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HAEMATOLOGICAL AND SERUM BIOCHEMICAL STUDIES IN BROILERS FED *GNETUM AFRICANUM* (WELW) LEAF MEAL

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ABSTRACT

In this study, the effect of diets supplemented with leaf meal of Gnetum africanum on hematology and serum biochemistry of broilers was determined. The leaves were collected, air-dried, and pulverized. Eighty broiler chicks used for this study were acclimatized for three days and randomly distributed into five groups (n=16). The birds were fed commercial starter and finisher ration into which the leaf meal was incorporated at various levels for 8 weeks. Birds in group 1 received feed without Gnetum africanum meal, while birds in groups 2, 3, 4, and 5 received: 0.5%, 1%, 2%, and 3% of Gnetum africanum supplemented meals, respectively. Blood was collected at the end of 8 weeks of feeding. Results showed that treated birds had significantly (P< 0.05) higher levels of packed cell volume, red blood cell, and white blood cell count than the control groups. Serum liver enzymes were significantly (P<0.05) reduced, while the serum kidney function markers, uric acid, and creatinine were significantly (P<0.05) higher when compared with the control group. We conclude that G. africanum induced hematopoiesis and could have been a stressor to the broilers which was seen as leukocytosis and elevated kidney function markers.

Keywords: *Erythrocytic profile, Gnetum africanum, kidney function, leukocytosis, liver function, stress*

INTRODUCTION

Gnetum africanum is a climbing vine in the tropical rainforest of West and Central Africa. The leaves are highly valued as a nutritious green vegetable and they have a high nutritional value, containing eight essential amino acids in significant amounts (Mialoundama, 1993). Medicinally, Gnetum Africanum is used in the treatment of a variety of illnesses. In Nigeria, the leaves are used for the treatment of enlarged spleen, sore throat and as a cathartic (Burkill, 1994). In Ubanji (DR Congo), it is used to treat nausea and it is considered to be an antidote to some form of poison (Burkill, 1994). It is anti-dyslipidaemic (Udeh *et al.,* 2018a) and anti-diabetic (Udeh *et al.,* 2018b). Acute and chronic toxicity studies using Wistar rats indicated the wide safety margin of *Gnetum africanum* (Udeh *et al.,* 2018c).

The poultry industry in developing countries is facing some challenges, one of which is an increase in the cost of feed because of high prices of protein and energy sources (Anosike *et al*., 2018). Livestock feed costs in developing countries are a continuing challenge. The high and increasing prices for animal feeds have compelled researchers to direct their attention to non-conventional feed sources, with particular emphasis on protein substitutes. The use of leguminous multipurpose trees and shrubs has been suggested to be a viable alternative source of protein, vitamins, and minerals for poultry feeding. Plant leaves are commonly processed into leaf meals for use as poultry feed (Ewa *et al*., 2018).

Gnetum africanum is presently being focused on globally as another promising leaf meal in livestock (Iweala *et al*., 2009). However, the effect of this plant on the health of broilers is yet to be determined. The present study therefore supplies information on the safety of *Gnetum africanum* feed meal in broilers.

MATERIALS AND METHODS

Location and duration of experiment

This study was carried out at the poultry farm of the Department of Veterinary Biochemistry and Animal Production, Michael Okpara University of Agriculture Umudike.

Preparation of *Gnetum Africanum* leaf meal

Fresh leaves of *Gnetum africanum* were sourced from a local market in Nsukka, Nigeria. The leaves were separated from the stalk, picked, air dried, and ground to powdered form, and stored in a transparent bucket for later use. The proximate composition of the ground leaves and the feed at the different inclusion levels was determined according to the procedure of AOAC (1975).

Formulation of Experimental Diet

Five experimental diets namely control diet (A) and *Gnetum africanum* supplemented diet (B, C, D, and E) were formulated. The diets were formulated from commercially available feed. The

control diet (A) was formulated without the inclusion of *Gnetum africanum* feed meal while broiler diets B, C, D, and E were incorporated with 0.5%, 1%, 2%, and 3% *Gnetum africanum* feed meal, respectively (Table 1).

