RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 5: 804-810 (May 2025)

Effect of Moringa, Subabul and Guava Leaves Either Sole or in Combination on Nutrient Utilization, Nitrogen Balance of Ram Lambs

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10.18805/IJAR.B-5388

ABSTRACT

Background: The use of alternate feed resources like tree leaves is key element in addressing the problems derived from climate change and food shortages that demand establishment of sustainable feeding system for ruminants.

Methods: A study was conducted on 36 Nellore cross ram lambs (6-7 months; 18.48±0.29 kg BW) randomly allotted to six dietary treatments to evaluate the effect of 20% dietary inclusion of moringa (MOR) subabul (SBL) and guava (GVL) leaves either sole or in combination (MS and MG mixture incorporated at 60:40 and 40:60, respectively) in concentrate mixture on nutrient utilization, plane of nutrition and nitrogen balance. The control group concentrate mixture contained maize, de-oiled rice bran, cotton seed cake and soybean meal as major ingredients and in the 5 experimental concentrate mixtures, de-oiled rice bran and soybean meal were partially replaced with moringa (MOR20), subabul (SBL20), guava (GVL20), moringa and subabul mixture (MS20) and moringa and guava mixture (MG20), by including them at 20%. Animals were fed with respective concentrate mixtures to meet about 80% of protein requirements and sorghum stover was offered *ad libitum* to meet the nutrient requirements of lambs for maintenance and growth as per ICAR (2013).

Result: Dietary inclusion of tree leaves had no effect on the intake of various nutrients *viz*. DM, OM, CP, EE, CF, NFE, NDF, ADF, Hemicellulose and cellulose and were comparable (P>0.05) among the six dietary groups. The crude protein and crude fibre digestibility improved (P<0.01) in tree leaves included diets compared to control, while ether extract (EE) digestibility was lower (P<0.01) in tree leaves included diets compared to CON. The DCP, TDN (%) and ME content was higher (P<0.05) in MS20, MG20 and MOR20 groups compared to CON. The N intake (g/d), excretion through faeces and urine (g/d), total N excretion and total N balance (g/d) was comparable among the different dietary groups.

Key words: Dietary inclusion, Lambs, Nitrogen balance, Nutrient utilisation, Tree leaves.

INTRODUCTION

Sheep are the most important species of economic value especially to the small and marginal farmers and to the landless laborers in the country. In India sheep are reared mainly on grazing and continuous depletion of grazing lands due to urbanization, pressure for food and cash crops on land, resulting in scarcity of fodder which is adversely affecting production potential of small ruminants. Supplementation with concentrates could overcome the fodder scarcity and improve the productive performance of small ruminants, but it is generally not a profitable practice due to high feeding costs as the available ingredients for concentrate production are limited (Salem and Smith, 2008). Success of livestock sector largely depends on quality feed and fodder (Kumar *et al*., 2010), thus it is important to develop alternatives which constantly supply of feed and fodder at low cost. Fodder trees provide one such option since they have been traditionally grown in many regions and could be an integral part of diets of ruminants. Previously experiments have been conducted with supplementing tree leaves or replacing part of dietary protein with few studies involving mixture of tree leaves (Patra *et al.,* 2006). However an adherence to fixed composition of leaf mixture is not feasible because of area specific distribution and

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How to cite this article: Raju, S., Nagalakshmi, D., Nalini Kumari, N., Rajanna, N., Swathi, B. and Roupesh, G. (2025). Effect of Moringa, Subabul and Guava Leaves Either Sole or in Combination on Nutrient Utilization, Nitrogen Balance of Ram Lambs. Indian Journal of Animal Research. 59(5): 804-810. doi: 10.18805/IJAR.B-5388.

Submitted: 23-04-2024 **Accepted:** 17-06-2024 **Online:** 22-07-2024

accessibility of plant species to farmers. Hence the present study was designed to investigate the effect of dietary inclusion of locally available tree leaves either sole or in combination on nutrient utilization, plane of nutrition and nitrogen balance in lambs.

