Indian Journal of Animal Sciences **94** (11): 979–986, November 2024/Article
https://doi.org/10.56093/ijans.v94i11.151029

Effect of dietary inclusion of tree leaves on growth, antioxidant status and blood biochemical constituents in ram lambs during summer stress

S RAJU[1], D NAGALAKSHMI[2], N NALINI KUMARI[1], N RAJANNA[3] and B SWATHI[4]

College of Veterinary Science (PVNRTVU), Hyderabad, Telangana 500 030 India

Received: 29 April 2024; Accepted: 4 October 2024

ABSTRACT

A growth trial of 120 days was conducted during summer on 42 ram lambs (5-6 months age; 18.48±0.29 kg BW) to evaluate effect of 20% tree leaf inclusion in concentrate mixture on their performance. The concentrate mixture of control group contained maize, de-oiled rice bran, cotton seed cake and soyabean meal as major ingredients and in the five experimental concentrate mixtures, de-oiled rice bran and soyabean meal were partially replaced either with moringa (MOR20), subabul (SBL20), guava (GVL20), moringa and subabul mixture (60:40) (MS20) or moringa and guava mixture (40:60) (MG20). Animals were fed with respective concentrate mixtures to meet about 80% of protein requirements and sorghum stover was offered *ad lib.* Tree leaf inclusion did not affect fortnightly body weights, dry matter intake, average daily gain, or feed efficiency. However, combination groups (MS20 and MG20) showed higher apparent ADG and feed conversion ratio (13% and 11%, respectively) compared to the control. Blood analysis revealed higher HDL concentrations and lower cortisol levels in lambs fed with tree leaves. Antioxidant enzyme activities increased, while lipid peroxidation decreased in tree leaf fed groups. It was concluded that dietary inclusion of tree leaves at 20% in concentrate mixture improved antioxidant enzyme activities, decreased serum cortisol levels with no adverse effect on feed intake, growth rate and feed efficiency in sheep.

Keywords: Antioxidant enzyme, Cortisol, Growth, Tree leaves, Ram lambs, Summer stress

Grazing animals are potentially exposed to multiple stressors such as heat stress, feed and water scarcity, and physical stress due to walking in hot semi-arid environments which adversely affects their production and reproduction (Sejian *et al*. 2013). Hence, it is important to develop alternatives which constantly supply feed and fodder at low cost. Fodder trees provide one such option and they have been traditionally grown in many regions and can be cultivated on degraded lands for supply of nutritious fodder to grazing animals (Kumar *et al.* 2019). Further, tree leaves are potential sources of phytochemicals that have been found to counteract free radicals due to their antioxidant activity (Simbaya *et al.* 2020). Experiments have earlier been conducted on inclusion of tree leaves like moringa, subabul as protein supplement or as supplementary feed in small ruminants (Redekar *et al*. 2019, Sushmitha 2019). Some researchers also evaluated functional properties of tree leaves (Zapata *et al.* 2021, Wankhede *et al*. 2022). Tree leaves, including moringa, subabul, and guava, are rich in diverse bioactive compounds, such as vitamins, polyphenols, flavonoids, and tannins (Khalid *et al*. 2020, Daing *et al*. 2021). Considering the differences in functional properties of these tree leaves, it was hypothesized that combination of tree leaves would be a better option to improve performance of lambs. Hence, the present study was designed to evaluate the effect of dietary inclusion of moringa, subabul and guava leaves on growth performance, blood biochemical constituents and antioxidant activity in ram lambs during summer season.

Present address: [1]Department of Animal Nutrition, College of Veterinary Science (PVNRTVU), Hyderabad, Telangana. [2]College of Fishery Science (PVNRTVU), Pebbair, Telangana. [3]Krishi Vigyan Kendra (PVNRTVU), Mamnoor, Telangana. [4]Department of Veterinary Physiology, College of Veterinary Science (PVNRTVU), Mamnoor, Telangana. ✉Corresponding author email: sagivetraju@gmail.com

MATERIALS AND METHODS

This study was conducted at Animal Experimentation Unit, Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad, India with approval of Institutional Animal ethics committee (no 27/25/C.V.Sc,Hyd.IAEC/2022-23;Dated:2/7/2022).

Procurement of feed ingredients: Moringa leaves were procured from Kesco Organics Exports Coimbatore, Tamil Nadu. Guava leaves were procured from ARS Herbals, Madurai, Tamil Nadu. The subabul leaves were procured from the trees available in college premises which were shade dried and ground. The feed ingredients and sorghum stover were procured from the local market in Hyderabad.

Experimental diets: A basal concentrate mixture was formulated with maize, de-oiled rice bran, cotton seed cake and soybean meal and in the five experimental concentrate mixtures, de-oiled rice bran and soyabean meal were partially replaced with moringa (MOR20), subabul (SBL20), guava (GVL20), moringa and subabul mixture (60:40) (MS20) and moringa and guava mixture (40:60) (MG20), by including them at 20%. All the concentrate mixture were iso-nitrogenous and iso-caloric and contained 20% CP and 68% TDN (Table 1).

