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Original Article

Effect of African black plum seed nut (*Vitex doniana)* meal diets on hematological and biochemical indices of finisher broiler chickens

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ABSTRACT

This study was conducted to determine the hematological and biochemical indices of finishing broiler chickens fed African black plum nut basal diets (ABPNBD). A total of 150 1-day-old, un-sexed Agrited birds, were reared for 56 days of the experiment. The birds were assigned to five dietary treatments with ten birds per treatment replicated 3 times. Experimental diets were formulated by supplementing the milled African black plum nut at (0, 5, 10, 12.5, and 15%) inclusion levels in the finisher broiler's ration designated as T_1 (control diet containing no ABPBD), Treatments (T2,3,4 and 5) contained ABPNBD at 5, 10, 12.5, and 15%, respectively, in a completely randomized design. The hematological and biochemical indices of the birds were determined. The result revealed that all hematological parameters measured were not significant ($P > 0.05$) except for packed cell volume (PCV), hemoglobin (HB), red blood cell (RBC), and mean corpuscular hemoglobin concentration. Increasing inclusion levels of ABPNBD decreased the PCV, HB, and RBC of the birds across the treatment groups. Birds fed 12.5 and 15% ABPNBD diets recorded the least of the parameters. The result of the serum biochemical indices showed significant ($P < 0.05$) influence for all parameters measured except for alanine transaminase (ALT), uric acid, and creatinine. The total protein (TP), albumin, globulin, and glucose values reduced across the treatment group as ABPNBD inclusion increases. Birds fed above 10% ABPNBD recorded the least value of TP, albumin, globulin, and glucose as compared with birds on control diet and other treatments. The uric acid ranged from 3.80 to 1.35 mmol/L while creatinine ranged from 61.00 to 44.00 mmol/l. ALT ranged between 18.50 u/l (5%) to 15.00 u/l (12.5 and 15%). In conclusion, up to 10% ABPNBD is safe to be incorporated in finisher broiler chickens diets without posing any threat to health, while above 10% showed negative effect on some blood parameters measured. Therefore, it should be supplemented with probably probiotics or subjected to different processing methods.

Keywords: African black plum seed, finisher broiler chickens, hematological and serum biochemical indices

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INTRODUCTION

Poultry products such as meat and egg provide vital nutrients of high protein quality: this is associated with low levels of fat with favorable concoctions of fatty acid. In addition, the short gestation period makes their production encouraging in bridging the wide gap of protein deficiency in Nigerians populace.[1] However, the profit margin in poultry business has been constrained by critical factors which are the quality of feedstuff and its cost implication.[2] Feed alone accounts for 70–87% total variable cost of producing a poultry bird and a profitable livestock enterprise depends on availability and affordability of feedstuff.[3] Previous work by Amaefule *et al*.[4] observed that monogastric animals can utilize non-conventional feed stuffs effectively due to their efficient transformation of raw ingredients, rich in starches, and proteins into poultry products. Consequently, evaluation of unconventional feed stuff as alternative feed sources to mitigate competition among human, animal, and industries will go a long way in poultry production.

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African black plum (*Vitex doniana)* is one of the non-conventional feedstuffs recently discovered and underutilized. It is an indigenous tropical plant distributed across tropical sub-Saharan, Africa's coastal savannas, and savanna wool land. *V. doniana* is commonly known as black plum (English), Dinya (Hausa), Oriri (Yoruba), and Uchakoro (Igbo) or African olive and the fruits are oblong drupe 2–3 cm long, green when not matured and turned purplish-black when ripped.[5] The leaves of *V. doniana* have been reported to have anti-malaria and anti-dysentery properties the stems bark as anti-hepatotoxic and blood tonic, anti-bacterial, and the fruit pulp as good source of phytochemicals and nutritional compounds.[6]