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) in the year 2022.

Procurement of Feed Ingredients

The 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 at college premises which were shade dried and ground. The concentrate feed ingredients (maize grain, de-oiled rice bran, cotton seed cake, soybean meal, salt, calcite powder and mineral mixture) 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 as major ingredients and in the 5 experimental concentrate mixtures, de-oiled rice bran and soybean 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% (Table 1). All the concentrate mixture were iso-nitrogenous and iso caloric and contain 20% CP and 68 % TDN.

Experimental animals and management

Thirty six Nellore cross lambs with an average body weight of 18.48±0.29 kg were purchased from local market. After initial quarantine and adaptation period for 15 days they were randomly divided in to 6 groups consisting of 6 animals each in completely randomized design. Animals were fed with concentrate mixture and *ad libitum* sorghum stover separately twice in a day such that concentrate mixture supplied around 80% of crude protein requirements and to meet the nutrient requirement for maintenance and growth (100g/day) per ICAR (2013).

Metabolic trial and analytical procedure

A metabolism trial with 5 days collection period was conducted after completing 120 days of feeding with experimental diets by shifting them to metabolic cages with facilities for individual feeding and watering. The feed, faecal and urine samples were analyzed for proximate constituents (AOAC, 2019).

Statistical analysis

The data was subjected to one way analysis of variance as per the procedure of Snedecor and Cochran (1994) using software (SPSS, Version 20). The difference between means was tested by using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of tree leaves

The chemical composition of moringa, subabul and guava leaves are presented in Table 2.

Table 1: Ingredient composition of concentrate mixtures containing Moringa, subabul and guava 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	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 B6 0.2 mg, Vitamin A 7500 IU, Vitamin D3 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.

Chemical composition of experimental diets

The chemical composition of concentrate mixtures containing twenty per cent (%) inclusion of moringa, subabul and guava leaves were similar for most of nutrients (Table 3), since only soya bean 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.

Nutrient intake and digestibility

The mean intakes of DM, CP, DCP, TDN and ME were not affected (P>0.05) in ram lambs with inclusion of moringa, subabul and guava leaves either sole or in combination at 20% in concentrate mixtures and was comparable to control diet fed animals. The comparable nutrient intake can be attributed to the fact that the diets were iso-caloric and iso-nitrogenous and also inclusion levels of tree leaves were within the levels that were not adversely affecting the intakes. In accordance with our findings Ramesh (2021) observed no effect on nutrient intake with inclusion of moringa and mulberry leaves up to 35% in concentrate mixture of lambs. Similarly dietary replacement of concentrate mixture with subabul leaves at 20% in chick pea straw based complete diets had no effect on the nutrient intake in rams (Sushmitha, 2019). Contrary to our results,Wankhede *et al.* (2022) reported higher (P<0.05) DCP and TDN intake in goat kids fed complete diets replacing wheat straw with moringa foliage at 25% compared to control which could be due to higher DM and CP intake in moringa supplemented group, in contrast to our study where intake of nutrient was similar in all dietary treatments.

The DM digestibility was higher (P<0.01) in MOR20, SBL20, MS20, MG20 dietary groups compared to control, while in GVL20 diet fed lambs the digestibility was

Table 2: Chemical composition (%DM basis) of Moringa, subabul and guava leaves.

Constituent	Moringa	Subabul	Guava
Dry matter	91.50	91.63	91.03
Organic matter	88.49	88.82	93.31
Crude protein	18.35	23.17	13.15
Ether extract	8.56	9.32	2.66
Crude fiber	6.71	13.67	16.47
Nitrogen free extract	54.84	42.67	61.04
Total ash	11.51	11.17	6.68
Neutral detergent fiber	40.56	47.97	56.08

Each value is average of duplicate analysis.

Table 3: Chemical composition (%DM basis) of Sorghum stover and concentrate mixtures containing twenty percent 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
Nitrogen free extract	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
Acid insoluble ash	3.12	2.34	2.56	3.84	2.94	2.65	0.65
Neutral detergent fiber	63.54	58.54	59.37	60.53	57.57	58.87	62.51
Acid detergent fiber	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 soya bean meal.

