sample was brought to the laboratory in properly marked, well stoppered sample bottle.

Analysis of feed and fodder

The ground samples of feed and feces were analyzed for different proximate constituents as per the methods described by AOAC (2000). Samples of feeds and faeces were analyzed for different fiber components as per the method given by Van Soest *et al.,* (1991). The extraction and estimation of total phenolics and tannins were done as per the methods of Makkar (2000).

Analysis of urine samples for purine derivatives and microbial protein synthesis

Allantoin in urine and plasma was determined by the colorimetric method of Young and Conway. Urine was analysed directly after filtration (0.22µm Millipore filter) and dilution (1:50). Uric acid was determined by the colorimetric method. In enzymatic method xanthine and hypoxanthine are converted to uric acid and thus determined as uric acid, which is monitored by its absorbance at 293 nm.

Total PD in urine

Total PD= Allantoin (mmol/d) + Uric acid (mmol/d) + Xanthine and Hypoxanthine (mmol/d)

Daily purine absorption

$X = Y- (0.217\ kgW^{0.75}\ e^{-0.25x})/0.84$

Where, Y is urinary PD excretion and W0.75 represents metabolic body weight (kg) of the animal.

Microbial nitrogen supply

Microbial nitrogen (g/day) = 0.727 X (where X is daily purine absorption)

DOMR: organic matter fermented in rumen

DOMR = Feed intake × DM content (%/100) × OM content (%/100) × OM digestibility (%/100) × 0.65

Immunological parameters

Bluegene Goat Immunoglobulin A ELISA kit was used in this study for quantitative estimation of IgA using serum. Goat Immunoglobulin G ELISA kit (Bethyl Laboratories, USA) was used in this study for quantitative estimation of IgG using serum as sample.

Antixodant profile

On each collection, Blood samples were collected aseptically from jugular vein of kids in 2 ml appendrop tube with acid citrate dextrose (@ 1.5ml/10 ml blood) as anticoagulant and centrifuges at 2000 rpm for 15 min at 4^0C, followed by separation of plasma and buffy coat. The resulting erythrocyte pellet was washed thrice with 250 mOsm/L (pH-7.4) phosphate buffer saline as per Yagi *et al.,* (1989). The erythrocyte pellet (packed RBC) obtained was mixed with an equal volume of PBS to form RBC suspension. 0.5 ml RBC suspension was mixed with 4.5ml stabilizing solution (EDTA, 2.7mM and 0.7 mM, β marcaptoethanol) to prepare a haemolysate of 1:20 dilution. The haemoglobin in RBC suspension was estimated by Cyanomethaemoglobin method. Reduced glutathione was estimated by DNTB method of Prins and Loos (1969). Catalaes was assayed in erythrocytes by spectrophotometric method as described by Bergmeyer (1983). Superoxide dismutase (SOD) activity of RBC haemolysate samples was measured using nitro blue tetrazolium as a substrate after suitable dilution according to method of Marklund (1974).

Statistical analysis

All the data generated in the above experiments were statistically analyzed using SPSS (2005) computer package. For comparison of groups, Generalized Linear Model ANOVA procedure and Duncan's multiple range tests were used (Snedecor and Cochran, 1994).

Results and Discussion

Chemical composition of experimental diets

The chemical composition of concentrate mixture, oak leaves (*Quercus leucotricophora*) and native grass (*Pennisetum clandestinum*) offered to goats (kids) was within the normal range (Ranjhan, 1998; Paswan *et al.,* 2008) (Table 1). Higher DM intake (DMI) through roughage (oak leaves) raised the total DMI in anthelmintic treated and oak fed groups $T_{1A+,}$ $T_{2A-,}$ $T_{2A+,}$ T_{3A-} and T_{3A+} than in grass fed group T_{1A-}. The nutritional effects of CT are related with their ability to bind with proteins (dietary and enzymes), structural carbohydrate polymers found in plant cell walls and minerals with an overall effect of depressing the bioavailability of nutrients at specific sites in the gastro-intestinal tract (Ndluvo, 2000). Moderate concentration of tannin (2-4.5% CT) from *L. corniculatus* reduce rumen forage protein degradation due to reversible binding to these proteins and reduce the populations of proteolytic rumen bacteria without reducing the amount of microbial protein synthesis, as a result, there was improvement in milk production, wool growth, ovulation rate and lambing percentage due to action of CT in increasing EAA absorption from small intestine (Min *et al.,* 2003).