Experimental animals and management: Forty-two Nellore×Deccani cross lambs (18.48±0.29 kg) were procured, quarantined as per guidelines of Department of Animal Husbandry Dairying and Fisheries (DAHDF), India and then randomly divided into six groups of seven animals each. They were fed a concentrate mixture (80% of crude protein requirements) and *ad lib.* sorghum stover twice daily, meeting their maintenance and growth needs (100 g/day) as per ICAR (2013).

Growth performance: The lambs were weighed individually at fortnightly intervals in morning before feeding and watering to observe the body weight changes for 120 days. The daily dry matter intake, average daily gain and feed conversion ratio were calculated using standard formulae.

Temperature-Humidity Index (THI): It was measured by calculating the air temperature (T) and relative humidity (RH) by using the following equation given by NRC (1971):

$$THI = (1.8 \times Tdb + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26.8)$$

where, Tdb, dry bulb temperature (°C) and RH, relative humidity (%).

Collection of blood: Blood samples were collected from the jugular vein of all rams on days 0, 60, and 120 into sterilized glass tubes. The tubes were tilted for 6 h to allow serum separation, centrifuged at 3000 rpm for 5 min. The serum was transferred to 5 mL Eppendorf tubes and stored at -20°C for further analysis

Analytical methods: Serum biochemical parameters (glucose, total protein, cholesterol, HDL, LDL) were estimated using commercial kits of Coral Clinical Systems (Goa, India). Serum corticosterone was measured using an ELISA kit (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd.). Glutathione Peroxidase (GPx) was assayed according to the method of Paglia and Valentine (1967); Glutathione Reductase (GRx) assayed according to method of Carlberg and Mannervik (1985); and Superoxide Dismutase (SOD) according to the method of Madesh and Balsubramanian (1998). Lipid peroxidation activity was measured by the method of Ohkawa *et al*. (1979).

Statistical analysis: The growth performance data was analyzed using one-way ANOVA general linear model (GLM) through SPSS software (version 20) in accordance with Snedecor and Cochran (1994). The statistical difference in mean blood constituents, antioxidants, and cortisol hormone between the groups and at different intervals of time were analyzed using two-way repeated measure ANOVA to investigate diet and period interaction effects. The difference between means was tested by using Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Chemical composition of concentrate mixtures: The chemical composition of concentrate mixtures containing 20% inclusion of moringa, subabul and guava leaves were similar for most of nutrients (Table 2), since only soyabean meal and de-oiled rice bran of control concentrate mixtures were partially replaced with moringa, subabul and guava leaves in experimental concentrate mixtures and rations were formulated to be iso-nitrogenous and iso-caloric.

Table 1. Ingredient composition of concentrate mixtures containing moringa, subabul and guava tree leaves

Ingredient	CON[2]	MOR20[3]	SBL20[4]	GVL20[5]	MS20[6]	MG20[7]
Maize	25	25	25	25	25	25
Soyabean meal	19	13.5	14	19	15	17
Deoiled rice bran	36	21.5	21	16	20	18
Cotton seed cake	18	18	18	18	18	18
Moringa leaves	0	20	0	0	0	0
Subabul leaves	0	0	20	0	0	0
Guava leaves	0	0	0	20	0	0
Moringa and subabul leaves (60:40)	0	0	0	0	20	0
Moringa and guava leaves (40:60)	0	0	0	0	0	20
Mineral and vitamin mixture[1]	0.2	0.2	0.2	0.2	0.2	0.2
Calcite powder	0.8	0.8	0.8	0.8	0.8	0.8
Salt	1	1	1	1	1	1
Total	100	100	100	100	100	100

[1], Mineral and vitamin mixture provided per kg diet: Calcium 2.5 g, Phosphorus 1.275 g, Magnesium 0.065 g, Iron 0.0175 g, Sulphur 0.092 g, Zinc 0.096 g, Copper 0.042 g, Manganese 0.015 g, Potassium 1.5 mg, Sodium 0.2 mg, Iodine 3.5 mg, Cobalt 1.5 mg, Vitamin B_6 0.2 mg, Vitamin A 7500 IU, Vitamin D_3 750 IU, Vitamin E 3 mg, Niacinamide 0.012 g; [2], Control concentrate mixture; [3,4,5,6,7], Moringa, Subabul, Guava leaves, Moringa and Subabul leaves (60:40 ratio) and Moringa and Guava leaves (40:60 ratio) included at 20% in the concentrate mixture by partially replacing de-oiled rice bran and soyabean meal.

