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Effect of Inclusion of Different Locally Available Tree Barks in Total Mixed Ration on Serum Biochemical Profile and Parasitic Load in Nellore Ram Lambs

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A B S T R A C T

An experiment was carried out to study the effect of inclusion of different locally available tree barks in total mixed ration on serum biochemical profile and parasitic load in Nellore ram lambs. A ninety day growth trail was conducted with twenty eight Nellore Jodipi ram lambs (13.72 ± 0.05 kg B.Wt) which were randomly (CRD) allotted to four treatment groups with seven animals per treatment and were fed total mixed ration T1 (control), T2 (10% Neem bark), T3 (10% tamarind bark), T4 (10% Acacia bark). All the four total mixed rations were iso-nitrogenous. Feed intake was recorded daily while, body weights were recorded at weekly intervals. Blood samples were collected early in the morning at day 0 and 90 of the study. Faecal samples were collected early in the morning at fort -nightly interval. Serum biochemical profile of ram lambs at the beginning of the experiment indicated non-significant ($P > 0.05$) difference for serum glucose, total protein, globulin, ALT, AST and ALT: AST, cholesterol, triglycerides except for albumin ($P<0.05$). Serum biochemical profile at the end of the experiment revealed non-significant ($P > 0.05$) difference for serum glucose, total protein, albumin, globulin, ALT, AST and ALT: AST, but the concentration of cholesterol and triglycerides significantly ($P< 0.05$) reduced in the treatment groups supplemented with different tree barks when compared to control group. There was no significant ($P > 0.05$) difference in the faecal egg count of ram lambs fed total mixed rations containing different tree barks at 10% level when compared with control group throughout the experimental period. The egg count is reduced towards at the end of the experiment in the treatment groups supplemented with different tree barks. While, the egg count is increased towards the end of the experiment in control group. Addition of different tree barks in the diet didn't have negative impact overall health status of the animal.

Introduction

In India, small ruminants are having important economic value to the small and marginal farmers and landless labourers. India has an estimated sheep and goat population of 74.2 million and 144.8 million respectively (20th Livestock census, 2019).Sheep with its multi-facet utility for wool, meat, milk, skins and manure, form an important component of rural economy. Despite of fabulous contribution towards economy, its profitability is hampered by poor availability of feed resources and disease management causing high morbidity and mortality and resulting in reduced production. Judicious use of locally available feed resources facilitates economic production. The tree bark is a by-product of paper and timber industry obtained from bark-stripping of trunks. Wood pulp and bark residues have been used as energy supplements during periods of critical feed shortage, but have never been generally recognized as alternatives to conventional feed stuffs under normal conditions (Scott *et al.,* 1969). Though wood and bark residues contributes little to the dietary energy needs of ruminants, they can serve as alternatives to conventional roughages. The use of bark in ruminant rations also helps to alleviate the disposal problem of timber industry.

A large number of pure organic chemicals includes flavonoids, tannins, essential oils, alkaloids, carbohydrates, inositols, terpenoids, glycosides, saponins, esters, steroids, gums, resins and fats can be isolated from bark. In addition, bark possess anthelminthic, anti-microbial, anti-diarrheal, anti-oxidant, hepato-protective, hypoglycaemic, anti-inflammatory, antiseptic and immune-stimulant properties (Mohammad *et al.,* 2014 and Rajvaidhya *et al.,* 2012). This provided a suggestion that tree bark may affect the ruminal ecosystem and could enhance the health status of the ruminants. Improved animal performance (Min *et al.,* 2012) and a positive impact on N-balance (Min *et al.,* 2015b) and meeting elevated calcium demands during lactation (Masaki *et al.,* 2004) were observed by using different tree barks. A direct anthelmintic effect of a bark extract high in condensed tannins (Quebracho) towards an adult T. colubriformis population has been demonstrated (Athanasiadou *et al.,* 2000).

However, research on different locally available tree barks under Indian conditions in the livestock feeding especially on blood biochemistry and their anthelmintic property is limited. Keeping this in view, the experiment was carried out to study the effect of inclusion of neem, tamarind and acacia tree barks in total mixed ration on serum biochemical profile and faecal egg count in Nellore ram lambs.

Materials and Methods

Tree barks of neem (*Azadirachta indica*), tamarind (*Tamarindus indica*) and acacia (*Acacia nilotica*) species were collected during the month of January and February from the intact mature trees. An incision was made down the tree trunk vertically with the knife. Bark was scrapped or unwrapped by pushing the knife between outer bark and the cambium. Outer bark was manually separated and naturally flaked dry pieces of bark were also collected, dried, ground and stored. Dried samples of tree bark were analysed for proximate principles (AOAC, 2005). The total phenolic compounds and non-tannin phenolic compounds present in the tree bark samples were estimated according to the procedure given by Makkar *et al.,* (1993). The amount of condensed tannins present in the sample was estimated according to the procedure described by Porter *et al.,* (1985).

