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CHANGES IN HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN RAM LAMBS FED ON HORSE GRAM TANNINS AND PROBIOTIC SUPPLEMENTATION DIETS

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ABSTRACT

An experiment was conducted to study the effect of probiotics supplementation and tanniferous diet inclusion on health status by estimating the hematological and biochemical diagnostic parameters in Deccani ram lambs. 18 Deccani ram lambs of uniform body weight (16.5±0.64 kg) were randomly allotted to 3 groups in a completely randomized design. The dietary treatments were *viz*., T_1: Basal diet (chopped sorgum stover + chopped green fodder as roughage source) + group 1 concentrate, T_2: Basal Diet + Group 2 concentrate (with horse gram), T_3: Basal diet + Group 3 concentrate supplemented with probiotics @ 4kg per100kg concentrate. Deccani ram lambs were fed the respective diets *ad lib.* to meet the nutrient requirements throughout 90 days of feeding trial. Blood glucose concentration was comparable among the experimental groups, however relatively higher (P<0.05) total proteins, cholesterol, albumin and globulin concentration in blood serum was observed with probiotic supplementation and horse gram inclusion. Significant (P<0.05) increase in the mean serum liver enzymatic activities was observed in growing Deccani ram lambs during experimentation than before start of experiment. Serum cobalt, copper, zinc, iron, magnesium and manganese values were comparable among the experimental groups, however a significant (P<0.05) increase was observed in the mean mineral values in growing Deccani ram lambs during experimentation than before start of experiment. It may be concluded that, inclusion of tanniferous protein had no detrimental effect on health as reflected in serum metabolites and serum minerals.

Key words: Horse gram, Probiotic, Serum biochemical parameters, Tannins.

Small ruminant rearing is one of the important livestock rearing in the developing countries contributing enormously towards promotion of livelihoods security and as an insurance cover [1] to cope with crop failures in the semi-arid, arid and mountainous regions. The small ruminants are favored because of low investments, easy to raise and manage, low feed requirement compared to cattle, ability to thrive on different flora, high disease resistance and superior market potential. But this sector is mostly in the hands of traditional rural poor farmers with poor management practices and little concern about providing concentrate feed to animal. The anticipated rise in demand for sheep and goat meat and meat products with the growing economy in the near future, will provide an avenue for resource-poor farmers to increase production, improve their livelihood, reduce malnutrition and thereby, contribute to the goal of overall poverty alleviation in rural areas.

Inspite of huge demand for mutton, the small ruminant sub-sector is unable to transform from pastoral subsistence levels to highly modern, commercial and efficient production systems. Further, dwindling grazing resources, quantitative

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and qualitative shortage of feed and fodder resources, disease epidemics and frequent droughts are the major constraints for sustaining a positive growth in the small ruminant sector, especially during dry season [2]. Condensed tannins (CT) at low-moderate level are not toxic to ruminants and exhibit both beneficial and detrimental effects on ruminants based on level and type. Tannins also chelate some of the macro (Ca and P) and micro minerals (Fe, Co, Zn etc.), influencing their apparent absorption in the digestive tract and subsequent utilization in the animal system depending upon level and type of tannin [3]. Immobilization of positively charged minerals by condensed tannin has been widely documented in non-ruminants and, to a lesser degree, in ruminants [4]. Whether directly or indirectly, tannin has the ability to have synergistic or antagonistic interaction with minerals based on level and type of tannin available in different feedstuffs, which may influence the productive performance of ruminants. There is a growing interest in the use of supplements and in the identification of efficient feed additives, particularly tanniferous feed ingredients and also probiotics in livestock production systems. Feeding animals for extended periods of non-conventional feeds containing anti-nutritional compounds, may affect their health status and ability to withstand diseases [5]. Blood constituents provide valuable media for clinical investigations and nutritional evaluations of an animal. The serum biochemical and haematological indices helps in clinical prediction of the health status of a particular animal. This is because one of the fastest means of ascertaining toxicity of ingested feed in animals is by assessment of the blood [6].