Table 2. Chemical composition (%DM basis) of sorghum stover and concentrate mixtures containing 20% level of moringa, subabul and guava leaves fed to growing lambs

Constituent	CON[1]	MOR20[2]	SBL20[2]	GVL20[3]	MS20[3]	MG20[6]	Sorghum stover
Dry matter	92.14	91.74	91.42	91.55	91.94	92.03	90.58
Organic matter	90.44	89.98	90.11	89.91	89.96	89.86	90.14
Crude protein	20.01	20.04	20.05	19.98	20.03	19.98	2.48
Ether extract	1.94	2.54	2.67	2.34	2.96	2.49	1.34
Crude fibre	16.02	13.56	14.12	15.56	12.34	14.52	28.54
NFE	52.47	53.84	53.27	52.03	54.63	52.87	57.78
Total ash	9.56	10.02	9.89	10.09	10.04	10.14	9.86
AIA	3.12	2.34	2.56	3.84	2.94	2.65	0.65
NDF	63.54	58.54	59.37	60.53	57.57	58.87	62.51
ADF	27.34	22.56	24.32	28.14	23.18	25.12	49.88
Hemicellulose	36.2	35.98	35.05	32.39	34.39	33.75	12.63
Cellulose	18.55	15.57	16.76	19.08	14.92	17.73	41.04
Lignin	5.46	4.46	5.22	4.94	4.61	4.64	3.88
Calcium	1.25	1.06	1.17	0.87	1.24	1.02	0.5
Phosphorus	0.91	0.54	0.58	0.44	0.68	0.57	0.18

Each value is the average of duplicate analysis. [1], Control diet; [2,3,4,5,6], moringa, subabul, guava, moringa and subabul (60:40 ratio), moringa and guava (40:60 ratio) leaves included at 20% in the concentrate mixture partially replacing de-oiled rice bran and soyabean meal. NFE, Nitrogen free extract; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; AIA, Acid insoluble ash.

Dry matter intake: The inclusion of moringa, subabul, and guava leaves (20% sole or combined) in concentrate mixtures did not affect dry matter intake (DMI) in growing lambs (Table 3). This suggests that these leaves can be added without impacting palatability. These findings align with previous studies (Jelali and Salem 2014, Okwori *et al*. 2016, Redekar *et al*. 2019, Sushmitha 2019, Ramesh 2021, Tadesse *et al*. 2022) that reported no effect on DMI with dietary inclusion of these leaves. However, some studies (Gebregiorgis *et al*. 2012, Rahman *et al*. 2016, Leketa *et al*. 2019, Wankhede *et al*. 2022) found improved DMI with supplementation or replacement of basal diets with tree leaves. The varying results may be attributed to differences in feeding of tree leaves; in some experiments, it was additional supplement to basal diet, in others it was replacing concentrate mixture.

Growth rate and average daily gain of lambs: The ADG and FCR were similar (P>0.05) among dietary groups, indicating no adverse effects from including moringa, subabul, and guava leaves (up to 20%) in concentrate mixtures (Table 3). The findings were in accordance with Redekar *et al*. (2019), Ramesh (2021), Tadesse *et al*. (2022), who reported no effects on ADG and FCR with inclusion of moringa and subabul leaves. However, other studies (Jiwuba *et al*. 2016, Okwori *et al*. 2016, Rahman *et al*. 2016, Mataveia *et al*. 2019, Wankhede *et al*. 2022)

Table 3. Overall performance of lambs fed diets containing concentrate mixtures with 20% inclusion of moringa, subabul and guava leaves

Attribute	CON[1]	MOR20[2]	SBL20[3]	GUL20[4]	MS20[5]	MG20[6]	SEM	P- value
Initial body weight (kg)	18.36±0.75	18.50±1.27	18.64±0.84	18.36±0.94	18.50±0.89	18.57±0.69	0.350	1.000
Final body weight (kg)	28.07±0.90	29.09±1.14	28.21±1.02	28.79±0.78	29.49±0.85	29.54±0.78	0.363	0.802
Average body weight gain (kg)	9.71±0.75	10.59±0.55	9.57±0.90	10.43±0.27	10.99±0.31	10.97±0.42	0.239	0.379
Average daily gain (g)	80.95±6.29	88.21±4.60	79.76±7.52	86.90±2.28	91.55±2.56	91.43±3.50	1.993	0.379
Dry matter intake (g per day)	943.14±29.24	944.86±48.92	965.57±34.66	945.57±36.18	968.00±34.87	970.00±35.18	14.267	0.987
Average dry matter intake (% of body weight)	4.01±0.01	3.98±0.01	4.02±0.01	3.99±0.01	3.99±0.01	3.98±0.01	0.004	0.080
Average dry matter intake (g per kg $W^{0.75}$)	88.17±0.70	87.74±1.32	88.86±0.87	87.92±0.93	88.45±0.87	88.37±1.04	0.377	0.972
Feed conversion ratio	11.99±0.82	10.91±0.87	12.71±1.16	10.97±0.64	10.63±0.54	10.67±0.48	0.324	0.347

Each mean is an average of 7 values. [1], Control diet (0% leaves); [2,3,4,5,6], moringa, subabul, guava, moringa and subabul (60:40 ratio) and moringa and guava (40:60 ratio) leaves included at 20% in the concentrate mixture partially replacing de-oiled rice bran and soyabean meal. SEM, Standard Error Mean; P-value, Probability value.