Microbial protein synthesis

Ruminants' feeds usually have a low purine content, most of which undergoes extensive degradation in the rumen as the result of microbial fermentation. Therefore, nucleic acids leaving the rumen are essentially of microbial origin. Absorbed nucleic acid purine are degraded and excreted in urine as their

derivatives, allantoin, uric acid, xanthine and hypoxanthine (Chen *et al.*, 1990). The urinary excretion of purine derivatives is directly related to the microbial purine absorption and increasingly being used as well accepted indicator for estimating microbial protein synthesis in the rumen (Fujihara *et al.*, 1987). Daily excretion (mmol/day) of purine derivatives (Allantoin, uric acid, xanthine and hypoxanthine) and total purine, microbial nitrogen supply in urine was significantly increased with CT supplementation as compared to grass fed groups (Table 2).

The DOMI was also significantly increased in oak leaves fed groups than grass fed groups. The DOMI, excretion of purine derivatives and microbial nitrogen supply was significantly decreased with PEG supplementation which might be due to binding of PEG with condensed tannin of oak leaves. In contrast, PEG has been used to reduce the negative effect of tannins in livestock which facilitate microbial protein synthesis *in vivo* (Landau *et al.*, 2000).

The 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). CT supplementation on rumen microbial protein synthesis was similar with the observations found earlier in sheep fed tannin containing *Acacia* pods and *F. infectoria* (Ngwa *et al.*, 2002). The findings suggested that the feeding of *Q. leucotrichophora* leaves as CT source having positive impact on microbial nitrogen synthesis.

Although tannins are generally regarded as antinutritional, certain tannins at low-moderate concentrations are known to enhance microbial protein synthesis (Bhatta *et al.*, 2002) and PEG supplementation is not useful with low-moderate level of CT as it decreases the microbial protein synthesis.

Immunological status

Table 3 showing that, IgG and IgA were decreased at 28th day of post infection and again improved significantly in anthelmintic treated groups (T_{1A+}, T_{2A+}, T_{3A+}) and group fed with oak leaves (T_{2A-}) while it decreased with PEG supplementation(T_{3A-}) and lowest in the non-treated grass fed group (T_{1A-}). The grass fed group of animals showed higher concentration of IgE throughout the experimental trial compared to anthelmintic treated groups. Highest concentration was observed on 14th to 21st day of post infection (PI). After 21st day, concentration showed a continuous decreased up to the end of experimental trial. With conformity of these findings Kooyman *et al.*, (1997) reported increased level of IgE specific to *H. contortus* 2-4 weeks PI. Increased level of IgG_1 has been reported to be followed by IgG_2, IgM and IgA (Schallig *et al.*, 1994). Among all the immunoglobulin isotypes, IgE is the most investigated in nematode infections and it has been found to increase the most during infection. In the present experiment, grass fed group of animals showed higher concentration of IgE throughout the experimental trial compared to anthelmintic treated and oak fed groups.

Antioxidant status

The antioxidant status is the representative of health status of the body as they are important part of body defense mechanism by scavenging free radicals (ROS: Reactive oxygen species) which damages the biological system (Padh, 1991). Haematological values of GSH, SOD and catalase are the representative of antioxidant status of body (Bisla *et al.*, 2002; Han *et al.*, 2004).

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 fiber	36.80	76.70	62.30
Acid detergent fiber	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 Effect of feeding tanniferous oak leaves on microbial nitrogen supply

Fortnight	T_{1A-}	T_{1A+}	T_{2A-}	T_{2A+}	T_{3A-}	T_{3A+}	SEM	P value
Allantoin ** (mmol/day)	4.25 ^{a}	4.38 ^{a}	5.00 ^{b}	5.00 ^{b}	4.25 ^{a}	4.50 ^{ab}	0.09	0.01
Uric acid * (mmol/day)	0.18 ^{a}	0.21 ^{ab}	0.23 ^{b}	0.24 ^{b}	0.17 ^{a}	0.20 ^{ab}	0.02	0.04
Xanthine and Hypoxanthine* (mmol/day)	0.25 ^{a}	0.31 ^{ab}	0.32 ^{ab}	0.34 ^{b}	0.28 ^{ab}	0.32 ^{ab}	0.02	0.02
Total PD*	4.68 ^{a}	4.89 ^{ab}	5.55 ^{b}	5.58 ^{b}	4.69 ^{a}	5.02 ^{ab}	0.10	0.02
PD absorption (mmol/day)	5.25	5.62	6.30	6.61	5.56	5.69	0.14	0.23
Microbial N** (g/day)	3.40 ^{a}	3.56 ^{a}	4.03 ^{b}	4.06 ^{b}	3.41 ^{a}	3.65 ^{a}	0.07	0.01
DOMR	10.52	16.86	20.28	21.14	18.97	19.21	1.87	0.66
DOMI**	9.92 ^{a}	10.51 ^{a}	11.85 ^{bc}	12.19 ^{c}	10.25 ^{a}	10.71 ^{ab}	0.23	0.01

