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Effect of Neem Leaf Meal (*Azadirachta indica*) Inclusion Levels on Haematology and Biochemical Parameters of Uda Rams

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Abstract

The research that investigated the influence of feeding graded amounts of neem leaf meal on Uda rams was undertaken at Professor Lawal Abdu Saulawa Livestock and Teaching Farm, Federal University Dutsin-Ma, Katsina State. Twenty Uda rams were used for the study and were allocated to four (4) treatments in a Completely Randomised Design (CRD). Each of the experimental animals served as a replicate. The experimental diets which served as the treatments contained the test ingredients in the following order: A (0%), B (5%), C (10%) and D (15%). The feeding trial lasted for 84 days after which samples of blood were gotten early in the morning before being to their being fed. The samples obtained from two animals that were chosen at random from each treatment were analysed for haematological and biochemical parameters. The outcomes of these were not significantly (P>0.05) different from each other among the treatments. The research concludes that the inclusion of neem leaf meal for up to 15% is not harmful to the sheep and their nutrient requirements were met.

Keywords: Leaf meal, ruminant, blood, Uda rams

Introduction

The importance of small ruminant production as a means of sustaining the livelihood of the rural and urban populace cannot be over-emphasized. Goat production is popular across every stratum of Nigeria's populace, and it plays a prominent role in the supply of animal protein and income generation. Aruwayo and Muhammad (2018) reported that goat production performs a crucial function in the provision of animal protein in Nigeria, in addition to possessing an outstanding capacity to mitigate the shortage. They are sources of meat for human intake with a vantage of short conception. Goat meat is widely accepted in numerous cultures and no religious prejudice against it. Goats thrive on feeds and crop residues that are of poor quality, they have short pregnancy cycles and are distinguished for multiple births (Aruwayo and Muhammad, 2018). Aruwayo et al. (2016) stated that ruminants especially goats can flourish on roughages with poor nutritive values and agricultural by-products that cannot nourish the non-ruminants. Nigerian goats have not been performing optimally. Aruwayo et al. (2024) stated that the potentials in goat production have not been optimally harnessed. This could have resulted from poor feeding. It is a well-known phenomena that the problem of dry season feeding of ruminants especially in the northern part of Nigeria poses enormous challenges to the production. One cause of the dearth in the provision of feed is the vagary of weather conditions (Aruwayo et al., 2016).

Shellee and Daniel (2017) stated that the main difficulty of goat production in Nigeria is insufficient nourishment occasioned by the paucity of feedstuff and the occurrence of roughages with inferior quality, particularly during the dry period. The expense of livestock production has immensely increased over the years. This could be due to rising inflation and human population increase. Aduku (1993) asserted that feed expenses amount to as much as 70% expenses of producing livestock. Ewuola *et al.* (2024) asserted that nutrition remains the most important factor in livestock management. This then obligates the necessity to explore options that can reduce the cost of feed and improve the utilization of available ones. Neem leaf is one of the considerations. The tree is considered one of the most important dry area trees that could grow in poor soils, and they are fast-growing evergreen trees (Kale *et al*., 2003). It has an abundance of crude protein and other chemical components that could influence improved digestibility and utilization. Neem leaves possess a large amount of crude protein (20.9%) in relative consideration with some other tree leaves (Ogbuewu *et al*., 2011). Neem leaves might be exploited in trying to seek a replacement for protein improvement in the ruminant's feed because it is not high fibre but comparably elevated in crude protein (Adjorlolo *et al.,* 2016). In view of the above, neem leaf meal was fed to Uda rams at graded levels to evaluate the effect on their haematology and biochemical parameters.

Materials and Methods

Experimental Site

The research was executed at the Professor Lawal Abdu Saulawa Livestock Teaching and Research Farm, Federal University Dutsin-Ma, Katsina State. The Farm is situated within latitude 2˚97N and longitude 17˚27E. Rainfall occurs between May and September with a peak in August at an altitude of 600 m above sea level of 700 mm annual rainfall within the Sudan savannah ecological zone (Abaje*,* 2014).