Reports showed that *V. doniana* fruit seed contains CP of 27.57%, moisture content of 8.25%, crude fats of 36.52%, and crude fiber of 4.84%, respectively.[5] Furthermore, phytochemical analysis revealed that *V. doniana* seed contain high content of toxic and anti-nutritional factors such as phenol, saponin, tannin, and flavonoids[5] yet could be a good source of economically valuable non-conventional feed stuff for livestock animals.[5] Blood serves a major function of transporting nutrients absorbed from the digestive system or released from storage in adipose tissues or liver. Hematological parameters are good indicators in determining the quality of feeds, anemia, and infection status of poultry birds. Furthermore, hematological and serum biochemical assay could be used to determine the clinical and physiological responsiveness and well-being of poultry birds.[7] The serum chemistry and hematological components are influenced by the quantity, quality, and the level of anti-nutritional factors present in the feedstuff.[8] Hence, there is need to explore utilization of *V. doniana* seed-nut in poultry production, in which information are scares. Similarly, there is need to ascertain the adequate inclusion level in broiler chickens' diet without negative effects on health of the birds. This research, therefore, evaluated the utilization of *V. doniana* seed-nut in blood parameters of finisher broiler chickens.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the Poultry Unit, Livestock Teaching and Research Farm, Federal University Dutse, Jigawa State. Dutse is located on longitude 9.34°E and Latitude 11.76°N and has an elevation of 431.36 m above sea level. There is usually a hot diurnal temperature and comparatively cooler at nights during the past 2–3 months of the dry season which is followed by a wet season between the months of June and September.[9]

Processing of Test Ingredient

Matured African black plum fruits were purchased in Dutse ultra-modern market, Jigawa State. The fruits were manually peeled to expose the pulp (edible part) which was removed to obtain the nut (contained the seed). The nut was sun dried, crushed, milled, and incorporated into broiler formulation. The finisher diet was formulated with African black plum nut basal diets (ABPNBD) to replace wheat offal at inclusion levels 0% (control diet, basal diet), 5, 10, 12.5, and 15% designated as T_1, T_2, T_3, T_4, and T_5, respectively.

Experimental Design and Animal Management

One hundred and fifty-day-old Agrited broiler chicks were randomly allotted to five dietary treatments comprising three replicates and ten chicks per replicate. The experimental diets were formulated with ABPBD in the diets of broilers at inclusion levels 0% (control diet and basal diet), 5, 10, 12.5, and 15% designated as T_1, T_2, T_3, T_4, and T_5, respectively. Feed and clean water were supplied *ad libitum*. The birds were reared intensively on deep litter (dried wood shavings) housing system. Normal vaccination program and medication schedule were strictly adhered to.

Experimental Diets

Five experimental diets were formulated for finisher [Table 2] phases of the study. The experimental diets were formulated with 0% (control diet and basal diet), 5, 10, 12.5, and 15% inclusion levels of ABPBD in the diets to meet the[10] nutrient requirements for broiler chickens. The chickens were allocated to five different treatments designated as T1, T2, T3, T4, and T5 fed with diets containing 0% (control diet, basal diet), 5%, 10%, 12.5%, and 15% inclusion levels of with ABPBD.

Data Collection and Parameters Measured

Blood parameter 1

At 56 days of the study, blood samples were collected from eight randomly selected birds per treatment (2 per replicate) to determine the blood profile of the birds. Blood collection was done through brachial vein puncture using needles and syringes.[11] Each blood sample was emptied into two sets of well-labeled sample bottles; the sample containing anti-coagulant was used for the analysis of hematological profiles while the plain bottle without anti-coagulant was used to analyze the serum biochemical profiles of the birds. Hematological parameters were determined using hematology analyzer model 6000. Analysis of the biochemical indices was conducted using the clinical chemistry semi-auto-analyzer and a commercial biochemical assay kit. Serum enzymes: Alanine transaminase (ALT) and aspartate serum transaminase (AST)

Table 1: Proximate composition of African black plum nut

Parameters	Raw ABPNBD
Crude protein (%)	17.51
Dry matter (%)	76.70
Ash (%)	6.00
Crude fiber (%)	3.70
Ether extract (%)	3.00

ABPNBD: African black plum nut basal diets

Table 2: Composition of experimental diets fed finisher broiler chickens (5–8 weeks) finisher phase

Ingredient (%)	0	5	10	12.5	15
Maize	53.90	53.90	53.90	53.90	53.90
Fish meal	3.00	3.00	3.00	3.00	3.00
Soya bean meal	14.70	14.70	14.70	14.70	14.70
African black	-	5.00	10.00	12.50	15.00
G/nut meal	9.00	9.00	9.00	9.00	9.00
Wheat offal	15.00	10.00	5.00	2.50	0.00
Bone meal	1.80	1.80	1.80	1.80	1.80
Oyster shell	1.50	1.50	1.50	1.50	1.50
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30
Salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Determined analysis					
ME (Kcal/kg)	2,906.09	2,875.09	2,866.00	2,863.00	2860.0
Crude protein (%)	20.00	19.77	19.52	19.4	19.07
Ether extract (%)	3.96	3.79	3.61	3.52	3.44
Crude fiber (%)	4.62	3.82	4.23	4.36	4.38
Ash (%)	3.72	3.91	3.91	3.91	3.91