Table 4. Blood biochemical constituents in lambs fed diets containing concentrate mixture with 20% inclusion of moringa, subabul and guava leaves

Period	CON[1]	MOR20[2]	SBL20[3]	GVL20[4]	MS20[5]	MG20[6]	Mean±SEM	P-value		
								D	P	D×P
Glucose (mg/dL)										
0d	83.22	86.67	85.67	84.33	86.11	85.80	85.30^{Y}±0.9	0.761	0.0001	0.998
60d	85.56	84.56	89.56	88.56	88.33	88.34	87.48^{Y}±1.13			
120d	91.11	92.89	93.39	93.89	94.50	94.74	93.42^{X}±1.23			
Mean±SEM	86.63±1.70	88.04±1.62	89.54±1.38	88.93±2.10	89.65±2.16	89.62±1.60				
Total protein (g/dL)								0.703	0.001	0.997
0d	7.24	7.14	7.02	7.16	7.15	7.13	7.14^{Y}±0.05			
60d	7.02	6.95	6.90	7.00	7.03	7.16	7.01^{Y}±0.04			
120d	7.25	7.29	7.21	7.36	7.37	7.33	7.30^{X}±0.05			
Mean±SEM	7.17±0.09	7.13±0.07	7.04±0.05	7.17±0.06	7.18±0.09	7.20±0.06				
Cholesterol (mg/dL)								0.53	0.07	0.86
0d	65.09	68.90	66.95	64.65	66.58	64.60	66.13±1.09			
60d	68.98	67.60	67.98	66.53	65.59	66.44	67.18±1.00			
120d	74.90	68.61	69.29	68.29	67.00	68.57	69.44±0.91			
Mean±SEM	69.66±2.02	68.37±1.95	68.07±1.34	66.49±1.17	66.39±0.68	66.53±1.03				
High density lipoprotein (mg/dL)								0.003	0.0001	0.490
0d	34.04	33.47	34.90	34.03	33.78	34.55	34.13^{Z}±0.50			
60d	38.85	41.75	43.64	44.35	44.19	42.78	42.59^{Y}±0.52			
120d	39.70	44.12	45.46	45.06	45.45	44.72	44.09^{X}±0.62			
Mean±SEM	37.53^{B}±0.92	39.78^{A}±1.33	41.33^{A}±1.34	41.15^{A}±1.38	41.14^{A}±1.37	40.68^{A}±1.30				
Low density lipoprotein (mg/dL)								0.082	0.0001	0.458
0d	16.29	21.04	17.91	16.52	18.32	16.19	17.71^{X}±1.20			
60d	13.77	10.67	9.12	7.24	6.43	8.90	9.35^{Y}±1.04			
120d	19.86	9.95	9.15	8.76	6.88	9.50	10.68^{Y}±1.26			
Mean±SEM	16.64^{A}±1.79	13.89^{AB}±2.51	12.06^{AB}±1.99	10.84^{B}±1.49	10.54^{B}±1.44	11.53^{B}±1.53				

^{ABC}, Means bearing different superscripts in a row differ significantly: P<0.01; ^{XYZ}, Means bearing different superscripts in a sub-column differ significantly: P<0.01; D, Diet; P, Period; D×P, Diet and Period interaction.[1], Control diet (0% leaves);[2,3,4,5,6], moringa, subabul, guava, moringa and subabul (60:40 ratio), moringa and guava (40:60 ratio) leaves included at 20% in the concentrate mixture respectively partially replacing de-oiled rice bran and soyabean meal. SEM, Standard Error Mean; P-value, Probability value

Table 5. Antioxidant indices and cortisol in lambs fed diets containing concentrate mixtures with 20% inclusion of moringa, subabul and guava leaves