The aim of the present study was to investigate the effect of inclusion of probiotic and replacement of protein source with a tanniferous protein meal on health by estimating hematological and serum biochemical parameters in growing Deccani ram lambs.

MATERIALS AND METHODS

Ethical Approval

Adequate measures were taken to minimize pain or discomfort in accordance with the International Animal Ethics Committee. The study was approved by the committee framed for the research by the university authority.

Study area, experimental design and management

Eighteen growing Deccani ram lambs aged 130±3.0 d with average body weight of 16.5±0.64 kg were selected for conducting a growth trial for a period of 90 days at Central Research Institute for Dry land Agriculture (CRIDA) Livestock farm, Hyderabad. These animals were randomly divided in to three groups of six animals in each in completely randomized design. All the experimental animals were housed in a well-ventilated animal shed with the provision for feeding and watering. Then lambs were weighed individually at fortnightly intervals before feeding and watering to observe the body weight changes for an experimental period of 90 days.

Experimental diets

The dietary treatments were *viz*., T_1: Basal diet (chopped sorghum stover as roughage source) (BD) + group 1 concentrate+ chopped green fodder (4kg), T_2: Basal diet + Group 2 concentrate (horse gram as tannin source + chopped green fodder (4kg), T_3: Basal diet + Group 3 concentrate supplemented with probiotics (*S. cerevisiae* 47@300 X 10^6 CFU /gm+ *S. boulardii*@50 X 10^6 CFU/gm + *L. acidophilus*@45 X 10^6 CFU + *P. freudenreichii*@50 X 10^6 CFU/gm) @ 4kg per100kg concentrate + chopped green fodder (4kg). Deccani ram lambs were fed the respective diets *ad lib.* to meet the nutrient requirements throughout 90 days of feeding trail.

Blood Analysis

Blood was collected from all individual animals at 45 days interval during experimental period to study the serum biochemical and mineral profile. Blood was collected at 0, 45 and 90 days of experimentation. Blood samples were collected aseptically from jugular vein of lambs with the help of sterilized needles and collected in vacutainers without anticoagulant. Samples were placed on ice, transferred to the laboratory and serum was separated carefully from the test tube to check

haemolysis. The collected serum samples were then centrifuged at 3000 rpm for 5 minutes and transferred to 5 ml eppendorf tubes and stored at -20°C for estimation of various biochemical constituents and mineral profileding trial. Concentration of total proteins, albumin, globulin, albumin: globulin ratio, glucose, cholesterol and alkaline phosphatase were estimated in blood. Serum samples are analyzed by kits.

Serum minerals were determined as per the method described by Standard Operation Procedure (Laboratory University of Wisconsin – Madison, 2005) using inductively coupled plasma optical emission spectrometry (ICP-OES) (GBC Scientific equipment, Australia). Accordingly, 1 ml of serum was digested with HNO_3 and perchloric acid on hot plate at 180-200°C till the dense color fumes of diacid mixture appeared. Then it was transferred to a 100 ml volumetric flask by several washings. Washing of each sample was done by double distilled water and made up to the final volume of 100 ml and then each sample was filtered and transferred to a separate sterilized plastic vials. Thereafter, the Ca, Co, Cu, Fe, Mg, Mn and Zn were estimated by ICP-OES (GBC Scientific equipment, Australia) with standard solution of different concentrations of elements in order to estimate the final concentration of each element. The concentration was expressed as parts per million (ppm) using blood biochemical semi auto analyser (ichem~168, Spain).

Statistical analysis

The data was subjected to one way analysis of variance. The differences between the means were tested by significance using Duncan's multiple range test [7]. All the statistical procedures were carried out as per the procedures of Snedecor and Cochran [8] by programming and processing in computer by using SPSS20.

RESULTS AND DISCUSSION

Serum Biochemical Constituents

The analyzed biochemical constituents in serum of Deccani ram lambs at 90 days of feeding trial are represented in Table 3. The serum total protein concentration increased during the growth trial among experimental animals.