Four iso-nitrogenous complete rations were formulated (CP 16.25% Average) and were

designated as T1, T2, T3 and T4. In T1 (control), Super Napier grass (Pennisetum purpureum) and groundnut straw (*Arachis hypogaea*) were incorporated at 30% level each, along with concentrate ingredients at 40% level. Whereas, T2, T3 and T4 comprised Super Napier grass and groundnut haulms at 25% level each, along with concentrate ingredients added at 40% level. Different tree barks *viz.*, Neem, Tamarind and Acacia were added at 10% level in total mixed rations T2, T3 and T4, respectively.

Twenty eight Nellore ram lambs (at Sheep Project Unit, Livestock Research Station, Palamaner) of 3-4 months age and of uniform conformation were selected and divided by completely randomized block design (CRD) into four groups of seven animals in each and were subjected to feeding trial of 90 days. Throughout the experimental period, the measured quantity of TMR was offered daily in the morning and evening to individual animals and leftover was collected the next morning to determine daily feed intake. Fresh drinking water was made available throughout the day. The body weight of the individual animal was recorded at fortnightly intervals in the morning before feeding and watering. Blood samples were collected on day 0 and 90 via the jugular vein in EDTA (3 mL) and non-EDTA (10 mL) containing Vacutainer tubes and were analysed for complete blood counts and packed cell volume (PCV). Plasma was then harvested by centrifugation (1500 rpm) and stored at −20°C for analyses of serum biochemical constituents. The parameters like RBC count, WBC count, haemoglobin, platelets, PCV, MCV, MCH, and MCHC were analysed using Mindray Vet 2800 Haematology analyser. The serum biochemical constituents (glucose by Glucose oxidase/peroxidise method, Total protein by Biuret method, Albumin by Bromocresol green method, Triglycerides by glycerol phosphate oxidase/peroxidase method, Total cholesterol by cholesterol oxidase/peroxidase method and the activity of enzymes (AST and ALT) were estimated using A15 automated biochemical analyser by Biosystems A 15 analyser kits.

Faecal samples were collected early in the morning to carry out faecal egg count. Fresh Faecal samples about 10 g were collected directly from rectum. Faecal samples were brought to the laboratory after affixing a proper identification label in zip-lock airtight polythene bags to slow down the egg development and hatching. Precautions were taken to avoid drying of faeces. Samples which could not be processed immediately were stored at 4^{o}C and examination was done within 4 days of collection. Faecal egg counts (FEC) were determined by modified Mc Master technique (Zajac and Conboy, 2012).

The data obtained were subjected to analysis through software (version 23.0; SPSS, 2015) by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test with a significance at $P< 0.05$ (Duncan,1955). The experimental protocols that were developed in this study were fully complied with the ethical principles of animal experimentation prepared by Institutional animal ethic committee (IAEC) with reference number 281/ go/ S/ 2000/ CPCSEA/ CVSc/ TPTY/ 012/ ANN/ 2020.

Results and Discussion

The chemical composition of total mixed ration indicated that DM, CP and EE (%) contents were similar (Table 1) among the different rations and were iso-nitrogenous with CP content of 16.25% (Average). The amount (%) of total phenolic compounds, condensed tannins and hydrolysable tannins found to be more in Acacia bark, followed by Neem, and Tamarind (Table 2).

Hemogram and blood serum chemistry of sheep at beginning and end of the period consuming different tree barks are presented in Tables 3, 4 and 5, respectively. These variables were used as a diagnostic tool for screening and monitoring animal health problems and abnormality. Serum concentrations of alanine transaminase, aspartate aminotransferase, α-glutamyl transpeptidase, alkaline phosphatase, and cholesterol are conventionally used for diagnosing human and domestic animal hepatic damage (Silanikove and Tiomkin, 1992).

In the present study, hemogram (Table 3) revealed a non-significant (P > 0.05) difference in Hb, WBC, RBC, PCV, MCV, MCH and MCHC between the groups except for platelet count. However, the values found to be within the normal range indicating that different tree barks included rations did not show any deleterious effect on blood profile.

Serum biochemical profile of ram lambs at the beginning of the experiment indicated non-significant (P > 0.05) difference for serum glucose, total protein, globulin, ALT, AST and ALT: AST, cholesterol, triglycerides except for albumin (Table 4). The concentration of albumin (g/dL) was significantly (P < 0.05) higher in the T4 followed by T1 or T3 and lowest (P < 0.05) in T2.