Period	CON[1]	MOR20[2]	SBL20[3]	GVL20[4]	MS20[5]	MG20[6]	Mean±SEM	P-value		
Lipid peroxidation (nmol MDA/mg protein)								D	P	DXP
0d	2.64^{a}	2.93^{a}	2.79^{a}	2.95^{a}	2.93^{a}	2.75^{a}	$2.83^{X}\pm0.07$	0.001	0.001	0.001
60d	2.74^{a}	1.89^{b}	1.81^{b}	1.78^{b}	1.79^{b}	1.81^{b}	$1.97^{Y}\pm0.06$			
120d	2.82^{a}	1.84^{b}	1.84^{b}	1.81^{b}	1.74^{b}	1.77^{b}	$1.97^{Y}\pm0.07$			
Mean	$2.73^{A}\pm0.06$	$2.22^{B}\pm0.15$	$2.15^{B}\pm0.12$	$2.18^{B}\pm0.14$	$2.15^{B}\pm0.11$	$2.11^{B}\pm0.06$				
Glutathione peroxidase (units/mL)								0.001	0.001	0.022
0d	551.16^{b}	533.66^{b}	531.59^{b}	540.79^{b}	539.25^{b}	534.38^{b}	$538.47^{y}\pm6.29$			
60d	566.01^{b}	646.48^{a}	635.99^{a}	647.42^{a}	651.43^{a}	647.94^{a}	$632.54^{x}\pm8.95$			
120d	539.16^{b}	643.62^{a}	630.81^{a}	650.08^{a}	664.56^{a}	625.28^{a}	$625.59^{x}\pm69.48$			
Mean	$552.11^{B}\pm1\ 0.91$	$607.92^{A}\pm15.69$	$599.46^{A}\pm15.13$	$612.76^{A}\pm15.68$	$618.41^{A}\pm16.42$	$602.53^{A}\pm15.20$				
Glutathione reductase (units/mL)								0.001	0.001	0.02
0d	459.54^{b}	437.69^{b}	441.68^{b}	435.46^{b}	447.59^{b}	442.38^{b}	$444.06^{Y}\pm5.57$			
60d	469.66^{b}	556.18^{a}	544.61^{a}	540.16^{a}	566.55^{a}	549.51^{a}	$537.78^{X}\pm9.56$			
120d	452.59^{b}	537.34^{a}	552.11^{a}	529.84^{a}	569.50^{a}	556.44^{a}	$532.97^{X}\pm8.94$			
Mean	$460.60^{B}\pm10.62$	$510.40^{A}\pm14.96$	$512.80^{A}\pm16.36$	$501.82^{A}\pm15.45$	$527.88^{A}\pm15.42$	$516.11^{A}\pm15.76$				
Superoxide dismutase (units/mg protein)								0.001	0.001	0.001
0d	3.64^{d}	3.62^{d}	3.55^{d}	3.62^{d}	3.56^{d}	3.65^{d}	$3.61^{Z}\pm0.036$			
60d	3.49^{d}	4.56^{c}	$4.68b^{c}$	4.74^{abc}	4.79^{abc}	4.84^{abc}	$4.51^{Y}\pm0.089$			
120d	3.40^{d}	4.71^{bc}	4.85^{abc}	4.89^{ab}	5.05^{a}	4.91^{ab}	$4.63^{X}\pm0.102$			
Mean	$3.51^{B}\pm0.04$	$4.25^{A}\pm0.13$	$4.36^{A}\pm0.14$	$4.42^{A}\pm0.15$	$4.47^{A}\pm0.16$	$4.47^{A}\pm0.14$				
Cortisol (ng/mL)								0.001	0.001	0.061
0d	8.78	9.06	8.36	9.18	8.82	8.90	$8.85^{X}\pm0.242$			
60d	9.44	7.54	7.22	7.56	7.28	7.58	$7.77^{Y}\pm0.204$			
120d	10.29	7.18	7.56	7.82	7.07	7.78	$7.95^{Y}\pm0.232$			
Mean	$9.50^{A}\pm0.38$	$7.93^{B}\pm0.26$	$7.71^{B}\pm0.19$	$8.18^{B}\pm0.36$	$7.73^{B}\pm0.27$	$8.09^{B}\pm0.32$				

^{ABC}, Means bearing different superscripts in a row differ significantly: P<0.01; xyz, Means bearing different superscripts in a sub-column differ significantly: P<0.01; ^{abc}, Means bearing different superscripts across row and column for each attribute differ significantly: P<0.05; P<0.01; D, Diet; P, Period; D×P, Diet and Period interaction. [1], Control diet (0% leaves); [2,3,4,5,6], moringa, subabul, guava, moringa and subabul (60:40 ratio), moringa and guava (40:60 ratio) leaves included at 20% in the concentrate mixture respectively partially replacing de-oiled rice bran and soyabean meal. SEM, Standard Error Mean; P-value, Probability value.

reported significant improvements in ADG and FCR with inclusion of these leaves, possibly due to variations in crude protein and fiber fractions in the diets compared to present study, where iso-nitrogenous and iso-caloric diets were offered to lambs.

Temperature humidity index: The daily temperature and humidity in animal shed were recorded to calculate temperature-humidity index. The calculated THI, ranged between 83.96 and 88.14 from 1st to 8th fortnight of trial. As per LPHSI (1990), the THI values <82 indicate absence of heat stress; 82 to 84 indicate moderate heat stress and the THI value between 84 and 86 show severe heat stress and over 86 signifies extremely severe heat stress. This indicated that the ram lambs were under severe to extreme heat stress and moderate heat stress during the entire course of study.