Blood glucose concentration was comparable among the experimental groups, however relatively higher blood glucose concentration was recorded in growing Deccani ram lambs during experimentation than before start of experiment. Thus, indicating a positive and high-energy balance in all the groups. Serum glucose level is the indicator of normal physiology [9,10]. Any alteration in glucose level is an indicator of stress to the animals. The serum Glucose levels within in the normal range are an indication that there is no adverse effect of inclusion of tannins and probiotics in lambs. The results of the present study are in agreement with the findings reported by Getachew *et al*. [11] who fed tannic acid (TA) (at 30, 60 and 90 g TA per kg DM) sprayed chopped alfalfa in sheep. Ding *et al*. [12] also reported comparable blood glucose concentrations in lambs supplemented with live yeast (LY) (20×109cfu/g) in the basal diet consisting of the mixed concentrate and roughage. Contrary to this, Antunovic *et al*. [13] reported non-significant higher levels of blood glucose concentrations in lambs with supplementation of probiotic BioPlus 2B containing *B. licheniformis* (DSM 5749) and *B. subtilis* (DSM 5750) spores in a 1:1 ratio (1.6 x 109 spores/g : 1.6 x 109 spores/g). Pathak *et al*. [14] reported the level of serum glucose, calcium, inorganic phosphorus, cholesterol and creatinine contents were similar among three groups. No significant difference was evident in serum enzymes irrespective of treatments.

There was an ($P<0.05$) increase in the mean serum total proteins values in growing Deccani ram lambs during experimentation than before start of experiment, however mean serum total proteins values were comparable among the experimental groups indicating that the protein requirements of lambs were met and surplus DCP was available in all the groups. Robert [15] concluded that total protein reflects availability of protein to the animal and concentration declines in the face of protein deficiency and occurs over a period of time, thus, indicating no adverse effect of tannins inclusion and probiotic supplementation in lambs. *Hossein-Ali Arab et al.* [16] reported decrease in the level of serum

total proteins, albumin, glucose, triglyceride and cholesterol significantly in lambs fed diets containing *Bacillus subtilis* and *Bacillus licheniformis.*

Total cholesterol concentration was significantly (P<0.05) higher in growing Deccani ram lambs during experimentation than before start of experiment. Further, values were comparable among the experimental groups indicating a positive high-energy and protein balance in all the groups. Similar cholesterol levels of all the animals indicate absence of hypocholesterolemia and agree with the findings of Olafadehan [17].

An (P<0.05) increase in the mean serum albumen and globulin values was observed during experimentation than before start of experiment. However, values were comparable among the experimental groups indicating available DCP was sufficient and meeting the protein requirements of growing Deccani ram lambs. The results are in agreement with Robert [15] who concluded that albumen reflects availability of protein to the animal and concentration declines within a month or two in the face of protein deficiency as albumin has relatively short half-lime than total proteins. The mean values of protein, albumin, globulin and A:G ratio of lambs under different treatments were within the normal physiological range indicating that tannin at low moderate level had no adverse effect on animals. The TP and globulin were significantly (p<0.001) higher in T group as compared to C group. The NC group was also comparable to T group. The albumin level was also found to be statistically lower (p<0.01) under T group as compared to C group. The level of albumin decreased from 30 days experimental period and onwards.

Mean serum urea level was comparable among the groups, however relatively lower values were observed at the end of experiment. It might be due to lower concentration of tannins in the rations. Serum urea level is an indicator of protein degradation in rumen and the findings in the present investigation corroborate the findings of Waghorn *et al.* [18]. Similarly BUN concentration was also comparable among the treatments and similar findings were reported by Waghorn *et al.* [18] when probiotics were supplemented.

The serum creatinine concentration in lambs was within the normal range in the ram lambs during the experimental period. Mean serum creatinine levels among the groups and periods were statistically non-significant (P>0.05) but lower at the end of experiment, which clearly indicated that CT in all the experimental rations are below the threshold levels and did not have any adverse effect on the kidney. The results are in agreement with findings reported by Belewu *et al*. [19] with tannins inclusion and Antunovicc *et al*. [13] with probiotics supplementation and suggest the absence of wasting or catabolism of muscle tissues, and that the animals were not surviving at the expense of the body reserve [17].