Serum biochemical profile at the end of the experiment revealed non-significant (P > 0.05) difference for serum glucose, total protein, albumin, globulin, ALT, AST and ALT: AST except for cholesterol and triglycerides (Table 5). The concentration of cholesterol and triglycerides (mg/dL) was higher (P < 0.05) in control when compared to Neem tree bark or Tamarind tree bark or Acacia tree bark included total mixed ration. A significant (P< 0.05) reduction in the concentration of serum cholesterol (59, 61 and 73.5% in neem, tamarind and acacia bark included rations) and triglycerides (37, 32 and 78% in neem, tamarind and acacia bark included rations) towards the end of the experiment. Similar to the present findings, El-Beshbishy*et al.*, (2006) reported a significant (P < 0.001) reduction (62 and 44%) in plasma total cholesterol and triglycerides in hyper-cholesteremic rats on oral supplementation of methanol fraction of *Morus alba* L. root bark extract (at 500 mg/ kg/day for 15 days) and Subash *et al.*, (2007) observed a significant decrease in serum total cholesterol and triglyceride concentrations in rats with cinnamon bark powder administration (20 mg/Kg B. Wt). Reduced serum cholesterol and triglycerides concentration in treatment groups receiving tree barks, could be attributed to various pharmacological properties of different tree barks.

Table.1 Chemical composition (%) of total mixed rations containing different tree barks*

Parameter	T1	T2	T3	T4
DM	94.68	94.67	94.64	94.64
OM	84.89	82.53	83.84	86.57
CP	16.30	16.27	16.21	16.23
CF	20.64	20.24	21.78	22.25
EE	1.23	1.16	1.12	1.15
TA	15.11	17.47	16.16	13.43
NFE	46.72	44.86	44.73	46.94

Table.2 Phenolic constituents present in different tree barks

Compound	Neem bark	Tamarind bark	Acacia bark
Total phenolic compounds (%)	0.322	0.267	0.841
Non-tannin phenolic compounds (%)	0.119	0.117	0.124
Pure tannins (%)	0.266	0.165	0.776
Condensed tannins (%)	0.09	0.02	0.20
Hydrolysable tannins (%)	0.176	0.145	0.576

Table.3 Blood profile of ram lambs fed total mixed rations containing different tree barks

Parameter	T1	T2	T3	T4
Hb (g/dl)	11.30±0.49	11.48±0.42	10.37±0.28	10.24±0.37
WBC ($x10^3$ /µl)	12.40±0.73	11.37±0.71	12.53±0.47	11.48±0.81
RBC ($x10^6$ /µl)	13.41±0.62	13.52±0.55	12.27±0.38	12.41±0.58
Platelets ($x10^5$ /µl)*	10.95^{ab}±0.89	10.98^{ab}±0.90	10.42^{b}±0.76	13.38^{a}±1.06
PCV (%)	32.23±1.39	32.70±1.18	29.80±0.70	29.85±0.98
MCV (fL)	24.12±0.30	24.28±0.63	24.37±0.47	24.22±0.66
MCH (pg)	8.38±0.10	8.47±0.20	8.42±0.10	8.22±0.14
MCHC (g/dl)	35.03±0.14	35.07±0.13	34.75±0.27	34.23±0.43

ab: Values in a row with superscripts differ significantly * (P<0.05)

Table.4 Serum biochemical profile (at the beginning of experiment) of ram lambs fed total mixed rations containing different tree barks

Parameter	T1	T2	T3	T4
Glucose (mg/dL)	114.5±8.4	96.66±8.40	119.0±10.95	188.83±72.36
Total protein (g/dL)	6.76±0.27	11.43±4.9	6.50±029	6.50±0.08
Albumin (g/dL)*	3.15^{ab}±0.10	3.05^{b}±0.14	3.40^{ab}±0.17	3.55^{a}±0.09
Globulin (g/dL)	3.61±0.31	8.38±4.94	3.15±0.36	2.95±0.16
ALT (IU/L)	95.05±8.29	76.80±15.46	80.55±7.82	123.71±41.5
AST (IU/L)	114.9±9.8	99.85±9.31	107.96±3.62	104.16±3.01
AST: ALT	1.22±0.71	1.46±0.21	1.39±0.11	1.14±0.20
Cholesterol (mg/dL)	157.83±4.74	154.33±9.27	156.50±8.9	234.33±77.7
Triglycerides (mg/dL)	110.3±1.83	109.33±2.27	110.83±3.15	129.83±18.34

a b: Values in a row with superscripts differ significantly * (P<0.05)

Table.5 Serum biochemical profile (at the end of experiment) of ram lambs fed total mixed rations containing different tree barks

