EFFECT OF NEEM (*AZADIRACHTA INDICA*) AND BITTER LEAF (*VERNONIA AMYGDALINA*) ETHANOLIC EXTRACTSON THE BLOOD PROFILE OF WEST AFRICAN DWARF SHEEP

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ABSTRACT
A study was aimed at investigating the impact of feeding neem (Azadirachta indica) and bitter leaf (Vernonia amygdalina) ethanolic extracts on WAD Sheep performance, aligning with the global move away from antibiotic growth promoters in animal nutrition. The sheep were subjected to four treatments in a completely randomized design: Distilled water (T1), Neem extract 50 mL/kg of feed (T2), Bitter leaf extract (T3), Neem + Bitter leaf extract (T4) at 50 ml/kg of feed. There was a significant ($p<0.05$) increase in RBC, hemoglobin, hematocrit, MCV, MCH, and MCHC in sheep on T3 and T4 compared with sheep in the control group, also there were more WBCs in sheep in T2 than in the other treatment groups. Total protein, albumin, and globulin were higher in T4 ($p<0.05$). Serum enzyme levels (AST, ALT, and ALP) were moderate, while cholesterol, triglyceride, and LdL concentrations were low. Administering neem + bitter leaf extracts at 50ml/kg DM feed enhanced growth performance and health status, suggesting their use as growth-promoting additives.
Keywords: Neem, Bitter Leaf, Blood, Extract, Blood, Sheep

INTRODUCTION

The rising human population has led to an increased demand for animal-derived food products to meet the minimum daily requirement of animal protein per individual (Nworgu, 2016). However, the average Nigerian's protein intake is estimated at only 45.5g per head per day, which falls short of the Food and Agricultural Organization's recommended minimum intake of 70g per head per day. Of this amount, half (35g) should come from animal sources (FAOSTAT, 2017). The inadequate supply of animal protein can be attributed to insufficient production capacity for common meat sources such as poultry, goat, cattle and sheep in Nigeria. Therefore, there is a need to increase the supply of animal protein by utilizing promising indigenous ruminant animals like sheep (Ogunbosoye *et al*., 2016).

Sheep are often overlooked despite their ability to provide a consistent source of high-quality meat and milk with essential minerals and fat-soluble vitamins (Kamal *et al*., 2021), making them ideal candidates for bridging the gap between supply and demand for animal protein in Nigeria (Abdulwaheed & Tsado, 2014). However, small ruminants' productivity levels are suboptimal due to poor growth rates resulting from poor health caused by poor nutrition (Ogunbosoye *et al*., 2018).

Growth performance and animal health have always been a major driving force in animal research (Babale *et al.*, 2018). The importance of growth performance and healthy wellbeing in animal production had led to the use of growth promoting substances (antibiotics) to promote and hasten growth to ensure quick turn over (Czech *et al.,* 2020). However, the long-term use raises concerns regarding cost implications, potential environmental impact as well as impacts on human health (Sarker *et al*., 2018) leading researchers to exploring medicinal plants as a safer alternative (Talukder *et al.*, 2017; Olagaray *et al*., 2019). This trend has spurred interest in natural solutions using medicinal plants include neem (*Azadirachta indica*), bitter leaf (*Vernonia amygdalina)*, ginger (*Zingiber officinale*), garlic (*Allium sativium*) and scent leaf (*Ocimum gratissimum*) have been reported to enhance performance in livestock (Akanmu *et al*., 2020).

Neem and Bitter leaves are two inexpensive options that could improve overall performance indices when added into feeding systems (Paul *et al.*, 2020; Sarkar *et al*., 2021)**.** These two contain secondary metabolites such as alkaloids, saponins, glycosides, tannins and others (Clement *et al.*, 2013), that confer pharmacological functions on them, thereby making them a potential replacement for antibiotics and a good growth promoter (Ijeh and Ejike, 2011; Nwogwugwu *et al.*, 2016). This study evaluates the response of WAD Sheep dosed with extracts derived-from neem and bitter neem leaves when administered as additives.

MATERIALS AND METHODS

Experimental Site

This study was carried out at the Small Ruminant Unit of the Kwara State University, Malete, Nigeria.

Experimental Animals

Sixteen (16) WAD sheep with an average body weight of 14 ±2 kg were used for this experiment.

Experimental Design and Diet Formulation

The animals were randomly allotted to four treatments in a completely randomized design (CRD) with four (4) sheep in each of the treatments. The four experimental treatments administered are: Treatment 1 (T1): Distilled water 50 ml/kg of feed (control), treatment 2 (T2): Neem leaf extract at 50 ml/kg of feed, treatment 3 (T3): Bitter leaf extract at 50 ml/kg of feed and treatment 4 (T4): Neem + bitter leaf extract at 50 ml/kg of feed.

Procurement and Preparation of Leaves and Extract from Neem and Bitter Leaves

Neem and bitter leaves were collected within the University farm in large quantity. They were air dried at room temperature, macerated, then milled into fine powder, sieved through 1 mm gauge and kept for subsequent use. Extraction was carried out following the procedures of Akanmu *et al.* (2020).

Extract Administration

The extracts (neem, bitter leaf, a combination of neem and bitter leaf) were administered to the sheep at 50 mL/kg of feed through drenching method as described by Akanmu (2018).

Determination of Haematological and Serum Biochemical Indices

Blood samples were collected sheep by the jugular-venipuncture on the last day of the study. An average of 10 mL of blood sample was transferred immediately into sterile sample bottles containing ethylene-diamine-tetra-acetic acid (EDTA) and plain ones for the haematological analysis and biochemical analysis respectively following the procedure of Okoruwa & Igene, (2015).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA), the means were separated using Duncan Multiple Range Test of the (SAS, 2002).