Table 4: Nutrient intake digestibility and plane of nutrition in ram lambs fed diets containing concentrate mixtures with 20% inclusion of moringa subabul and guava leaves.

Attributes	CON[1]	MOR20[2]	SBL20[2]	GVL20[3]	MS20[3]	MG20[6]	SEM	P value
Nutrient intake (g/day)								
Dry matter (g)	911.66±21.27	921.21±21.45	927.63±14.17	910.49±24.96	915.76±15.70	926.79±11.63	6.932	0.975
Crude protein (g)	105.29±2.53	105.48±2.55	106.99±1.59	104.93±3.35	105.19±1.68	106.44±1.14	0.832	0.984
Digestible crude protein(g)	65.27±4.46	71.53±2.97	69.19±2.99	67.34±0.91	72.90±3.93	70.88±2.84	1.281	0.579
Total digestible nutrients (g)	472.95±30.60	520.71±26.92	509.16±20.44	488.11±13.68	543.19±29.44	523.94±13.61	9.787	0.358
Metabolisable energy (Mcal)	1.71±0.11	1.88±0.95	1.84±0.07	1.76±0.04	1.96±0.10	1.89±0.04	0.035	0.363
Nutrieint digestibility (%)								
Dry matter	59.97c±0.51	63.76b±1.05	62.83b±1.16	61.43bc±0.74	66.45a±0.69	63.96b±0.70	0.469	0.001
Organic matter	62.74d±0.64	66.33bc±1.36	64.00cd±0.65	63.93cd±0.48	68.97a±0.57	66.48b±0.73	0.462	0.001
Crude protein	61.75e±0.46	67.74ab±1.12	64.60cd±0.68	64.35d±0.60	69.19a±0.64	66.56bc±0.62	0.488	0.006
Ether extract	72.03a±0.57	64.73b±0.84	65.33b±1.16	64.70b±0.73	65.20b±0.75	65.01b±0.66	0.526	0.001
Crude fibre	52.65c±0.83	58.78a±1.10	57.84ab±0.85	55.54b±0.91	59.10a±1.19	57.97ab±0.66	0.522	0.001
Nitrogenfree extract	54.74c±0.98	60.02b±1.57	58.38bc±1.01	57.09bc±1.06	63.88a±1.20	60.93ab±1.42	0.676	0.001
Neutraldetergent fiber	55.38d±0.85	61.99a±1.06	61.03ab±0.88	57.56cd±0.85	60.76ab±0.71	58.98bc±1.04	0.515	0.0001
Acid detergent fibre	45.94c±0.67	50.36ab±0.89	48.13bc±0.76	46.54c±0.79	51.56a±1.01	47.61c±0.75	0.460	0.0001
Hemicellulose	67.29b±1.13	71.48a±1.56	72.20a±1.24	72.05a±1.29	73.08a±1.11	73.58a±1.19	0.589	0.022
Cellulose	59.20b±0.93	60.15b±0.85	59.59b±0.095	58.99b±0.69	64.26a±1.30	61.04b±0.88	0.473	0.005
Nutitive value of ration (%)								
CP (%)	11.54±0.11	11.45±0.12	11.53±0.14	11.52±0.13	11.48±0.08	11.48±0.09	0.044	0.916
DCP (%)	7.14c±0.19	7.74ab±0.10	7.45abc±0.13	7.41bc±0.16	7.94a±0.19	7.64ab±0.16	0.073	0.020
TDN (%)	51.79c±1.42	56.41ab±1.10	54.82bc±1.12	53.77bc±1.36	59.22a±21.45	56.51ab±0.86	0.612	0.004
ME (Mcal/kg DM)	1.87c±0.09	2.03ab±0.05	1.98bc±0.04	1.94bc±0.05	2.14a±0.07	2.04ab±0.04	0.024	0.018

Each value is an average of six observations.