Blood biochemical constituents: Inclusion of moringa, subabul, and guava leaves (20% sole or combined) in lamb diets did not affect serum glucose, total protein, and cholesterol levels (Table 4). Corroborating with these findings Jelali and Salem (2014) and Ramesh (2021) reported no effect on serum glucose, total protein, and cholesterol with moringa leaves in ram lamb diets. Similarly, Ebadi *et al*. (2017) and Sushmitha (2019) observed no effect on serum cholesterol with subabul leaves in goat and ram diets. Okwori *et al*. (2016) reported no effect on serum glucose and total protein with guava leaf meal in goat diets. Contrary to findings of p[resent study, other research workers (Al Mufarji *et al*. 2022, Wankhede *et al*. 2022) reported higher serum glucose, total protein with inclusion of moringa leaves in diets of ewes and goat. Similarly, other researchers (Babiker *et al.* 2017, Wafa *et al.* 2017, Wankhede *et al*. 2022) reported lowered serum cholesterol in ewes and goats fed moringa leaves.

The HDL concentration was higher in tree leaves fed lambs as compared to CON group. Irrespective of the diets, serum HDL increased (P<0.01) from 0 day to 60 day which further increased on 120 day, while LDL content decreased (P<0.01) from 0 to 60 day and at 120 day, it was comparable to day 60. The significant decrease in LDL and increase in HDL concentration in lambs fed tree leaves diets could have resulted from functional effects associated with phenolics, tannins, flavonoids and saponin contents in tree leaves. Saponins reduces cholesterol synthesis and its absorption resulting in lower cholesterol (Saxena *et al.* 2013). Similarly other researchers (Wafa *et al.* 2017, Ramesh 2021, El-Kashef 2022a) reported decrease in serum triglycerides, LDL and increase in HDL content with inclusion of moringa leaves in diets of lambs and rabbits.

Effect on serum antioxidant activity: Lipid peroxidation (LPx) differed significantly (P<0.01) among lambs of various dietary groups and also at different time intervals of collection (Table 5). The interaction of diet and day of collection was also significantly (P<0.05) different which indicates that impact of diet on LPx changed over time. The highest LPx value was observed in the control group (CON), while tree leaves-fed lambs had the lowest levels. LPx decreased (P<0.05) with tree leaves inclusion at 20% on days 60 and 120, but remained high in CON diet lambs. In accordance with findings of present study, lowered serum TBARS content in ewes and goats was observed with feeding of moringa leaves (Babiker *et al.* 2017, Al-Juhaimi *et al.* 2020). Pathak *et al*. (2017) reported lowered lipid peroxidation with *Ficus infectoria* and *Psidium guajava* leaf meal supplementation. Similarly, Daing *et al*. (2021) reported lowered (P<0.05) lipid peroxidation with supplementation of guava leaf in diet of broilers. However, El-Harairy *et al.* (2016) found no effect on malondialdehyde concentration in ram semen with *Moringa oleifera* leaves extract.

Antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GRx), and superoxide dismutase (SOD) play a crucial role in scavenging ROS and reducing oxidative stress (Slimen *et al*. 2014). The serum GPx and GRx activities (units/mL) and SOD activity (units/mg protein) were significantly (P<0.01) different among various dietary groups and also at different intervals of collection (Table 5). The interaction of diet and day of collection was also significantly (P<0.05) different revealing that impact of diet on antioxidant activity varied significantly over time. Present study found significantly improved GPx, GRx, and SOD activities in lambs fed tree leaves-based diets compared to the control group. The increased enzyme activities are attributed to the total phenolics and tannins present in tree leaves. Pathak *et al*. (2017) reported improved GPx, reduced glutathione, and SOD activities with condensed tannins supplementation. Present results also corroborate well with previous reports (Shokry *et al.* 2020, Ramesh 2021, El-Gindy *et al*. 2022). Supplementation of guava leaf meal in diets of broilers significantly improved catalase, glutathione-S transferase, reduced glutathione, superoxidase dismutase activities compared to control group (Daing *et al*. 2021).

Effect on cortisol: Serum cortisol concentration, a key stress indicator, was significantly lower (P<0.01) in lambs fed tree leaves compared to the control group. Irrespective of diets, cortisol levels decreased over time, with the highest levels on day 0 and comparable levels on days 60 and 120 (Table 5). The reduction in cortisol is attributed to the presence of phenolics and tannins in the leaves. These findings are similar to previous studies which reported lowered cortisol levels with supplementation of phytogenic feed additives in buffalo calves (Lakhani *et al*. 2019) and moringa leaf powder supplementation in heat-stressed rabbits (Khalid *et al*. 2020).

This study concluded that dietary inclusion of moringa, subabul, guava leaves and mixture of moringa-subabul (60:40) and moringa-guava (40:60) at 20% in concentrate mixture decreased serum cortisol and lipid peroxidation, improved antioxidant enzyme activities in ram lambs with no adverse effect on feed intake, growth rate and feed efficiency.

