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Effect of Feeding Oak (*Quercus leucotrichophora*) Leaves as Alternative Control of Gastro Intestinal Nematode Infection of Goat in Kumaon Hills

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A B S T R A C T

The present study was undertaken to explore the anthelmintic effect of feeding oak leaves (*Quercus leucotricophora*) having 3.35% condensed tannin 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.) (T_{A+}). Experimental feeding was similar in all 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. 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+}). The fecal egg count was lower ($P<0.01$) in animals fed oak leaves and anthelmintic treated groups. It was concluded that feeding of oak leaves improved the feed intake as well as showing anthelmintic property.

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. 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 severely suffer from heavy gastrointestinal parasitic infection in temperate hills of Himalaya, 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). *H. contortus, an* adult parasite can ingest 0.05 ml of blood/helminth/day (Rowe *et al.*, 1988) which leads to marked anemia in the animal and decrease their growth and

production (Hayat *et al.*, 1996). 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 anthelmintics 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 a modulation of the biology of adult worm populations, affecting particularly the egg excretion (Shaik *et al.*, 2006) through direct and indirect effect. 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. *Q. leucotrichophora* contain moderate level of CT (3-4%) and shows positive effect on voluntary feed intake, nutrient utilization, live weight gain in animals and reduces the GI nematode load in kids (Raju *et al.*, 2015). 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 as an alternative to control *H. contortus* 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 anthelmintic effect of feeding oak leaves (*Quercus leucotricophora*) having 3.35% condensed tannin on nutrient utilization (especially protein, macrominerals), and the comparative effect of oak (*Quercus leucotricophora*) leaves with or without polyethylene glycol (PEG) in goats infected with *H. contortus* for its anthelmintic property.

Twenty four local adult male goats of about 6-7 months of age were selected at goat farm, Surmane, Mukteshwar, Nainital, Uttarakhand 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. The goats were kept under uniform managemental conditions throughout the experimental period of 120 days by housing them in well ventilated pukka shed with facilities for individual feeding. Ample clean drinking water was provided to all the animals. Proper health management and sanitation was also provided during the course of experimentation. 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 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. 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. Area Specific mineral mixture developed at IVRI, Mukteshwar was supplemented @ 2% of concentrate mixture throughout the experimental period. The animals were fed a weighed quantity of concentrate and roughage daily. The weight of residues was recorded in the next morning. The feed samples and residues were collected and sampled for DM estimation to assess DM intake in animals during the whole feeding trial of 120 days at fortnight's intervals. 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).

Faecal samples were collected directly from rectum of the animals and put into the faecal bags. The faecal nematode egg count was performed by a modified McMaster technique (Anon, 1977). 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

The present study was undertaken to assess the anthelmintic effect of feeding oak leaves (*Quercus leucotrichophora*) having condensed tannin (3.35 %) to control *H. contortus*, nutrient utilization and growth performance of goat, and also to assess the comparative effect of oak (*Quercus leucotrichophora*) leaves with or without polyethylene glycol (PEG) in goats infected with *H. contortus* for its anthelmintic property during a period of 4 months. Results of the study are summarized as follow-

Chemical composition of experimental diets

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 (Paswan *et al.,* 2008) (Table 1). The organic matter (OM) and ether extract (EE) content of oak leaves were comparatively more than the concentrate mixture and grass. Similarly, fiber fraction (NDF and ADF) of oak leaves and grass was more than concentrate mixture which was attributed to high cell wall constituents in roughage (Dubey *et al.,* 2011). However, comparatively lower total ash and NDF content of oak leaves than grass was an indication of better quality of nutrients in oak leaves. 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 (Makkar and Becker, 1998). Climate, environmental factors like temperature and precipitation, radiation, nutrient availability, soil pH and other factors such as age of plant and maturity of leaves and other potentially defensive traits such as toughness, fibrosity and thorns are known to influence tannin level (Makkar and Becker, 1998) in oak leaves.

Feed intake

Table 2 showing, 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 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* (Raju *et al.,* 2015) and cattle fed on *Q.leucotricophora* based diet (Paswan and Sahoo, 2012 and Sharma *et al.,*2008). Higher voluntary intake

of moderate (1-4% CT) level of CT containing diets was reported by many workers (Dey *et al.,* 2008). However, Singh *et al* (1996) reported no difference in DMI *Q. semecarpifolia* leaves and oats hay fed to Pashmina kids. CT can prevent efficient nutrient utilization by limiting digestibility and feed intake (McSweeney *et al.,* 2001). CT may have some detrimental effect on animal's appetite and feed intake when given in the diet at a level > 3% of CT (Bengaly *et al.,* 2007). Similarly, tannin tends to affect the nutritive value of ruminant feeds by reducing voluntary feed intake and digestibility (Barry and McNabb, 1999). The relative increase in DMI and voluntary feed intake in oak leaves fed groups than the grass fed group may be attributed to a number of factors like contribution of higher neutral detergent soluble or non-structural carbohydrates (23.3% in *P. clandestinum* and 37.7% in *Q. leucotricophora*) through oak leaves, better ruminal environment contributed possibly due to CT from oak leaves and relatively better immune and antioxidant status of kids under oak leaves fed groups than the grass fed group.