Vitamin/Minerals premix supplied per kg diet; vita, 8,000 iu; vit D3, 1440 iu; VitE, 21.6 mg; VitK 3, 2.7 mg, VitB 1, 1.8 mg; VitB 2, 3.6 mg: Vit B 6, 2.7 mg; Niacin, 21.6 mg; VitB 12, 0.018 mg; Folic Acid, 0.54 mg; Pantothenic acid, 9.0 mg; Biotin, 0.036 mg; Choline chloride, 270 mg; Zinc, 27 mg; Mn, 108 mg; Fe, 18 mg; I 2, 0.72 mg; Se, 0.072 mg; Cu, 1.44 mg; Co, 0.14

were analyzed using the commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201–04).

Data Analysis

Data generated were subjected to one-way analysis of variance using[12] significant means at 5% level of probability which was separated using Duncan's Multiple Range Test of the same statistical package.

Statistical Model

$$Yij = U+Ti +eij$$

Where;

Yij = The observed response

U = The overall mean

Ti = The fixed effect of ith treatment (n = 0%, 5%, 10%, 12.5%, and 15%)

∑ ij = Random residual error.

RESULTS

Proximate Analysis

The result of the proximate composition of African black plum nut (ABPN) is shown in Table 1. The dry matter content of ABPN was 76.70%. The crude protein and crude fiber had values of 17.51 and 3.70%, respectively. The ether extract and ash content of ABPN ranged between 3.00% and 6.00%.

The effect of ABPNBD on hematological parameters of broiler chickens is presented in Table 3. The ABPNBD inclusion did not show significant ($P > 0.05$) difference fed the measured factors/traits except for packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC). Broiler chickens fed above 10% ABPNBD had significantly ($P < 0.05$) lower PCV value than those fed 0, 5, and 10% ABPNBD diets. Values for Hb and RBC were significantly ($P < 0.05$) increased in birds fed up to 10% ABPNBD diet when compared with those on 12.5 and 15% ABPNBD diet. A significantly ($P < 0.05$) high mean corpuscular hemoglobin concentration (MCHC) was observed in birds fed ABPNBD with control diet, while the least was recorded for birds fed 12.5% ABPNBD. Birds fed 10% ABPNBD diet and control diet recorded the highest MCHC that compared favorably with that of birds fed 5% ABPNBD but differed significantly ($P < 0.05$) from birds fed 12.5 and 15% ABPNBD diets. The white blood cell, mean cell volume (MCV), and MCH measured were not significantly ($P > 0.05$) influenced by the treatment imposed. Bird fed control diet recorded numerically higher white blood cells, while the lowest was recorded for birds on T4 diet. The MCV and MCH values ranged between 76.60 and 65.60 and 23.45

and 18.10, respectively. The differential blood counts which are monocytes, neutrophil, and lymphocyte did not show significant ($P > 0.05$) difference among the treatment groups. Birds fed 5 and 12.5% recorded the highest numerical values of neutrophil and lymphocyte counts. The monocytes value ranged between 17.00 and 9.00.

Serum Biochemical Indices

Effect of ABPNBD diet on biochemical indices of finisher broiler chickens is shown in Table 4. The result of biochemical indices revealed significant ($P < 0.05$) influence of ABPNB diet on all measured parameters except for ALT, uric acid, and creatinine. Birds fed above 10% ABPNBD had significantly ($P < 0.05$) reduced total protein (TP), albumin, globulin, and glucose compared with those fed 0, 5, and 10% ABPNBD. Birds fed 0 and 5% recorded the highest TP and albumin that compared favorably with birds on 10% ABPNBD but differed significantly ($P < 0.05$) from those on 12.5 and 15% ABPNBD. The globulin of birds fed control was significantly ($P < 0.05$) higher and statistically similar with birds fed 5% and 10%, while the least values were recorded for birds fed 12.5 and 15% ABPNBD, respectively. The glucose value decreased with increasing inclusion level of ABPNBD. Birds fed control diet, 5 and 10% ABPNBD recorded the highest similar glucose values while birds fed 12.5 and 15 recorded the lowest glucose value. AST of birds fed 12.5 and 15% was highest compared to birds on control diet and 5% ABPNBD. However, the values obtained for uric acid ranged from 3.80 to 1.35 mmol/l while creatinine value ranged from 61.00 to 44.00 mmol/l. A numerically higher value was recorded for ALT when birds were fed 5% and control diets as compared to other treatments.