ACKNOWLEDGEMENT

The authors wish to acknowledge the financial assistance received from European funded ECLIPSE-CIRAD project for carrying out the present work.

REFERENCES

Al-Juhaimi F Y, Alsawmahi O N, Abdoun K A, Ghafoor K and Babiker E E. 2020. Antioxidant potential of moringa leaves for improvement of milk and serum quality of Aardi goats. *South African Journal of Botany* **129**: 134–37

Al Mufarji A, Mohammed A A, Al Masruri H and Al Zeidi R. 2022. Modulation impacts of *Moringa oleifera* on thermo tolerance parameters and blood indices in subtropical ewes under heat stress. *Advances in Animal and Veterinary Sciences* **10**(7): 1641–8.

Babiker E E, Juhaimia F A L, Ghafoora K and Abdoun K A. 2017. Comparative study on feeding value of Moringa leaves as a partial replacement for alfalfa hay in ewes andgoats. *Livestock Science* **195**: 21–26.

Carlberg I and Mannervik B. 1985. Glutathione reductase. *Methods in Enzymology* **113**: 484–90.

Daing M I, Pathak A K, Sharma R K and Zargar M A. 2021. Growth performance, nutrient utilization, blood indices and immunity of broiler chicks fed diets with graded level of condensed tannins containing *Psidium guajava* leaf meal. *Animal Nutrition and Feed Technology* **21**(2): 327–40.

Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**(1): 1–42.

Ebadi M, Abadi T M, Vakili S T, Chaji M and Mirzadeh K. 2017. The substitution effect of subabul (*Leucaena leucocephala*) with alfalfa on digestibility, ruminal fermentability, chewing behavior and blood metabolites of Najdi goat. *Iranian Journal of Animal Science Research* **9**(2): 158–69.

El-Gindy Y M, Zahran S M, Ahmed M H, Adegbeye M J, Salem A Z and Salam M Y. 2022. Enhancing semen quality, antioxidant status and sex hormones of V-line rabbit bucks fed on supplemented diets with dried moringa leaves. *Animal Biotechnology* **20**: 1–10.

El-Harairy M A, Abdel-Khalek A E, Khalil W A, Khalifa E I, El-Khateeb E Y and Abdulrhmn A M. 2016. Effect of aqueous extracts of *Moringa oleifera* leaves or *Arctium lappa* roots on lipid peroxidation and membrane integrity of ram sperm preserved at cool temperature. *Journal of Animal and Poultry Production, Mansoura University* **7**(12): 467–73.

El-Kashef M. 2022a. Impact of using moringa oleifera leaves meal in growing rabbit diets on productive performance, carcass traits and blood biochemical changes un-der heat-stress conditions. *Egyptian Journal of Rabbit Science* **32**(2): 141–62.

Gebregiorgis F, Negesse T and Nurfeta A. 2012. Feed intake and utilization in sheep fed graded levels of dried moringa (*Moringa stenopetala*) leaf as a supplement to Rhodes grass hay. *Tropical Animal Health and Production* **44**: 511–17.

ICAR. 2013. *Nutrient Requirements of Sheep, Goat and Rabbit*. Indian Council of Agricultural Research, New Delhi. pp. 5.

Jelali R and Salem H B. 2014. Daily and alternate day supplementation of *Moringa oleifera* leaf meal or soyabean meal to lambs receiving oat hay. *Livestock Science* **168**: 84–88.

Jiwuba P C, Ahamefule F O, Okechukwu O S and Ikwunze K. 2016. Feed intake, body weight changes and haematology of West African dwarf goats fed dietary levels of Moringa oleifera leaf meal. *Agricultura* **13**(1-2): 71–77.

Khalid A R, Yasoob T B, Zhang Z, Yu D, Feng J, Zhu X and Hang S. 2020. Supplementation of *Moringa oleifera* leaf powder orally improved productive performance by enhancing the intestinal health in rabbits under chronic heat stress. *Journal of Thermal Biology* **93**: 102680.

Kumar A, Kadam S S, Yadav R P and Singh S K. 2019. Tree fodder as an alternate land use option for sustaining forage security in India. *International Journal of Chemical Studies* **7**(2): 202–07.

Lakhani N, Kamra D N, Lakhani P and Alhussien M N. 2019. Immune status and haemato-biochemical profile of buffalo calves supplemented with phytogenic feed additives rich in tannins, saponins and essential oils. *Tropical Animal Health and Production.* **51**: 565–73.

Leketa K, Hassen A, Donkin E F and Akanmu A M. 2019 Substitution of Leucaena hay for oil seed cake meal in total mixed rations for goats. *South African Journal of Animal Science* **49**(5): 934–43.

LPHSI. 1990. *Livestock and Poultry Heat Stress Indices Agriculture Engineering Technology Guide*. Clemson University, Clemson, SC 29634, USA.

Madesh M and Balasubramanian K A. 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry and Biophysics* **35**(3): 184–88.