Table 1: Proximate Composition of *Gnetum africanum* feed meal

Sample (%)	Moisture (%)	Crude protein (%)	Ash	Crude fiber (%)	Crude fat (%)	Nitrogen-free extract (%)	Metabolizable energy (%)
0.5	9.18	22.25	9.10	6.01	8.12	45.34	343.44
1	9.16	22.51	8.88	5.79	7.85	45.81	343.93
2	9.17	22.75	8.79	5.83	7.41	46.06	341.89
3	9.19	22.85	8.70	5.95	7.25	46.19	341.41

Experimental Design and Management of Experimental Birds

A Completely Randomized Design (CRD) was used for this study. Eighty-day old white marshal broilers were procured from a reputable local commercial hatchery at a day old and were reared on deep litter. They were grouped into five treatment groups with 16 birds each. The five groups were A, B, C, D and E fed diets with varying levels of *Gnetum africanum* (0%, 0.5%, 1%, 2%, and 3%) respectively. In phase one, there was a 3-day acclimatization period to cater for any mortalities that might occur as a result of transportation before the birds were randomly assigned to their various treatment groups. A vaccination programme was planned and followed. The birds were vaccinated against Newcastle, Gumboro, and Fowl Pox on day 1, day 10, and day 21 respectively; with the second dose of Newcastle and Gumboro vaccines repeated on day 14 and day 28 respectively. They were given vitamins and prophylactic antibiotics (Gendox®) and anticoccidial (AmproCox®) on day 2, day 6, and day 14 respectively. The research was conducted following the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Sample Collection and Analysis

At the end of the 8 weeks of feeding, 3 ml of blood was collected from seven (7) birds from each of the groups from the wing vein using a sterile disposable needle and syringe. Before bleeding, a cotton swab soaked in 70% ethanol was used to dilate the vein and prevent infection. Blood samples were collected into labeled sample bottles with drops of ethylene-diamine-tetra-acetic acid

(EDTA) as an anticoagulant. Blood for serum was collected in plain bottles and allowed to clot. They were centrifuged and serum harvested.

Hematological analysis

The hemoglobin (HB) concentration, packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined using automated hematology analyzer (OX-360, Balio diagnostics, France).

Determination of biochemical parameters

Total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid, and creatinine levels were determined in the serum using a commercially available Randox reagent kit (Randox Diagnostics, UK).

Statistical analysis

Data generated were presented as mean ± SEM and subjected to one-way analysis of variance (ANOVA) using the statistical package for social scientists (SPSS) software. The variant means were separated using the Duncan New Multiple Test method. The differences in means were considered significant at $P < 0.05$.

RESULTS

The effect of *G. africanum* feed meal on haematological indices is shown in Table 2. Haematological indices generally increased with an increase in the percentage substitution of feed with *Gnetum africanum*. Broilers on 5% substitution had the highest levels of Hb (8.80±0.28) PCV (28.42±4.42); RBC (4.07±0.13) and WBC (11.72±0.54). These were significantly different ($P<0.05$) when compared to the control which was 8.11±0.22, 25.28±0.95, 3.21±0.06 and 7.31±0.35 respectively.

Table 2: Effect of *Gnetum africanum* leaf meal on hematology of broilers

Group	Hb (g/dl)	PCV (%)	RBC ($\times 10^{12}$/l)	WBC ($\times 10^{9}$/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)
A	8.11±0.22	25.28±0.95	3.21±0.06*	7.31±0.35*	86.82±4.2	25.26±1.07	32.11±1.14
B	8.45±0.57	25.71±2.62	3.47±0.09*	10.23±1.02*	80.99±7.74	24.37±2.01	33.37±5.55
C	8.05±0.39	27.42±3.86	3.54±0.24*	10.37±0.43*	84.43±7.43	22.85±1.88*	29.97±5.37
D	7.97±0.26	27.28±3.25	3.69±0.21*	10.54±0.53*	80.50±9.81	21.62±1.21*	29.48±2.74
E	8.80±0.28*	28.42±4.42*	4.07±0.13*	11.72±0.54*	75.61±12.95	21.64±1.20*	31.41±3.48

*P<0.05

Hb: hemoglobin PCV: packed cell volume, RBC: red blood cell, WBC: white blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Table 3 shows the effect of *G. africanum* feed meal on liver and kidney function markers. All par ameters were affected by the feed meal, especially at the 5% substitution level. Total protein, AL P, and AST were significantly (P<0.05) lower in broilers fed *G. africanum* feed meal at 5% subst itution (3.9±0.06; 58.23±3.07 and 40.00±2.72) compared to the control (4.66±0.03; 64.46±0.47 a nd 64.46±0.47. The levels of both uric acid and creatinine increased with an increase in levels of substitution with *G. africanum* and these were significantly different (P<0.05) and higher than th e control.