Preparation of test ingredient and experimental diets

Neem leaves were harvested within the premises of Professor Lawal Abdu Saulawa Livestock Teaching and Research Farm. These leaves were air-dried within 7-8 days for them to turn brittle while their greenish colour was still being preserved. The dried neem leaves were pulverized with the use of a hammer mill and then included in the experimental diets at 0, 5 %, 10 %, and 15 %. Other feed ingredients that were used are maize offal, cowpea husk, cotton seed cake, bone meal, and salt as shown in **Table I**.

Experimental Design

The study was carried out in a Completely Randomized Design (CRD) that involved the use of twenty (20) Uda rams that were randomly allotted into four (4) experimental treatments namely A, B, C, and D and each of them constitute a replicate.

Experimental Animals and Management

A total of twenty (20) Uda rams of approximately the same age and size were purchased from Dutsin-Ma local market. These rams were quarantined for two weeks to acclimatize to the environment and observed for any incidence of parasite infestation and disease infection. Prophylactic treatment was given using oxytetracycline (a broad-spectrum antibiotic), Levamisole and Ivermectin were administered to get rid of any possible internal parasite and ectoparasite respectively during which roughages and concentrates were fed to them.

Table 1: Experimental Diet

Ingredients %	A(Control)	B	C	D
Maize offal	32.5	30	33	35
Cowpea husks	30	30	30	21.5
Cotton seed cake	20.50	18	14	11.50
Groundnut hay	15	15	15	15
Neem leaf meal	0	5	10	15
Bone	1	1	1	1
Salt	1	1	1	1
Total	100	100	100	100

Table 2: Chemical Composition of the Test Ingredient and Experimental Diet

Parameters (%)	Treatments				
	T1	**T2**	**T3**	**T4**	**Neem leaf meal**
Dry Matter	87.2	87.4	87.2	87.8	88.72
Ether extract	4.97	5.02	4.94	4.93	2.36
Crude Protein	15.27	15.19	15.52	15.48	18.22
Crude Fibre	23.21	22.75	23.11	23.97	16.25
Ash	5.13	7.00	7.28	7.21	6.00
NFE	51.63	50.01	49.15	50.41	45.96
ADF	58.28	59.05	58.17	58.05	54.75
NDF	73.3	74.64	73.44	72.21	71.24

NFE= Nitrogen free extract, ADF= Acid detergent fibre, NDF= Nitrogen detergent fibre

Table 3: Haematological Parameters of Uda rams fed Neem leaf-based diets

Parameter	Treatments				
	A	**B**	**C**	**D**	**±SE**
Packed cell volume (%)	33.40	33.50	33.50	33.60	2.67
Haemoglobin (g/dl)	10.90	11.55	9.71	10.95	0.97
Red blood cell (10^{12}l)	9.50	9.69	9.99	9.87	0.78
White Blood cell (10^{9}l)	4.60	4.05	4.50	4.80	0.82
Neutrophil	31.00	34.50	29.50	29.50	4.44
Lyhpocutes	69.00	64.50	67.50	76.00	7.54
Monocytes	1.00	0.50	1.00	1.50	0.76
Eosinophil	0.50	0.50	0.00	0.50	0.52
Basohil	.50	0.00	0.00	0.00	0.36

Means not followed by the same superscripts are significantly different (P<0.05) along the row.

Management of Pens

The pens were cleansed and sanitized seven days prior to the commencing of the study. Each experimental pen contained feeding and water troughs that were able to contain the feed and water without wastage. These feeding and water troughs were cleaned daily prior to offering feed and water. The experimental animals were offered the treatment diets without restriction and twice in a day, early in the morning and afternoon to avoid wastage. Feed consumption was recorded all throughout the feeding trial.

Blood collection and analysis

Ten (10) ml blood samples were obtained from three representative animals from each treatment at the end of the feeding trial through bleeding of the jugular (Coles, 1986) in the morning before offering feed. About 3 ml of the blood samples were poured into a bottle that contained Ethylene Diamine Tetra acetic acid (EDTA) for haematological studies while the remaining 7 ml was put in an ordinary bottle, kept for about 2 hours at room temperature and then centrifuged at 700 x g for 15 minutes. The serum was separated, poured out and cold stored for serum biochemistry analysis.