Serum electrolytes Na, K and Cl concentrations were comparable among the experimental groups, however non-significantly (P>0.05) lower sodium and potassium and higher chloride concentrations were observed at the end of experiment than at the start of experiment. This could be due to increased excretion of electrolytes through urine and loss of electrolytes output in sweat and also through panting [20] as HLI and THI values were slightly above 75 in the month of May during the experiment. The results of the present experiment were similar to the results reported by Getachew *et al*. [11] who observed no effect on serum electrolytes in sheep with feeding tannic acid (TA) (30, 60 and 90 g TA per kg DM) sprayed chopped alfalfa. Lower plasma sodium concentration [21], higher concentration of potassium and lower chloride concentration in the blood serum of rams supplemented with probiotics was also observed [13].

Horse gram inclusion and probiotic supplementation had no significant effect on blood calcium levels but increased blood phosphorus levels among the groups during the growth period. This could be due to proper functioning of homeostatic mechanism in Deccani ram lambs in addition to bio-availability of these minerals in required quantities from the offered feed and fodder. These results are in agreement with Robert [15] who concluded that concentrations of macro minerals

like calcium are not reflective of dietary status when the homeostatic system is functioning properly and Olafadehan [22] who reported normal serum mineral concentration in Red Sokoto male goats fed with tannins and concluded that tannins did not interfere with dietary mineral availability and absorption. Similar findings were reported by Antunovic *et al*. [13] in lambs supplemented with probiotic PIONEER PDFM® (2x10[11] CFU.kg-1 of *Enterococcus faecium*).

Serum Enzyme Activity

Serum enzyme activities provide an insight into the functioning of liver and kidney. The analyzed serum enzymatic parameters are represented in Table 3. Liver enzymatic activities alkaline phosphatase, SGPT, SGOT were comparable among the experimental groups, however a significant (P<0.05) increase in the mean serum liver enzymatic activities in growing Deccani ram lambs during experimentation than before start of experiment was observed. The insignificantly varied cholesterol values for all the ram lambs further confirmed the absence of hepatocellular damage. The results obtained in the present study were in accordance with the results suggested by Dey *et al*. [9] who reported a non-significant effect on serum enzymes (ALT and AST) in lambs fed with *Ficus infectoria* at 0, 1.0, 1.5 and 2.0% levels of CT.

Serum Minerals

The effect of horse gram meal inclusion and probiotic supplementation on serum cobalt, copper, zinc, iron magnesium and manganese in Deccani lambs during 90 days of feeding trial are presented in table 4. Serum cobalt, copper, zinc, iron, magnesium and manganese values were comparable among the experimental groups, the results are in agreement with Raju Kushwaha *et al.* [23], who reported the concentration of plasma minerals such as Cu, Fe, Mn and Zn remained similar amongst the dietary groups with Babul (*Acacia nilotica*) pods in pregnant crossbred goats. However a significant (P<0.05) increase was observed in the mean mineral values in Deccani ram lambs during experimentation than before start of experiment. This could be due to more bio-availability of these minerals in required quantities from the offered feed and fodder and not much effect of precipitation by tannins.

Table 1. Chemical composition of experimental feeds (%DM) offered to Deccani ram lambs

Nutrient	Basal diet		Concentrate mixture		
	Green fodder (HN-CO4)	Dry fodder (Sorghum straw)	Group 1 (control)	Group 2 (Horse gram inclusion)	Group 3 (Probiotic supplement)
Proximate principles					
Dry matter	20.38	98.59	98.03	98.35	97.74
Organic matter	87.22	92.29	91.05	91.72	91.07
Crude protein	11.75	3.02	17.96	17.94	17.96
Crude fibre	35.86	40.45	14.96	14.94	14.96
Ether extract	2.64	2.49	6.34	6.63	6.36
NFE	36.97	46.34	49.61	48.93	49.59
Total ash	12.78	7.71	8.95	8.28	8.93
Cell wall constituents					
NDF	71.34	83.27	48.12	58.29	48.17
ADF	41.58	52.34	19.44	19.96	19.48
Hemicellulose	29.76	30.93	28.67	38.33	28.66
Cellulose	33.78	42.93	11.71	14.4	11.74
Anti-nutritional compounds					
Total Phenolic compounds	-	-	0.21	0.48	0.21
Condensed Tannins	-	-	0.09	0.14	0.09