DISCUSSION

The crude protein of ABPNBD was 17.51% which is higher compared to values (8.24%), (10.00%) obtained by these authors[13] and[14] for Africa black plum fruit. The crude fat value of 3.00% was similar to 3.00% reported by Agbede and Ibitoye.[15] for *V. doniana* fruit but extremely lower to value 34.62% observed by[13] for *V. doniana* fruit. The crude fiber 3.70% reported here is higher than the report of[13] but lower to values obtained by[14] for Africa black plum leave. The ash content has been deduced as a means to determine the total mineral content of a food.[13] The ash content here is 6.00% which was at close range with 5.27% reported by Agbede and Ibitoye[15] meanwhile; it was lower compared to 11.50% documented by.[13] The variation noticed generally in the proximate composition

Table 3: Effect of African black plum nut (*Vitex doniana*) meal diets on hematological parameters

Ingredient (%)	0	5	10	12.5	15	SEM
PVC (%)	31.00^{a}	28.50^{ab}	27.50^{ab}	25.00^{b}	22.50^{b}	1.05
Hb (g/dL)	9.40^{a}	9.30^{a}	8.05^{a}	7.10^{b}	7.00^{b}	0.80
RBC (*10^{12}/L)	4.20^{a}	4.10^{a}	3.90^{a}	3.25^{b}	3.10^{b}	0.49
WBC (*10^{9}/L)	9.95	7.60	6.90	5.75	7.05	0.63
MCV (fl)	70.75	74.20	65.60	75.95	76.60	6.03
MCH (pg)	22.55	22.15	18.10	23.45	23.20	2.54
MCHC (g/dL)	31.90^{a}	29.90^{ab}	30.75^{a}	27.55^{b}	26.20^{b}	0.85
Neutrophil	57.50	64.00	47.30	42.50	41.50	11.80
Lymphocyte (%)	36.50	42.00	41.50	44.50	44.50	9.13
Monocytes (%)	11.00	9.00	17.00	13.00	9.00	5.00

^{abc}Means on the same row having different superscripts are significantly different ($P<0.05$). PVC: Packed cell volume (%), Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

Table 4: Effect of African black plum seed (*Vitex doniana*) meal diets on serum biochemical indices

Parameters	0	5	10	12.5	15	SEM
Total protein (g/dL)	77.50^{a}	73.00^{a}	66.5^{ab}	57.00^{b}	50.50^{b}	0.04
Albumin (g/dL)	39.00^{a}	40.50^{a}	38.00^{a}	34.00^{b}	30.00^{b}	2.63
Globulin (g/dL)	38.50^{a}	32.50^{ab}	28.50^{ab}	23.00^{b}	20.50^{b}	4.38
Glucose (mg/dL)	184.78^{a}	182.33^{a}	179.01^{a}	174.13^{b}	171.50^{b}	5.70
ALT (u/l)	17.50	18.50	16.00	15.00	15.00	1.88
AST (U/l)	10.50^{c}	10.50^{c}	13.50^{b}	21.00^{a}	26.50^{a}	2.88
Uric acid (mmol/L)	2.00	3.80	1.35	3.60	3.20	0.46
Creatinine (mmol/L)	58.50	44.00	53.50	59.00	61.00	14.13

^{abc}Means on the same row having different superscripts are significantly different. ($P<0.05$) ALT: Alanine aminotransferase AST: Aspartate aminotransferase

could be attributed to environmental factors and maturity of the fruit seed (nut). Hematological indices such as red blood count, white blood count, PCV, and Hb concentrations are used to diagnosis disease and feed quality.[16] The result of the hematological parameters showed figures that were within normal values across the dietary treatments except for birds fed above 10% ABPNBD inclusion levels. Meanwhile, the hematological parameters of the birds fed above 10% ABPNBD revealed deleterious effect on health status of the birds. The increased inclusion levels of ABPNBD decreases the PCV, HB, and RBC of the birds. Birds fed 12.5 and 15% ABPN diets were below the normal range (PCV: 24.9–45.2%, Hb: 7.40–13.10 g/dL, and RBC: 1.58–3.8 × 10^{12}/l) for healthy birds.[17]

The implication of reduced PCV is that blood was diluted probably due to poor feed utilization and pointer to iron deficiency. Furthermore, reduced Hb and RBC are indications that the birds had low hemoglobin or hematocrit. Hemoglobin is the main protein in the RBC which carries oxygen and delivers it throughout the system. The reduced PCV, Hb, and RBC could be attributed to the presence of anti-nutritional factors present in ABPNBD which resulted to poor dietary protein quality. This corroborated[6] who had also reported *V. doniana* leaf meal to contain tannin, saponins, and flavonoids. Meanwhile, the improved PCV observed in birds fed ABPN inclusions up to 10% suggests that ABPN up to 10% did not inhibit oxygen carrying capacity of the animal cells.[18] Similar result was reported by Salami *et al*.[19] when fed up to 10% *V. doniana* leaf meal to cockerels.