[abc]Means with different superscripts in a row differ significantly: P<0.05.

[1]Control diet (0% leaves).

[2,3,4,5.6]Moringa, subabul,guava,moringa and subabul (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.SEM: Standard error mean; P value: Probability value.

comparable to control diet lambs (Table 4). The OM digestibility (OMD) improved (P<0.05) with inclusion of MOR20, MS20 and MG20 diets in concentrate mixture compared to CON. Whereas with inclusion of SBL20 and GVL20 diets no improvement in OMD was observed. Corroborating with our findings Sanchez *et al.* (2006), Giang *et al.* (2016) reported improved DM and OM digestibility in cows with feeding of moringa and subabul leaves. In contrast to our findings, Patra *et al.* (2006) reported that partial replacement (50%) of protein by *Leucaena leucocephala–Morus alba–Azadirachta indica* (2:1:1) leaf meal mixture in goats had no effect on digestibility of DM and OM. Similarly, Ali *et al.* (2018) had observed no effect on digestibilities of OM and DM in goats with replacement of cotton seed cake with moringa leaves, while Montoya-Flores *et al.* (2020) reported that digestibility of OM in cross bred heifers decreased with increasing levels (12, 24 and 36%) of DMI of subabul leaves. These variations in results obtained from various studies may be attributed to differences in composition, inclusion levels of moringa and subabul tree leaves and other nutrients in diet.

In present study, the digestibility of CP was higher (P<0.01) with inclusion of moringa, subabul and guava leaves at 20% in concentrate mixture of lambs compared to control (Table 4). Corroborating with the observations of Sanchez *et al.* (2006), Gebregiorgis *et al.* (2012), Ramesh (2021) where CP digestibility increased with supplementation of moringa leaves. Similarly, Montoya-Flores *et al.* (2020) reported increased CP digestibility in heifers with inclusion of subabul leaves at 12 and 24% of DM compared to control. The improved CP digestibility observed in present study could be attributed to tannin content in tree leaves as tannins binds to dietary protein, thus protecting it from rumen degradation and increasing protein flow to the small intestine of the host (Salem *et al.,* 2013). Contrary to results of our study, Sushmitha (2019) reported no effect on CP digestibility with inclusion of subabul leaves in concentrate mixture at 20% in chick pea straw based complete diet of rams.

In general, the inclusion of moringa, subabul and guava leaves at 20% in concentrate mixtures improved (P<0.01) the digestibility of CF and fiber fractions (NDF, ADF, hemicellulose and cellulose) in the diet and digestibility of these was either comparable or higher than the control group (Table 4). The higher fiber digestibility in tree leaves fed lambs could be due to better utilization of CP (Table 4) and was in synchrony with soluble carbohydrate availability (Sanchez *et al.,* 2006). Similarly, Zaher *et al.* (2020), Ramesh (2021) reported higher fibre digestibilities with inclusion of moringa and mulberry leaves in concentrate mixtures of rams. In contrast to the present study no effect on fibre fractions digestibility was reported by many workers with replacement of concentrate mixture with moringa at 25, 50 and 75% in sheep (Gebru *et al.,* 2017), replacement of cotton seed cake with moringa leaves in goats (Ali *et al.,* 2018). Similarly, Sushmitha (2019) reported no effect on digestibility of fiber fractions in lambs with inclusion of subabul leaves in concentrate mixture at 20% in chick pea straw based complete feed diets.