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

Fig.1 Faecal egg counts (Egg per gram) in different groups of kids

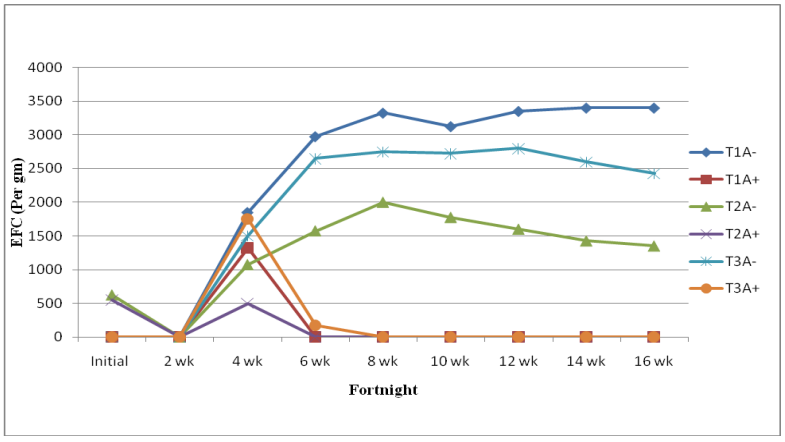

Table.2 Feed Intake

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 faecal egg counts

Fortnight	T_{1A-}	T_{1A+}	T_{2A-}	T_{2A+}	T_{3A-}	T_{3A+}	Mean	SEM	T	P	T*P
Initial	0.00	0.00	625.00	550.00	0.00	0.00	195.83[a]	119.22	0.01	0.01	0.01
2 wk	0.00	0.00	0.00	0.00	0.00	0.00	0.00[a]				
4 wk	1850.00	1325.00	1075.00	500.00	1500.00	1750.00	1333.33[b]				
6 wk	2975.00	0.00	1575.00	0.00	2650.00	175.00	1229.17 [b]				
8 wk	3325.00	0.00	2000.00	0.00	2750.00	0.00	1345.83 [b]				
10 wk	3125.00	0.00	1775.00	0.00	2725.00	0.00	1270.83 [b]				
12 wk	3350.00	0.00	1600.00	0.00	2800.00	0.00	1291.67 [b]				
14 wk	3400.00	0.00	1425.00	0.00	2600.00	0.00	1237.50 [b]				
16 wk	3400.00	0.00	1350.00	0.00	2425.00	0.00	1195.83 [b]				
Mean	2380.56[d]	147.22[a]	1269.44[b]	116.67[a]	1938.89[c]	213.89[a]					
SEM	97.34										

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

Faecal egg count

Table 3 showing Mean faecal egg counts (FECs) of kids in oak leaves fed groups (T_{2A-}, T_{2A+}) were significantly lower in comparison to PEG supplemented groups (T_{3A-} and T_{3A+}) and highest in grass fed group (T_{1A-}). In consistent with the present findings, Sahoo *et al.,* (2004) also reported reduced parasitic counts in calves fed with tanniferous oak leaves. The findings are in agreement with the previous reports (Barry *et al.,* 2001; Min *et al.,* 2003), who reported that dietary supplementation of CT may be used an alternative parasite management strategy. Several studies in the small ruminant species have shown that the consumption of a tannin-rich feed was associated with a modulation of the biology of adult worm populations, affecting particularly the egg excretion (Shaik *et al.,* 2006).

On the other hand, some recent *in vitro* evidence has shown that a contact with condensed tannin-rich extracts affects the establishment of third-stage larvae by disturbing the exsheathment (Brunet *et al.,* 2007). Reduced FEC have been attributed to both direct reduced fecundity and killing of adult worms; Shaik *et al* (2006) and indirect, the dietary supplementation of CT improved immune function against GIN through enhanced tissue protein supply (Niezen *et al.,* 2002).

Pathak *et al.,* (2013) also observed that CT extracts from various tree leaves can disrupt the life cycle of *H. contortus* by preventing their eggs from hatching and by preventing larval development to the infective stage and by direct killing of adult *H. Contortus*. As drug resistance has become an important issue in small ruminant husbandry because of repeated use of chemical anthelmintics (Pandey *et al.,* 2001) leading to anthelmintic failure (Kaplan, 2004). Alternative parasite management strategies using forages containing CT have recently been suggested (Min *et al.,* 2003). However, CT from all plant sources may not be effective in suppressing GIN infection (Naumann *et al.,* 2013). In the present findings, PEG supplementation in oak leaves based diet increased the FEC which clearly indicates that PEG binds with tannin and nullifies the anthelm

intic property of tannin. Hence, PEG should not be used with low-modarate level of CT which are beneficial for the host. Similarly, results from *in vitro* study with CT deactivating agent like PEG (Akkari *et al.,* 2008) or polyvinylpyrrolidone (Alonso-Diaz *et al.,* 2008), inhibit completely CT effect on 3rd instar larvae of gastro-intestinal nematodes. PEG suplementation to goats grazing tanniferous shrubs resulting in increased FECs.

In the present study, feeding of oak leaves decreased the faecal egg count by 42% (Fig 3.1) without any adverse effect on animal and normal animal performance in the face of larval challenge, corroborated with the findings of Min and Hart (2003) who reported a 76% reduction in total adult worm burden in Tracer goats grazed on *L. cuneata* compared to control. They further reported that *L. cuneata* diet resulted in a 94% reduction in *H. contortus* adults, a 100% reduction in *Teladorsagia* spp., and 45% lower numbers of *Trichostrongylus*. Similar results were also reported by Moore *et al* (2008) for goats fed *Sericea lespedeza* compared with *Burmuda grass* hay.

In conclusion, feeding of oak leaves (3.35% CT) improved the feed intake and growth performance of goats showing anthelmintic property and significantly lowered the worm load without any adverse effect in the animal health and performance.

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