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NUTRIENT DIGESTIBILITY AND BLOOD METABOLITES OF GROWING SHEEP AS INFLUENCED BY SUPPLEMENTARY FEEDING OF GLIRICIDIA (*Gliricidia sepium*) WITH NEEM (*Azadirachta indica*) LEAVES

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ABSTRACT

Twelve growing West African dwarf ewes, aged 7 – 8 months with an average weight of 7.00 ± 0.32kg were used in a 12 week feeding trial, to study the influence of dietary supplementation of Gliricidia sepium with neem leaves meal with a view to estimate digestibility and blood metabolites. Three levels of supplementation were used to obtain three treatment diets that included; diet 1 (50% Panicum maximum and 20% Gliricidia sepium which served as the control group), diet 2 (45% Panicum maximum and 25% Gliricidia sepium) and diet 3 (40% Panicum maximum and 30% Gliricidia sepium). Concentrate of 30% was added as fixed to all treatment diets, why 3 and 6grams of neem leaves was added to diets 2 and 3 respectively. The sheep were divided into three groups of four animals and each group was randomly allotted to one of the three diets in a completely randomized design. Results showed that ash digestibility (80.21%), serum urea (6.68mg/dl) and sodium concentration (129.12Mmole/L) were significantly ($P < 0.05$) higher in diet 1, while ether extract digestibility (82.07%) and serum cholesterol (69.01mg/dl) were higher in diet 2. Dry matter (86.25%), crude protein (90.53%), crude fiber (78.63%) and nitrogen free extract (67.80%) digestibility's, total protein (8.06g/dl), glucose (69.04mg/dl) and potassium (6.26Mmole/L) were significantly ($P < 0.05$) higher in diet 3 than diets 1 and 2. A non significant ($P > 0.05$) difference was observed with serum creatinine, calcium and phosphorus. It can thus be concluded that 40% Panicum maximum and 30% Gliricidia sepium with 30% concentrate plus 6grams of neem leaves has the potential to improve digestibility and blood metabolites of ewes.

Keywords: Blood, digestibility, foliages, ewe

Introduction

Livestock farmers in the tropics have faced a lot of challenges in generating income from their stock due to slow feed digestibility rate, reproductive inefficiencies and unstable weight gain related to seasonal imbalance of feeds. Small ruminant farmers suffer from this constraint heavily, particularly during the dry season, when the quantity of forages available is limited and deficient in nutrient. At such period, most available forages become fibrous and have low digestibility, leading to poor ruminant livestock performance. Though sheep depends on forages for their nutritional requirement, the quantity and quality are usually very poor and the nutrient is not enough to cover sheep maintenance and productive requirements. (Ajayi *et al.*, 2010). Thus, sheep experience starvation from poor nutrition that emanate from scarcity and reduction in nutrient content of pastures. The situation is further aggravated by overgrazing of pastures in most areas where forages are grown. The need to address this problem of inadequacy in nutrient supply by forages to sheep is very important to researchers. Hence, one of the sustainable ways of improving the feeding value of poor pasture in the tropics could be through supplementation strategy with shrub tree foliages to maximize nutrient digestibility and good health status to enhance performance and productivity in ruminants.

Gliricidia sepium and neem (*Azadirachta indica*) leaves have been identified as such shrub tree foliages that could serve as potential supplements for sheep on grass based diet. These foliages are characterized by high nutrient quality that can supplement forages in order to alleviate the prevailing problems of low quality forage intake in sheep (Aye and Adegun, 2010; Ogbuewu *et al.,* 2011 ; Oduguwa and Adu, 2010). Ruminant animals cannot meet their maintenance needs on grass alone during the off-season, hence the use of foliages particularly those of shrub / tree foliages known for all year round quality forage. However, limitations of effective utilization of gliricidia and neem leaves are mainly the odour, bitter taste. Hence, some levels of harnessing are required to increase the acceptability and digestibility that strengthen the health status and improve sheep performance. The study was therefore designed to determine the effect of gliricidia with neem leaves supplementation on nutrient digestibility and blood metabolites of growing ewes.