The haematological parameters determined are hemoglobin (Hb) content using cyanmethemoglobin method (Coles, 1986). Red blood cell, packed cell volume (PCV), white blood cells, erythrocytes, leucocytes, basophils and eosinophils were also determined in accordance with the methods of Coles (1986) while biochemical parameters are the blood urea concentration that was assessed by Nessler's reaction (Tanis and Maylor, 1968), total proteins by the Biuret method as described by Henry and Stobel (1957), albumin by BromoCresol Green Method (Grant, 1987) while globulin was determined by differences between total protein and albumin. The bilirubin was determined by the Colometric method based on the method described by Jendrassik and Grof (1938) while direct (conjugated) reacted with diazotized sulphuric acid alkaline medium to form a blue-coloured complex. Creatine was determined by Jaffe reaction using a photometric Colometric test for endpoint measurement. Alanine transaminase and Aspartate transaminase were determined by Kinetic Technique while Sodium and Potassium will be determined by Flame Photometric Technique.

Chemical analysis of test ingredient and treatment diets

The test ingredients and the treatment diets were analysed for proximate composition using AOAC (2000) and crude fibre fractions by the Van Zoest method.

Statistical Analyses of Feed Samples

The data generated was analysed with analysis of variance (ANOVA) in a Completely Randomized Design (CRD) using SAS Package (2000). Duncan Multiple Range Test (DMRT) (Duncan, 1955) was used to separate the means. The following model will be utilized:

$Yij = \mu + Tj + eij$

Where:

μ = Overall mean

Tj = Effect of the jth treatment diet (j = 1… 5)

eij = Random error.

Table 4: Liver function Test of Uda rams fed neem leaf-based diets

Parameters	Treatments				
	A	B	C	D	±SE
Total Protein (g/dl)	70.50	72.00	69.50	71.50	2.03
Albumin (g/dl)	30.50	27.50	44.00	32.00	11.12
Globulin (g/dl)	40.00	39.50	25.50	39.50	0.71
Total bilirubin (mg/dl)	5.05	3.10	4.55	2.60	1.25
Conjugated biliribin (mg/dl)	1.55	.90	1.15	.80	0.40
Alanine transamin. (iu/litre)	32.50	32.55	30.20	30.90	2.02
Alanine amino Phosph. (iu/l)	50.01	52.85	60.60	59.35	1.63
Alkaline Phosphatase (iu/l)	106.05	104.90	83.10	95.30	13.11

Means not followed by the same superscripts are significantly different (P<0.05) along the row

Table 5: Renal function Test of Uda rams fed Neem leaf-based diets

Parameters	**Treatments**				
	A	**B**	**C**	**D**	**±SE**
Sodium (mmo/L)	141.00	138.00	142.50	138.50	4.37
Potassium (mmo/L)	5.05	4.45	4.15	3.45	7.09
Chloride (mmo/L)	101.50	102.50	105.00	124.00	11.65
Bicarbonate (mmol/L)	27.00	28.00	26.00	25.00	1.77
Urea Nitr. conc. (mmol/l)	4.45	6.6	5.85	4.70	1.44
Calcium ((mg/dL)	94.00	101.00	88.00	86.5	7.37

Means not followed by the same superscripts are significantly different (P<0.05) along the row

Results and Discussion

The Experimental Diets' and Test Ingredient Chemical Composition

The proximate composition and crude fibre fractions of the test ingredient and experimental diet are shown in **Table 2**. The proximate composition shows a dry matter content of 87.2 to 87.8%, crude protein (15.27 to 15.52%) and 22.75 to 23.21% of crude fibre. Ether extract value of the diets ranged from 4.93 to 5.02% and that of ash was 5.13 to 7.28%. Acid detergent fibre and nitrogen detergent fibre recorded values that ranged from 58.05 to 59.05% and 72.21 to 73.3% respectively. The proximate composition as depicted in **Table 2** showed that the nutrient requirements of the Uda sheep were satisfied. The dry matter content of the experimental diet is adequate for a growing male sheep, although it is lower than the 93.05 to 96.01% reported by Aruwayo *et al*. (2022). The crude protein was within the 15 to 18% recommended for a growing sheep (ARC, 1990) and was therefore adequate for their nutritional requirement. High CP could increase voluntary feed intake as reported by Chriyaa (1997).