Parameter	T1	T2	T3	T4
Glucose (mg/dL)	53.83±7.19	44.5±2.2	45.16±6.03	51.16±8.79
Total protein (g/dL)	6.95±0.16	7.08±0.15	6.75±0.21	6.76±0.91
Albumin (g/dL)	3.63±0.11	3.78±0.60	3.86±0.10	3.86±0.76
Globulin (g/dL)	3.31±0.21	3.3±0.17	2.88±0.24	2.9±0.10
ALT (IU/L)	31.98±3.65	30.28±2.01	32.55±3.01	27.41±1.66
AST (IU/L)	146.16±11.04	140.55±7.35	139.06±8.90	124.43±2.22
AST: ALT	4.81±0.52	4.79±0.5	4.42 ±0.40	4.63±0.31
Cholesterol (mg/dL)*	70.33^{a} ±1.99	63^{b}±1.87	60.66^{b}±1.54	62.16^{b} ±2.80
Triglycerides (mg/dL)*	83.83^{a} ±1.06	71.16^{b}±2.21	74.83^{b} ±2.80	66^{b} ±4.4

a b: Values in a row with superscripts differ significantly * (P<0.05)

Table.6 Faecal egg count per gram (EPG) and percentage reduction in faecal egg count in ram lambs fed different tree barks compared with control group

Day	T1 (control)		T2 (Neem bark)		T3 (Tamarind bark)		T4 (Acacia bark)	
	Mean count ($\times10^2$)	% reduction	Mean count ($\times10^2$)	% reduction	Mean count ($\times10^2$)	% reduction	Mean count ($\times10^2$)	% reduction
0	2.33±0.83	-	2.17±0.88	-	2.30±0.94	-	2.10±0.85	-
15	2.12±0.86	-	2.02±0.82	4.99	2.07±0.84	2.56	2.04±0.83	4.14
30	2.77±1.11	-	2.03±0.83	26.69	2.35±0.82	15.40	2.47±1.00	10.96
45	2.45±1.05	-	1.85±0.66	24.58	2.05±0.91	16.42	1.70±0.69	30.35
60	2.40±1.20	-	1.89±0.77	21.11	2.00±0.73	16.77	1.89±0.75	23.45
75	2.55±1.18	-	1.92±0.78	19.60	2.15±0.77	15.68	1.75±0.61	31.37
90	2.45±1.18	-	1.85±0.75	24.45	2.05±0.83	16.23	1.70±0.64	30.61

Faecal egg count ($\times10^2$) values were 2.33, 2.17, 2.30 and 2.10; 2.45, 1.85, 2.05 and 1.70 at the beginning and end of the experiment for T1, T2, T3 and T4, respectively (Table 6). There was no significant (P > 0.05) difference in the faecal egg count of ram lambs fed total mixed rations containing different tree barks at 10% level when compared with control group throughout the experimental period but the FEC values tend to be lower in groups receiving bark when compared to control. During the 90 days experimental period the percentage reduction in faecal egg count in group supplemented with Neem bark (T2) was maximum on 30^{th} day with 26.69% reduction, while it was 16.77% in group supplemented with Tamarind bark (T3) on 60^{th} day and 31.37% in group supplemented with Acacia bark (T4) on 75^{th} day of experimental period.

The statistical difference between the groups could not be expressed because of the fact that there was high individual variation found and which is normally observed in FEC analysis (Benavides *et al.*, 2016). The high variability in FEC within the group might be due to the difference in terms of physiological condition of each animal and individual ability to utilize the pharmacological properties of different barks.

Condensed tannins present in different plant parts acts as a protector of protein in the diet, allowing higher availability for the ruminant, and aiding in immune response and reduce parasitic infection (Cenci *et al.,* 2007). In present study, the different tree barks were incorporated at 10% level in total mixed ration. The concentration of condensed tannins present in the tree barks (Neem, Tamarind and Acacia) is quite minimal (0.09, 0.02 and 0.2%, respectively) and the amount of condensed tannins consumed through DMI was also less (0.001 to 0.02 g) compared to other studies cited in the literature, which is insufficient to reduce faecal egg count to larger extent in ram lambs. In addition, the duration of experimental period also need to be considered. Present trial was relatively short, lasting for 90 days while acute parasitic infections may become more severe in course of time (Lima *et al.,* 2019). The differences in results observed in various reports might be due to the varying chemical nature of different tannins, amount of condensed tannins and distinct mechanism of action on parasites.

The current study indicated that the addition of tree barks at 10% inclusion level in total mixed rations did not show any negative impact on serum biochemical profile indicating normal health status of the ram lambs and different tree barks showed antiparasitic effect to a limited extent and can be an alternative in the treatment of this ailment, may be with greater inclusion levels.

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