Minerals					
Calcium	0.40	0.34	1.12	1.18	1.12
Phosphorus	0.16	0.24	0.82	0.78	0.82

Table 2. Ingredient composition of concentrate mixtures (parts per 100) offered to Deccani ram lambs

Ingredients	Group 1 (control)	Group 2 (Horse gram inclusion)	Group 3 (Probiotic supplementation)
Maize	40	40	40
Rice bran	32	29	32
Soya meal	25	00	25
Horse gram meal	00	28	00
Mineral mixture	02	02	02
Common Salt	01	01	01

Table 3. Serum biochemical parameters affected by feeding experimental rations in Deccani ram lambs

Parameter	Period	Group I	Group II	Group III	SEM	P
Total Protein (g/dl)	0 day	$7.46^{ab} \pm 0.11$	$6.94^{b} \pm 0.21$	$7.82^{a} \pm 0.21$	0.13	0.01
	45 day	6.37 ± 0.15	6.53 ± 0.17	6.45 ± 0.12	0.08	0.73
	90 day	$8.70^{a} \pm 0.27$	$7.77^{b} \pm 0.18$	$8.16^{ab} \pm 0.22$	0.15	0.03
Serum Albumin (g/dl)	0 day	$3.72^{ab} \pm 0.09$	$3.68^{b} \pm 0.07$	$3.95^{a} \pm 0.07$	0.05	0.06
	45 day	$3.78^{a} \pm 0.07$	$3.38^{b} \pm 0.08$	$3.63^{ab} \pm 0.11$	0.06	0.02
	90 day	5.28 ± 0.29	4.64 ± 0.15	5.00 ± 0.20	0.14	0.16
Serum Globulin (g/dl)	0 day	$3.74^{b} \pm 0.11$	$3.26^{ab} \pm 0.19$	$3.87^{a} \pm 0.18$	0.11	0.04
	45 day	2.58 ± 0.18	3.15 ± 0.22	2.82 ± 0.14	0.11	0.12
	90 day	3.42 ± 0.16	3.13 ± 0.22	3.16 ± 0.24	0.12	0.56
Albumin: Globulin	0 day	1.00 ± 0.04	1.15 ± 0.08	1.03 ± 0.04	0.03	0.15
	45 day	$1.50^{a} \pm 0.11$	$1.11^{b} \pm 0.09$	$1.31^{ab} \pm 0.10$	0.07	0.05
	90 day	1.57 ± 0.14	1.53 ± 0.14	1.65 ± 0.20	0.09	0.87
Glucose (mg/dl)	0 day	63.28 ± 2.32	65.02 ± 1.12	72.78 ± 4.34	1.87	0.08
	90 day	72.74 ± 1.74	71.22 ± 1.38	73.78 ± 1.66	0.90	0.54
Cholesterol (mg/dl)	0 day	56.00 ± 1.15	60.00 ± 3.01	54.40 ± 4.38	1.80	0.45
	0 day	125.50 ± 5.46	130.80 ± 8.05	110.75 ± 2.29	3.74	0.07
Creatinine (mg/dl)	90 day	0.93 ± 0.04	1.20 ± 0.06	1.14 ± 0.09	0.05	0.03
	0 day	0.84 ± 0.05	0.84 ± 0.03	0.86 ± 0.03	0.02	0.93
Urea (mg/dl)	90 day	31.17 ± 2.46	32.60 ± 4.13	29.67 ± 3.22	1.83	0.83
	0 day	22.33 ± 1.73	29.40 ± 1.02	29.00 ± 3.89	1.58	0.12
Ca (mg/dl)	90 day	7.35 ± 0.19	7.13 ± 0.16	7.34 ± 0.25	0.11	0.69
	0 day	12.10 ± 1.02	12.38 ± 0.81	12.04 ± 0.33	0.42	0.95
P (mg/dl)	90 day	7.15 ± 0.13	6.51 ± 0.21	7.45 ± 0.13	0.13	0.00
	0 day	9.41 ± 0.18	10.32 ± 0.56	10.73 ± 0.41	0.26	0.10
Na (mEq/l)	0 day	138.39 ± 0.83	138.43 ± 0.97	137.02 ± 1.21	0.57	0.55
	90 day	142.40 ± 1.30	141.98 ± 1.39	143.24 ± 1.00	0.68	0.77