Haematological parameters of Uda rams fed Neem leaf-based diets

The outcomes of the haematological parameters of Uda rams fed graded levels of leaf meal are shown in Table 3. The packed cell volume (PCV) values of 33.40 to 33.60% obtained were significantly similar (P>0.05) and fell within the limits of 10.46 to 10.66%, 27.81 to 35.64%, 32.40 to 34% and (24-45%) reported by Fanta (2024), Aruwayo *et al*. (2016), Aruwayo *et al*. (2009) and Cole (1986). Haemoglobin concentration and red blood cell values remained significantly comparable (P>0.05) with ranges of 10.90 to 11.55(g/dl) and 9.87 to 9.99 (10^{12}l) respectively. These values stayed in the standard haematological values of sheep communicated by RAR (2009). From the standard limits of 9-13 g/L, 27-45%, 9-15 x 10^{12} /L and 4-12 x 10^{9} / L for Hb, PCV, RBD and WBC respectively in sheep (The Merck Veterinary Manual, 2010), it can then be implied that the leaf meal in the diets did not pose any threat to the health of the animals and the experimental diets satisfied their nutrient requirements.

The white blood cell (WBC) obtained in the study which ranged from 4.05 to 4.80 (10^{9}l) did not vary significantly (P> 0.05) from each other. The results compared with the ones reported by Aruwayo *et al* (2009) and Fanta (2024). The differential counts obtained from the investigation implied that the Uda rams used were of safe healthiness. In **Table 3**, the differential counts parameters did not indicate any significant variation (P>0.05) and compared with the values of 10-50% neutrophil, 40-75% lymphocytes, 1-5% monocytes, 1-8% eosinophil and 0-3% basophil documented by Coles (1986) and that of Heath and Olusanya (1988) values of 30% neutrophils, 62% lymphocytes, 25% monocytes, 5% eosinophil and 0.5% basophil in sheep. There was no threat of parasitic infestation and disease infection. Frandson (1981) reported that differential counts that are not within the reference range indicate health problems.

Liver function Test of Uda rams fed neem leaf-based diets

The outcome obtained for the liver function test displayed in **Table 4** with all the parameters contained in it not showing

any significant difference. Total protein values ranged from 69.50 to 70.50(g/dl) and showed similarity (P>0.05) among the treatments. A Similar tendency was observed for other parameters. The report of these biochemical parameters compares with that Aruwayo *et al.* (2016) values of total protein 69.5-75.0 g/l, Albumin (29.5-35.5 g/l), Globulin 40.0-50.0g/l, Alanine transaminase (29.5 - 35.3 iu/litre), Alanine amino Phosphate (53-78) and Alkaline Phosphatase (38.0 - 54.0iu/litre).

Renal function Test of Uda rams fed Neem leaf-based diets

The kidney function test of the experimental animals fed leaf-based diets are displayed in **Table 5**. The values were not significantly different among the treatments. Sodium recorded 138.00 to 142.50(mmo/L), potassium (3.45 to 5.05 mmo/L) and chloride (101.50 to 124.00(mmo/L). Bicarbonate and Urea nitrogen concentration values varied from 25.00 to 28.00 and 4.45 mmo/L to 6.60 (mmol/l) respectively. All these parameters were within the safe ranges reported in the literature. Peter *et al*. (2022) reported sodium (45–152 mmo/L), Potassium (3.9–5 mmo/L), Chloride (95–103mmo/L), Bicarbonate (21–28 mmo/L) and urea nitrogen concentration (1.0–3.5 mmol). Aruwayo *et al.* (2009) and Cole (1986) also reported similar values to what was obtained in this study. It can be inferred from this experiment that leaf meal inclusion in the sheep did not impede the renal function of the animals.

Conclusion

The haematological and biochemical parameters of the animals used in this research conform to those of healthy sheep and leaf meal inclusion in the feed was not deleterious to them. The nutrient requirements of the experimental animals were satisfied. It can then be concluded that the diets are safe and nutritionally adequate for ruminant consumption.

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