The EE digestibility was significantly (P<0.01) lower in tree leaves leaves included diets compared to control fed lambs. The lower EE digestibility with inclusion of tree leaves could be attributed to differences in fat composition and tree leaves may contain more ether soluble components than true fat compared to soya bean meal and de-oiled rice bran in concentrate mixture. In accordance with our findings, Ramesh (2021) reported decreased (P<0.05) EE digestibility in lambs with inclusion of moringa and mulberry leaves in concentrate mixture at 30 and 35%. In contrary, Kholif *et al.* (2015); Ali *et al.* (2018); Sushmitha (2019); Wankhede *et al.* (2022) reported no effect on EE digestibility with dietary inclusion of tree leaves. The digestibility of NFE increased (P<0.01) with inclusion of moringa, subabul and guava leaves either sole or in combination at 20% in concentrate mixtures in the diet and digestibility of these was either comparable or higher than the control group. The higher NFE digestibility in lambs

Table 5: Nitrogen balance in ram lambs fed diets with concentrate mixtures containing twenty per cent (%) inclusion of moringa, subabul and guava leaves.

Attribute	CON[1]	MOR20[2]	SBL20[3]	GVL20[4]	MS20[5]	MG20[6]	SEM	P value
Metabolic body weight	12.12±0.22	12.15±0.22	12.21±0.14	12.08±0.30	12.11±0.14	12.23±0.10	0.073	0.994
N intake(g/d)	16.84±0.41	16.88±0.40	17.12±0.25	16.79±0.53	16.83±0.27	17.03±0.18	0.133	0.984
N excretion (g/d)								
Faecal	6.23±0.38	5.43±0.11	6.05±0.23	6.01±0.50	5.17±0.37	5.69±0.36	0.149	0.296
Urinary	2.46±0.40	2.92±0.22	2.94±0.26	2.74±0.31	2.99±0.23	2.79±0.18	0.108	0.786
Total	8.68±0.20	8.35±0.31	8.99±0.46	8.75±0.73	8.15±0.21	8.48±0.50	0.172	0.812
N balance								
g/day	8.17±0.31	8.53±0.56	8.13±0.69	8.04±0.25	8.68±0.47	8.55±0.57	0.189	0.918
% of intake	48.44±1.0	50.42±2.39	47.36±3.37	48.15±2.92	51.47±2.00	50.16±3.14	0.987	0.862

Each value is an average of six observations.

[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 soya bean meal. SEM: Standard error mean, P value: Probability value.

observed in present study with inclusion of moringa, subabul and guava leaves could be attributed to better synchrony in utilization of CP, fiber fractions and fermentable carbohydrates. In accordance to our findings Asaolu *et al.* (2010), Ramesh (2021) reported increased NFE digestibility with inclusion of moringa leaves in diet. In contrary Ali *et al.* (2018) Sushmitha (2019) and Wankhede *et al.* (2022) reported no effect on NFE digestibility with inclusion of tree leaves in diet.

Nutritive value and plane of nutrition

The crude protein (CP) (%) in lambs fed diets containing different concentrate mixtures was comparable whereas DCP content of diets increased with inclusion of moringa either sole or in combination with subabul or guava at 20% and similar trend was observed with regard to TDN and ME content (Table 4). The comparable CP% among diets could be attributed to similar DM intake and iso-nitrogenous concentrate mixtures fed to lambs. The higher DCP,TDN and ME content observed with MOR20, MS20 and MG20 included diets could be attributed to higher CP, CF, NFE digestibility which resulted in high energy content in diets.

Nitrogen balance

The N intake (g/d), excretion through faeces and urine (g/d), total N excretion and total N balance (g/d) was comparable among the different dietary groups (Table 5). The lambs in all the dietary groups were on positive N balance. The positive N balance among the treatment groups is indication of energy and N supply much higher than the maintenance requirement of lambs. Corroborating with our results, Sushmitha (2019); Ramesh (2021) reported no effect on N retention and N balance in lambs fed with subabul and moringa leaves. Contrary to our results, Sultana *et al.* (2015); Pineiro-Vazquez *et al.* (2017) reported significantly ($P<0.05$) higher N retention with feeding of moringa and subabul leaves.

CONCLUSION

Inclusion of moringa, subabul and guava leaves either sole or in combination at 20% in concentrate mixture of lambs significantly improved the protein and fiber digestibilities with no effect on nutrient intakes and nitrogen balance.

ACKNOWLEDGEMENT

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

Conflict of interest

The authors have no conflict of interest to declare.

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