K (mEq/l)	0 day	4.89 ± 0.10	4.80 ± 0.16	4.98 ± 0.21	0.09	0.74
	90 day	4.25 ± 0.08	4.07 ± 0.12	4.17 ± 0.13	0.06	0.53
Cl (mEq/l)	0 day	99.10 ± 0.79	101.77 ± 0.44	98.65 ± 2.03	0.77	0.21
	90 day	103.26 ± 1.16	101.09 ± 0.88	102.58 ± 0.56	0.57	0.55
SGOT (U/l)	0 day	92.20^{a} ± 2.81	77.50^{b} ± 5.35	75.75^{b} ± 1.58	2.65	0.01
	90 day	91.40^{a} ± 3.31	90.00^{a} ± 6.51	110.20^{a} ±3.57	3.39	0.01
SGPT (U/l)	0 day	14.40^{b} ± 0.54	17.42^{a} ± 1.25	19.78^{a} ± 0.97	0.75	0.00
	90 day	30.42 ± 2.84	29.78 ± 1.14	29.48 ± 0.72	0.99	0.93
ALP (U/l)	0 day	134.60^{b} ± 3.50	124.33^{b} ±1.41	164.33^{a} ±10.23	5.35	0.00
	90 day	403.50^{a} ± 27.34	303.20^{b} ±25.26	405.75^{a}± 24.14	18.10	0.02

Table 4. Serum minerals profile affected by feeding experimental rations in Deccani ram lambs

Parameter	Period	Group I	Group II	Group III	SEM	P
Zn (ppm)	0 day	1.62 ± 0.03	1.58 ± 0.05	1.56 ± 0.02	0.005	0.02
	45 day	1.71 ± 0.01	1.70 ± 0.03	1.65 ± 0.01	0.003	0.06
	90 day	1.80 ± 0.03	1.80 ± 0.02	1.70 ± 0.02	0.004	0.09
Fe (ppm)	0 day	4.52 ± 0.03	4.56 ± 0.05	4.54 ± 0.02	0.004	0.71
	90 day	4.22 ± 0.02	4.28 ± 0.03	4.16 ± 0.03	0.005	0.06
Co (ppm)	0 day	0.58 ± 0.02	0.60 ± 0.02	0.58 ± 0.04	0.003	0.07
	90 day	0.46 ± 0.01	0.48 ± 0.02	0.42 ± 0.01	0.002	0.09
Cu (ppm)	0 day	0.84 ± 0.03	0.88 ± 0.04	0.88 ± 0.03	0.004	0.067
	90 day	0.96 ± 0.02	0.98 ± 0.03	0.92 ± 0.03	0.004	0.15
Mg (ppm)	0 day	2.08 ± 0.04	2.02 ± 0.07	2.06 ± 0.03	0.005	0.67
	90 day	2.30 ± 0.07	2.40 ± 0.06	2.40 ± 0.07	0.008	0.58
Mn (ppm)	0 day	0.62 ± 0.03	0.64 ± 0.02	0.62 ± 0.04	0.003	0.58
	90 day	0.58 ± 0.03	0.60 ± 0.02	0.54 ± 0.03	0.003	0.19

CONCLUSION

The present study indicated that probiotic supplementation and horse gram meal inclusion had positive influence on haematological and biochemical parameters in growing deccani ram lambs and there was no detrimental effect of inclusion of horsegram tannin inclusion on health of rams lambs as reflected in serum metabolites and serum minerals.

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