RESULTS AND DISCUSSION

The haematological indices of sheep given neem and bitter leaf extracts are presented in Table 1.

Table 1: Haematology Parameters of WAD sheep Administered with Extracts of Neem an Bitter leaf

Parameters	Treatments				S.E.M	*P*-value
	T1	T2	T3	T4		
WBC ($\times10^3/\mu L$)	8.69^c	12.63^a	9.55^b	7.37^d	0.56	1.6824
RBC ($\times10^6/\mu L$)	7.38^b	6.64^c	7.68^b	7.97^a	0.45	1.3935
HGB (g/dL)	8.10^b	7.25^c	8.53^b	9.23^a	0.45	1.3895
HCT (%)	0.44^c	0.47^b	0.52^b	0.62^a	7.89	0.2430
MCV (*fl*)	59.50^d	64.45^c	80.93^b	85.50^a	14.34	44.1719
MCH (*pg*)	10.87^c	11.00^b	11.60^b	12.33^a	0.79	2.4487
MCHC (g/dL)	17.13^b	15.97^c	19.30^b	21.50^a	2.91	8.9529
PLT ($\times10^3/\mu L$)	$11,700^b$	$12,130^a$	$11,673^b$	$10,793^c$	726.12	7.3949

WBC= white blood cells; RBC= red blood cells; HGB= haemoglobin; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; PLT= platelet count. T1=Feed at 5% of body weight + Distilled water 50ml/kg of feed (control), T2= Feed at 5% of body weight + Neem extract 50ml/kg of feed, T3=Feed at 5% of body weight + Bitter leaf extract 50ml/kg of feed, T4= Feed at 5% of body weight + Neem +bitter leaf extract 50mL/kg of feed, ^{abcd} means with different superscript across the rows differed significantly ($p < 0.05$).

The results showed significant ($P<0.05$) differences between the treatments for all parameters measured. The values for each parameter ranges within the normal range values reported for healthy sheep (Merck Veterinary manual, 2010). It is worth noting that the sheep on T2 had slightly highly

WBC than the recommended range. This is an indication that the sheep must have experienced certain level of infections which could warrant high production of WBC to combat the foreign bodies in the system. High percentage of WBC and lymphocytes are associated with the ability of the animal to express high immunity and combat infections within the body system (Mitruka and Rawnsley, 1997).

The values obtained for platelets are however greater than the recommended range value of (342.02-535.82) for healthy sheep. The values of platelet count were higher in the current study as compared to values recorded by Okonkwo *et al.* (2011) and Habibu *et al.* (2017) in goat. The physiological variation might be as a result of the differences in climate, breed and season, and exposure to cold environment was also reported to increase platelet count in human (Keigo *et al*., 2017).

The serum biochemical indices of sheep given neem and bitter leaf extracts are shown in Table 2.

Table 2: Serum Biochemistry Parameters of WAD sheep drenched with Neem and Bitter Leaf Extract

	Treatments					
Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**	***P*-value**
Total protein(g/dL)	5.48^{c}	6.14^{b}	6.15^{b}	6.29^{a}	0.46	0.6209
Albumin(g/dL)	2.59^{c}	2.74^{b}	2.84^{a}	2.87^{a}	0.12	0.4421
Globulin(g/dL)	2.63^{b}	3.14^{ab}	3.27^{ab}	3.69^{a}	0.46	0.4972
ALP(U/L)	15.09^{ab}	13.12^{b}	17.75^{a}	19.03^{a}	1.22	0.2828
AST(U/L)	117.89^{b}	113.59^{b}	153.33^{a}	155.25^{a}	12.28	0.1818
ALT(U/L)	22.65^{c}	24.30^{b}	25.10^{b}	30.51^{a}	2.90	0.3189
Cholesterol(mg/dL)	68.13^{b}	83.92^{a}	83.24^{a}	59.85^{c}	12.81	0.6089
Triglyceride (mg/dL)	118.77^{c}	104.08^{d}	130.46^{b}	142.92^{a}	2.07	0.0034
HdL(mg/dL)	18.57^{c}	11.56^{d}	21.84^{b}	38.30^{a}	0.78	0.2952
LdL(mg/dL)	19.68^{b}	35.97^{a}	16.68^{b}	4.94^{c}	2.91	0.2976
Urea(mmol/l)	1.08^{ab}	1.10^{ab}	1.10^{ab}	0.61^{b}	0.15	0.7509

ALP = alkaline phosphatase, ALT = alanine transaminase, AST = aspartate transaminase, HDL = high density lipo-protein, LDL = low density lipo-protein, SEM = standard error of mean, T1 = Feed at 5% of body weight + Distilled water 50ml/kg of feed (control), T2 = Feed at 5% of body weight + Neem extract 50ml/kg of feed, T3=Feed at 5% of body weight + Bitter leaf extract 50ml/kg of feed, T4 = Feed at 5% of body weight + Neem + bitter leaf extract 50ml/kg of feed. 1mmol/equivalent to 18 mg/dL.

^{abcd} means with different superscript across the rows differed significantly ($p < 0.05$).

All parameters measured were significantly ($P<0.05$) influence by the treatments. The ranges recorded for all parameters measured were within the normal range recorded by Mitruka and Rawnsley (1997). Generally, the enzymes are also within the normal physiological range suggested for sheep (Pathak *et al.*, 2013).

CONCLUSION

The study concludes that the combination of neem and bitter leaf extracts positively impacted sheep health, with no abnormalities in blood parameters. Sole administration of bitter leaf extract also showed positive effects. Recommends use of herb combination as additives for better sheep health.

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