Materials and Methods

Study Area*:* The study was carried out in the Small Ruminant Unit of the Teaching and Research Farm, Ambrose Alli University, Ekpoma – Nigeria. The location of the farm is latitude 6.42^0N and longitude 6.09^0E with a unimodal rainfall pattern that starts from April and end in October. The area has a prevailing tropical climate with mean temperature and rainfall of about 31^0C and 1556mm respectively.

Sourcing and preparation of experimental diets*:* *Gliricidia sepium* foliage and *Panicum maximum* were sourced within the Teaching and Research Farm. They were allowed to wilt for 12 to 24hours before being chopped manually with cutlass about 5cm sizes. The neem leaves that were obtained fresh within Ekpoma were air-dried under shade for about 7days before milled into meal. The concentrate consisted of 70% wheat offal, 20% dried brewery grain, 8% rice bran, 0.75limestone, 0.25% bone meal, 0.75% and 0.25% vitamin. Hence, the diets comprised different proportion of *Panicum maximum*, *Gliricidia sepium* and neem leaves with fixed quantity of concentrate diet at 30%. The three experimental diets that were prepared consisted of diet 1 (50% *Panicum maximum* and 20% *Gliricidia sepium* with 30% concentrate), diet 2 (45% *Panicum maximum* and 25% *Gliricidia sepium* with 30% concentrate) and diet 3 (40% *Panicum maximum* and 30% *Gliricidia sepium* with 30% concentrate). Diet 1 that served as the control did not contain any neem leaves meal but diets 2 and 3 contained 3 and 6grams of neem leaves meal per animal per day respectively.

Management of experimental animals*:* Twelve growing West African dwarf ewe-lambs, aged between 7 and 8months and with average body weight of 7.00 ± 0.32kg were raised for this study. The animals were randomly allotted to the three dietary treatments of four animals each with four replicates of one animal each in a completely randomized design. The animals were housed in individually demarcated pens. The pens were adequately ventilated and cleaned daily. The diets were offered the animals at 5% of their body weights once daily. The experimental diets were manually mixed to ensure voluntary consumption among the animals. Clean water was provided *ad libitum* with all routine management practices and medications carried out strictly. Animals were allowed out for exercise early in the morning on days with favourable weather. The study lasted for 12weeks after 2weeks adjustment period.

Digestibility trial

At the end of the experimental trial before termination, four sheep per treatment (totalling 12 sheep) were taken and placed in individual metabolic cages designed with facilities for feeding, watering with separate collection of faeces and urine. The animals were allowed 7-days to adjust to the environment and experimental diets before 7-days feeding trial that required daily weighed quantities of feed offered and total collection of faeces. Faecal collection from each animal was oven dried at 85^0C for 24hours, weighed, bulked and aliquot (10%) was pooled for chemical analysis. The procedure of Vogtmann *et al.* (1975) was used in calculating the digestibility for fat, crude protein, crude fibre, ash and nitrogen free extract.

$$\text{Apparent digestibility coefficient} = \frac{\text{Nutrient in feed} - \text{Nutrient in faeces}}{\text{Nutrient in feed}} \times \frac{100}{1}$$

Blood collection and constituents assay

At the end of 10^{th} week of the feeding trial, blood samples were collected in the morning from four sheep per treatment prior to feeding. About 8ml of blood sample was collected from each sheep through jugular venipuncture, using hypodermic needle and sterile disposable syringes.

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Free flow of the blood into labeled sterile universal bottles without anti-coagulant was used for determination of serum biochemical indices and plasma electrolytes. Blood samples were allowed to clot and the serum was separated immediately by centrifugation at 3500rpm for 10minutes. Serum biochemical parameters and plasma electrolytes assessed were according to the procedure reported by Sowande *et al.* (2008a).

Chemical and statistical analyses

The proximate composition of the test ingredients (*Gliricidia sepium* and neem leaves) and the experimental diets (Table 1) were determined by the method of AOAC (1990)

Data obtained from nutrient digestibility and blood metabolites were subjected to analysis of variance (ANOVA) and significant means were separated by Duncan multiple range test (SAS, 2002).