Mataveia G A, Garrine C M L P, Pondja A, Hassen A and Visser C. 2019. Impact of sup- plementation of *Moringa oleifera* and *Leucaena leucacephala* tree fodder on the production performance of indigenous goats in Mozambique. *Black Sea Journal of Agriculture* **2**(2): 93–102.

Mendieta-Araica B, Sporndly R, Sanchez N R and Sporndly E. 2011. Moringa (*Moringa oleifera*) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. *Livestock Science* **137**: 10–17.

National Research Council. 1971. *A Guide to Environmental Research on Animals*. National Academy of Science, Washington, DC

Ohkawa H, Ohishi N and Yagi K.1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* **95**(2): 351–58.

Okwori A I, Abu A H, Ahemen T and Ojabo L. 2016. Growth performance, haematological and serum biochemical profiles of West African dwarf goats fed dietary guava leaf meal. *International Journal of Agriculture and Biosciences* **5**: 188–91.

Paglia D E and Valentine W N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine* **70**(1): 158–69.

Pathak A K, Dutta N, Pattanaik A K, Sharma K, Banerjee P S and Goswami T K. 2017. The effect of condensed tannins supplementation through *Ficus infectoria* and *Psidium guajava* leaf meal mixture on erythrocytic antioxidant status, immune response and gastrointestinal nematodes in lambs (*Ovis aries*). *Veterinarski Aarhiv* **87**(2): 139–56.

Rahman M Z, Ali M Y, Talukder M A, Ershaduzzaman M and Akter M S. 2016. Effect of feeding tree forages on productive performances on growing sheep. *Asian Journal of Medical and Biological Research* **1**(3): 648–53.

Ramesh P. 2021. 'Assessment of anti-oxidant capacity of moringa and mulberry leaves and their effect on growth, nutrient utilization and reproductive traits in ram lambs during summer.' PhD thesis submitted to PVNRTVU, Hyderabad, India

Redekar M H, Bhalerao S M, Khanvilkar A V, Patodkar V R and Doiphode A Y. 2019. *Moringa oleifera* leaf meal supplementation on the performance of growing sheep. *The Indian Veterinary Journal* **96**(06): 43–45.

Saxena M, Saxena J, Nema R, Singh D and Gupta A. 2013. Phytochemistry of medicinal plants. *Journal of Pharmacognosy Phytochemistry* **1**: 168–82.

Sejian V, Maurya V P, Kumar K and Naqvi S M K. 2013. Effect of multiple stresses (thermal, nutritional and walking stress) on growth, physiological response, blood biochemical and endocrine responses in Malpura ewes under semi-arid tropical environment. *Tropical Animal Health and Production* **45**: 107–16.

Shokry D M, Badr M R, Orabi S H, Khalifa H K, El-Seedi H R and Abd Eldaim M A. 2020. *Moringa oleifera* leaves extract enhances fresh and cryo preserved semen characters of Barki rams. *Theriogenology* **153**: 133–42.

Simbaya J, Chibinga O and Salem A Z M. 2020. Nutritional evalution of selected fodder trees: Mulberry (*Morus alba Lam.*), Leucaena (*Leucaena luecocephala Lam de Wit.*) and Moringa (*Moringa oleifera Lam.*) as dry season protein supplements for grazing animals. *Agroforest Systems* **94**: 1189–97.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. Oxford and IBM Publications New Delhi. pp. 265.

Sushmitha T. 2019. 'Effect of feeding chickpea straw based complete feed blocks with incorporation of tree leaves on nutrient utilization in native sheep.' MVSc thesis, PVNRTVU, Hyderabad, India

Tadesse A, Melesse A and Rodehutscord M. 2022. Partial substitution of concentrate mix with dried *Leucaena leucocephala* leaf reduced in vitro methane production in rams without affecting the nutrient intake and performance traits. *Tropical and Subtropical Agroecosystems* **25**(2): 22.

Wafa W M, El-Nagar H A, Gabr A A and Rezk M M. 2017. Impact of dietary *Moringa oleifera* leaves supplementation on semen characteristics, oxidative stress, physio- logical response and blood parameters of heat stressed buffalo bulls. *Journal of Animal and Poultry Production, Mansoura University* **8**(9): 367–79.

Wankhede S D, Dutta N, Tambe M B, Kaur N, Jadhav S E and Pattanaik A K. 2022. Effect of dietary inclusion of *Moringa oleifera* foliage on nutrient metabolism, metabolic profile, immunity and growth performance of goat kids. *Emerging Animal Species* **3**: 100005.

Zapata C, Salinas J, Moran-Martínez J, De Santiago A, Veliz F G, García J E and Mellado M. 2021. Growth rate, scrotal circumference, sperm characteristics, and sexual behavior of mixed-breed goat bucks fed three leguminous trees. *Spanish Journal of Agricultural Research* **19**(4): e06.