Table 3: Serum biochemical markers of broilers fed *Gnetum africanum* feed meal

Group	Total protein (mg/dl)	ALP (IU/l)	AST (IU/l)	Uric acid (mg/dl)	Creatinine (mg/dl)
A	4.66±0.03*	64.46±0.47	42.50±2.0	3.79±1.66*	0.55±0.22*
B	4.42±0.01*	64.06±0.50	41.75±1.16	5.93±1.47*	0.84±0.32*
C	4.21±0.07*	62.73±0.92*	41.50±0.75	8.19±0.11*	1.32±0.30*
D	4.9±0.15*	60.06±0.99*	40.50±1.92*	11.73±0.19*	2.45±0.04*
E	3.9±0.06*	58.23±3.07*	40.00±2.72*	12.54±0.44*	3.51±0.05*

*P<0.05; ALP- Alkaline phosphatase; AST-aspartate aminotransferase

DISCUSSION

The increases in both red blood cell and white blood cell indices suggest a haematopoetic effect of the plant on broilers. The increase in haemoglobin, packed cell volume red blood cell count observed in groups of broilers fed *G. africanum* feed meal, especially at the highest inclusion level suggests that it could enhance erythropoiesis in broilers. This has been reported in rats (Nubila *et al*., 2013, Ufelle *et al*., 2017, Udeh *et al*., 2018a). Most green leafy vegetables, including *G. africanum,* contain iron and folate needed for erythropoiesis. Erythropoiesis is the process of production of *de novo* red blood cells. These new erythrocytes replace senescent ones that are phagocytosed and destroyed each day. Folate, vitamin B12, and iron have crucial roles in erythropoiesis. Erythroblasts require folate and vitamin B12 for proliferation during their differentiation. Deficiency of folate or vitamin B12 inhibits purine and thymidylate syntheses, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anemia from ineffective erythropoiesis (Koury and Ponka, 2004). As a result of the higher RBC count in the treated groups, MCH which are indices of the RBC count were significantly lower in these groups. However, there was no increase in MCV and MCHC in the treated groups. The increase in total white blood cell count in broilers fed *G. africanum* meal could be attributed to stress-induced leukocytosis. Stress is perceived by the brain and results in adaptive responses in other organ systems through neural and neuroendocrine pathways. Stress responses are readily identifiable for many routinely evaluated parameters, especially leukocyte counts (Everds *et al*., 2013). High transaminase activity

is indicative of hepatic injury (Kim *et al.*, 2015). The ability of *the Gnetum africanum* meal to bring about significant reductions of serum AST and ALT in broilers fed *G. africanum* meal could be an indication of its possible hepatoprotective activity. However, there were significant elevations in levels of serum uric acid and creatinine levels in all the different supplemental levels of *G. africanum* fed to broilers when compared with the control groups, which are markers for renal injury. Creatinine is a breakdown product of creatine phosphate from muscle and protein metabolism and is released at a constant rate in the body. It is then excreted via the kidneys so that higher serum creatinine levels indicate impaired kidney function (Kim *et al*., 2015). These elevations can be attributed to stress caused by the introduction of a new feed supplement to broilers as stress has been reported to cause an increase in creatinine and blood urea nitrogen levels in broilers (Huang *et al*., 2018).

CONCLUSION

Intake of *Gnetum africanum* meal led to an increase in red blood cell count, packed cell volume, hemoglobin concentration, and white blood cell count in broilers. This suggests that *Gnetum africanum* can induce erythropoiesis. Also, safety should be considered due to the increase of kidney markers recorded.

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