Results and Discussion

Presented in Table 1 are analysed proximate composition of feedstuffs and the experimental diets. Dry matter values that ranged from 67.03% in *Gliricidia sepium* to 92.42% in neem leaves were quite high, indicating good quality of the feed ingredients in nutrient retention. Crude protein value of diet 1 (15.87%) was lower than that of diets 2 (16.92%) and diet 3 (17.43%). The relative proportion of inclusion of *Gliricidia sepium* and neem leaves could be responsible for the difference observed. Okah and Anita (2016) observed that crude protein content of browses and tree foliages are higher than forage grass. However, the average percentage of 16.74% crude protein of the experimental diets fed to sheep was within the 14 – 18% recommended for reproductive performance of small ruminants as reported by NRC (1981). This shows that crude protein content in these diets were within recommended limits for good performance and without nutritional disorders. The crude fibre content of the experimental diets that ranged between 15.11% and 26.74% was highest in diet 1 and lowest in diet 3. The higher crude fibre content of guinea grass could possibly influence the content on diet 1. Values for ash that ranged from diet 3 (8.34%) to diet 1(8.77%) were similar in content. The difference observed in ether extract (1.28% to 1.60%) and nitrogen free extract (33.00 to 35.91%) gave an idea of the amount of oil and energy content present in the diets. The proximate composition of neem leaves, *Gliricidia sepium and Panicum maximum* obtained in this study were comparably to the values reported by Ogbuewu *et al.* (2011); Aye and Adegun (2010); Agangan and Tshwenyane (2004) respectively.

Table 1. Proximate composition (%DM) of feedstuffs and experimental diets for growing sheep.

	Feedstuffs				Diets		
Parameter	NL	GS	PM	CD	1	2	3
Dry matter	92.42	67.03	78.43	86.48	88.57	86.03	85.99
Crude protein	19.68	28.31	7.00	20.03	15.87	16.92	17.43
Crude fibre	16.61	20.06	37.00	14.01	26.74	25.98	25.11
Ash	7.10	6.67	10.00	7.99	8.77	8.66	8.34
Ether extract	4.16	3.00	0.90	1.90	1.28	1.50	1.60
NFE	44.91	49.02	45.11	56.92	35.91	33.00	33.42

NL= Neem leaves, GS= *Gliricidia sepium*, PM= *Panicum maximum*, CD= concentrate diet, NFE= Nitrogen free extract

Nutrient digestibility coefficient of West African dwarf sheep fed *Gliricidia sepium* with neem leaves supplement is depicted in Table 2. The results showed that apparent nutrient digestibility coefficient values were significantly different ($P < 0.05$) among the treatment diets. The mean percentage dry matter digestibility values tended to be higher in diet 3(86.25%) and decreased as the proportion of *Panicum maximum* inclusion levels increased in the diets 2 (82.67% and 1(70.32%). This suggested that increase in inclusion of *Gliricidia sepium* with neem leaves supplement to *Panicum maximum* basal diet could promote dry matter digestibility. Okah and Anita (2016) confirmed this observation when the dry matter digestibility increases as the proportion of selected browse plants supplement increases in guinea grass based diet for goats. Crude protein digestibility that ranged between 71.99% and 90.53% followed the same trend as in dry matter digestibility. This implies that combination of *Gliricidia sepium* with neem leaves as supplement to *Panicum maximum* positively influenced digestibility of crude protein. This is in line with the report of Babayemi and Bamikole (2006) who found that supplement of browse to a basal diet of *Panicum maximum* will improve dietary protein as well as digestibility in goats. The values obtained for crude protein digestibility in this work were relatively comparable with the mean value of 76.77% reported by Ukanwoko et al. (2009) for goats fed poultry waste and cassava peel based diets. Digestibility of crude fibre decreased with increased levels of grass in the diets. The significant ($P < 0.05$) difference in crude fibre digestibility observed in goats fed