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

Table.3 Effect of feeding tanniferous oak leaves on immunoglobulins

Fortnight	T_{1A-}	T_{1A+}	T_{2A-}	T_{2A+}	T_{3A-}	T_{3A+}	Mean	SEM	T	P	T*P
IgA (µg/ml)											
Initial	91.78	79.10	86.97	84.41	83.31	84.46	85.00^{c}	2.26	0.05	0.04	0.23
2 wk	84.75	82.63	82.87	80.67	77.91	72.96	80.30^{b}				
4 wk	73.49	72.08	76.95	72.67	73.53	67.76	72.75^{a}				
6 wk	76.61	74.58	75.17	78.00	75.68	72.26	75.38^{b}				
8 wk	75.17	76.86	73.34	78.50	75.73	75.17	75.79^{b}				
10 wk	67.67	76.83	75.17	77.67	68.25	76.50	73.68^{ab}				
Mean	78.25^{ab}	77.01^{b}	78.41^{bc}	78.65^{c}	75.73^{a}	74.85^{b}					
SEM	2.03										
IgG (µg/ml)											
Initial	2420.00	2371.96	2343.28	2405.00	2365.00	2280.00	2364.21^{c}	77.55	0.01	0.02	0.11
2 wk	2060.00	2034.40	2295.00	1825.00	1905.00	1875.00	1999.07^{b}				
4 wk	1569.85	1992.62	1780.00	1480.00	1823.02	1775.00	1736.75^{a}				
6 wk	1830.00	1990.00	2015.00	2210.00	1920.00	2005.00	1995.00^{b}				
8 wk	1945.00	2270.00	2305.00	2505.00	2005.00	2325.00	2225.83^{bc}				
10 wk	2005.00	2490.00	2390.00	2770.00	2255.00	2520.00	2405.00 ^{c}				
Mean	1971.64^{a}	2191.50^{b}	2188.05^{b}	2199.17^{b}	2045.50^{a}	2130.00^{b}					
SEM	67.59										

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

Table.4 Effect of feeding tanniferous oak leaves on antioxidant status

Treatments	Days post experimental feeding			Mean	SEM	P values		
	0	60	120			T	P	T*P
GSH (µmol/ml haemolysate)								
T_{1A-}	1.34	1.35	1.36	1.35[a]	0.021	0.15	0.88	0.43
T_{1A+}	1.41	1.41	1.42	1.4[a]				
T_{2A-}	1.51	1.51	1.5	1.5[b]				
T_{2A+}	1.56	1.54	1.53	1.54[b]				
T_{3A-}	1.44	1.44	1.46	1.44[ab]				
T_{3A-}	1.49	1.49	1.48	1.49[ab]				
Mean	1.46	1.46	1.46					
SEM	0.11							
SOD (mmol MTT formazon formed/g Hb)								
T_{1A-}	1.55	1.49	1.51	1.52[a]	0.02	0.01	0.29	0.64
T_{1A+}	1.53	1.68	1.49	1.56[a]				
T_{2A-}	1.39	1.75	1.71	1.61[ab]				
T_{2A+}	1.7	1.71	1.75	1.72[b]				
T_{3A-}	1.7	1.7	1.74	1.71[b]				
T_{3A-}	1.7	1.71	1.7	1.70[b]				
Mean	1.59	1.67	1.65					
SEM	0.23							
Catalase (mK/L haemolysate)								
T_{1A-}	1.19	1.2	1.2	1.19[a]	0.16	0.04	0.08	0.47
T_{1A+}	1.2	1.2	1.2	1.20[a]				
T_{2A-}	1.24	1.23	1.24	1.24[b]				
T_{2A+}	1.25	1.25	1.27	1.26[b]				
T_{3A-}	1.21	1.22	1.26	1.23[b]				
T_{3A-}	1.25	1.24	1.27	1.25[b]				
Mean	1.22	1.22	1.24					
SEM	0.17							

[a,b,c,ab] Means bearing different superscripts in a column differ significantly*P<0.05,**P<0.01

Table 4 shows the antioxidant status from GSH and SOD was significantly increased (P<0.05) in oak leaves fed groups (T_{1A+}, T_{2A-}, T_{2A+}, T_{3A+}) than grass fed groups (T_{1A-}, T_{1A+}) which might be due to anthelmintic property of tannin of oak leaves reducing the worm load and indirectly improved the antioxidant status of animals fed oak leaves. However, the catalase activity in different treatments was similar. The GSH level was not altered with PEG supplementation in oak fed groups (T_{3A-}, T_{3A+}), might be due to binding of tannin of oak leaves by PEG. It is assumed that dietary condensed tannins may display their first antioxidant defence in the digestive tract, by limiting ROS formation and scavenging them. Similar to the findings Dubey *et al.*, (2011) reported increased GSH, SOD and catalase activity in kids feeding with 1-2% CT.

It was concluded that feeding of oak leaves having CT upto 1.6% in diet improved the

microbial nitrogen supply, immunoglobulin status and antioxidant status of growing kids without any adverse effect in the animal health and performance.

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