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the experimental diets could be attributed to the crude protein content of the diets. The higher digestibility values for crude fibre in diets 3(78.63%) and 2(77.26%) suggest that during the dry season because of high cell wall contents, forages quality is too low to sustain animals and a balanced selection of browse for use as supplement could increase digestibility of crude fibre. However, the poorly digested crude fibre in diet 1(66.87%) might be linked with high percentage of fibre and residual protein inhibitor in the diet. Ososanya *et al.* (2013) reported that crude fibre and protein digestibilities increase with increasing levels of crude protein content in the diets. It was also noticed by Sowande *et al.* (2008b) that maximum dietary crude fiber digestibility in the rumen occurs when dietary crude protein is between 12 and 16% in the diet, in increasing the level of crude protein in the diets beyond 16% had no further much effect on fiber digestion. Ether extract digestibility that ranged from 70.16% to 82.07% did not follow any specific trend with the dietary treatments. The superiority of the ether extract digestibility exhibited by diet 2 significantly ($P < 0.05$) proved that goats on this group optimally utilized the oil to maximize digestibility and productivity compared with diets 1 and 3. Ash digestibility was significantly ($P < 0.05$) higher in sheep on diet 1(80.21%), followed by diets 2(70.08%) and 3(70.19%). The observed difference in ash digestibility could probably be a true reflection of the component of the diets, since nutrient digestibility among other factors could depend on the proximate composition of a ration as reported by Okoruwa and Adewumi (2010). Nitrogen free extract digestibility values of 59.64, 62.78 and 67.80% were recorded for diets 1, 2, and 3 respectively. These values were significantly ($P < 0.05$) different and increased as the forage grass in the diets tended to decline with increase in experimental foliages. This agrees with Okah and Antia (2016) who reported that feeding either grass or browse plant alone may not support adequate nutrient intake and digestibility but a combination of the two in appropriate ratio will enhance nutrient digestibility and utilization for the overall productivity of the animals.

Digestibility of feed is classified as high when values are greater than 60%, medium when values are between 40% and 60% and low when values are lesser than 40% (FAO, 1995). In this study, the apparent nutrient digestibility of dry matter, crude protein, crude fibre, ether extract and ash were generally high except for the medium value obtained for nitrogen free extract in ewes on diet 1.

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Table 2. Apparent nutrient digestibility of West African dwarf sheep fed *Gliricidia sepium* with neem leaves supplement

Parameters	Diets			SEM±
	1	2	3	
Dry matter	70.32^{c}	82.67^{b}	86.25^{a}	0.28
Crude protein	71.99^{c}	88.79^{b}	90.53^{a}	0.15
Crude fibre	66.87^{b}	77.26^{a}	78.63^{a}	0.86
Ether extract	70.16^{c}	82.07^{a}	79.88^{b}	0.37
Ash	80.21^{a}	76.08^{b}	70.19^{c}	0.66
Nitrogen free extract	59.64^{c}	62.78^{b}	67.80^{a}	0.82

^{a,b,c} Means ion the same row with different superscripts differ significantly ($P < 0.05$).
SEM= Standard error of mean.

Table 3 shows the blood metabolites of sheep fed *Gliricidia sepium* with neem leaves supplement. Parameters observed in blood metabolites were significantly ($P < 0.05$) affected by the treatment diets except for creatinine, calcium and phosphorus. Total protein values were similar between diets 2(8.02g/dl) and 3(8.06g/dl) but they were significantly ($P < 0.05$) higher than sheep on diet 1(7.89g/dl). Total protein being the most abundant compound in serum is of important diagnostic significance because they are involved in enzymes hormones and anti-body synthesis and serves a reserve source of nutrient for body tissues and muscles. The statistical ($P > 0.05$) similarity of total protein values for sheep in diets 2 and 3 indicates that the quality of protein in the test diets were adequate and the consumption by the sheep did not result in stress, diseases, starvation or malnutrition. The values recorded were within the optimum levels reported by Taiwo and Ogunsanmi (2003) for sheep. Glucose concentration values recorded were also significantly ($P < 0.05$) influenced by the treatment diet with sheep in diet 3 (69.04 mg/dl) being highest and those in diet 1(60.01mg/dl) the lowest. The disparity in values observed could be connected to the nutritional adequacy in terms of energy and safety of the test diets. This corroborates the finding of Okoruwa et al. (2016) who found that glucose synthesis in the blood is directly proportional to the amount of energy absorb and utilize in diets by the animals. There were significant ($P<0.05$) variations in cholesterol concentration among animals on the treatment diets. Animals on diet 2 (69.01mg/dl) recorded higher in serum cholesterol

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concentration than those on diets 3 (66.39mg/dl) and 1 (60.93mg/dl). Falola and Olufayo (2017) reported that cholesterol level of 180mg/dl and below is safe and may not result in arteriosclerosis. The cholesterol concentration obtained in this study therefore, suggests a safe concentration among the animals. Creatinine levels in the serum indicate the extent of muscle degradation in animals. When the values are high, it shows a high degradation of muscle phospho-creatinine to form creatinine or vice versa. This lays credence to the work by Okosun and Eguaoje (2017) who reported that creatinine is an indirect measure of protein utilization in livestock. However, the low creatinine levels (0.89 – 0.99mg/dl) and relative non-significant ($P > 0.05$) values recorded in this study suggest favourable protein utilization and non muscle wastage in the sheep. Serum urea concentration was significantly ($P < 0.05$) higher in sheep on diet 1(6.68mg/dl) compared with those on diets 2(4.89mg/dl) and 3(4.21mg/dl). Serum urea test measures the amount of nitrogen in the serum, high serum urea level is probably an indication of kidney dysfunction, because protein intake and kidney functioning are affected by quantity of blood urea nitrogen. The values recorded in this study were within the normal reference range of 2.03 to 7.01mg/dl for sheep as reported by Konlan et al. (2012), implies that the kidney was unaffected. Thus, there was a better utilization of protein, since blood urea can be attributed to protein breakdown. However, a contrary view was observed from the finding of Okoruwa et al. (2016) who reported serum urea levels of 2.83 to 7.83mg/dl as better urea nitrogen utilization. The significant ($P < 0.05$) variation of sodium levels (121.99 to 129.12 Mmole/L) observed in sheep on the treatment diets could be a reflection of varied dietary intake of salt and loss of sodium ion in the diets. Sodium concentration values recorded in this study were comparable with the values (113.02 to 139.17Mmole/L) reported by Taiwo and Ogunsanmi, (2003) for healthy sheep. The potassium concentration level recorded in sheep on diet 3 (6.26Mmole/L) was significantly ($P < 0.05$) higher, followed by diets 2(5.33Mmole/L) and 1(3.93Mmole/L). However, it might perhaps be logical to infer that inferior potassium level in diet 1 compared with diets 2 and 3 explains the tendency for infection. Calcium and phosphorus concentration levels that ranged from 4.59 to 4.74mg/dl and 4.37 to 4.93mg/dl respectively were not significantly ($P > 0.05$) affected by the treatment diets. This could probably explain the higher intake of available calcium and phosphorus contents in the diets that were optimally absorbed and utilized by the sheep.

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Table 3. Blood metabolites of sheep fed *Gliricidia sepium* with neem leaves supplement.

Parameters	Diets			SEM±
	1	2	3	
Total protein (g/dl)	7.89[b]	8.02[a]	8.06[a]	0.18
Glucose (mg/dl)	60.01[c]	67.37[b]	69.04[a]	0.23
Cholesterol (mg/dl)	60.93[c]	69.01[a]	66.39[b]	0.36
Creatinine (mg/dl)	0.99	0.96	0.89	0.04
Urea (mg/dl)	6.68[a]	4.89[b]	4.21[b]	0.07
Sodium (Mmole/L)	129.12[a]	123.03[b]	121.99[c]	0.36
Potassium (Mmole/L)	3.93[c]	5.33[b]	6.26[a]	0.08
Calcium (mg/dl)	4.74	4.62	4.59	0.05
Phosphorus (mg/dl)	4.37	4.04	4.93	0.02

[a,b,c] Means on the same row with different superscripts differ significantly ($P < 0.05$).
SEM = Standard error of mean.

Conclusion

The results obtained in this study, show that digestibility and health of West African dwarf sheep status were improved when *Gliricidia sepium* with neem leaves were used as supplement to a basal diet of guinea grass. It is an indication that the nutritive – potential of these tree foliages can go a long way in solving the problem of dry season feeds in ruminants.

However, digestibility and blood metabolites in sheep were better pronounced when 40% *Panicum maximum* with 30% *Gliricidia sepium* and 30% concentrate were fed with 6grams of neem leaves meal. This suggests that optimum protein-energy ratio for microbial synthesis was achieved with this dietary combination of forage grass and tree foliage.

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