The increased Hb and RBC values observed in birds fed ABPNBD within 10% ABPN are an indication of normal production of RBC (erythropoiesis) for high oxygen carrying capacity for improved health status.[20] This reflects the nutritional potent and safety of ABPNBD at 10% inclusion levels in finisher broiler's diets to pose no threat to health. This result agreed with[21] who reported that albino rats fed aqueous root back of *V. doniana* not to be anemic. The work is in line with[19] who observed significant increase of Hb and RBC with 10% *V. doniana* leaf meal to cockerel. The white blood cells values are within the normal range as postulated by Bounous *et al*.[22] It implies the diet was not toxic to trigger its production in the body. The high significant MCHC observed in birds fed up to 10% ABPN with control diet were within the reference range (26–35 g/dL) reported by Bounous *et al*.[22] This proved that there was sufficient iron production in the blood and health of the birds was not endangered.

In addition, the non-significant of MCV and MCH could be a pointer to better protein quality of the diet. MCV is used to calculate the average erythrocyte size, the MCH to measure Hb number per blood cell, and the MCHC to know the amount of Hb relative to the cell per RBC. In addition, mean cell Hb and mean corpuscular Hb concentrations are useful in determining feed toxicity. Similarly, when mean cell Hb and MCV are abnormally high and mean corpuscular Hb concentration is abnormally low, it indicates poor quality protein test feed.[23] The result of the MCV and MCV reflects that ABPNBD protein quality was not bad as supplement in broiler chicken ration formulation since values of these parameters were within normal figures (90.00–140.00) fL for healthy birds as recommended by Bounous *et al*.[22] The differential blood count, monocytes, neutrophil, and lymphocyte which are agranulocytes of white blood cell, was in the normal range of reference for healthy birds.[24] This portrays that ABPN supplementation caused no threat to health of the broiler chickens.

Serum Biochemical Assay

TP which comprises albumin and globulin is essential in maintaining colloid osmotic pressure, mobility of dietary nutrients, minerals, and hormones and also the biosynthesis of enzymes and immune system.[25] The TP, albumin, globulin, and glucose decreased with increased inclusion levels of ABPN with the lowest values of these parameters at 12.5% and 15%. The reduced TP, albumin, globulin, and glucose values recorded for birds above 10% ABPN as compared with birds on other treatments could be attributed to low protein digestibility of the feed since high protein in serum is an indicative of protein adequacy.[6] High concentration of albumin usually portrays dehydration while a low concentration may be due to inadequate function of the liver due to malnutrient and infection.[26] The reduced serum glucose with increasing inclusion levels of ABPN noticed though the values were within normal range (152 mg/dL–182 mg/dL) reported by Mitruka and Rawnsley[27] for healthy chickens that could be indicative of normal energy metabolism to release adequate and stable substrate (glucose) needed for physiological work and maintenance of the body.

The inclusion of ABPNBD showed no significance for ALT but recorded high AST for birds fed above 10% ABPNBD. These liver enzymes are important in determining the proper functioning of the liver.[28] The high value of these enzymes reflects damaged or sick liver. Meanwhile, the reduced AST observed in the work could means proper functioning of the liver as shown in birds fed ABPN within 10% inclusion.

The non-significance observed with uric acid and creatinine could be that there was no muscle wastage. Uric acid in the blood is produced as a result of protein metabolism meanwhile, increased protein metabolism; stress and dehydration influence the concentration of uric acid in the blood.[29] Creatinine is used to determine the kidney function to excrete waste products resulting from protein metabolism and muscle contraction.[30]

CONCLUSION AND RECOMMENDATIONS

The study revealed that feeding finisher broiler chickens up to 10% ABPNBD pose no threat on health, while above 10%

showed negative effect on some blood parameters measured. Therefore, inclusion above 10% of Africa black plum nut meal should be supplemented with probably probiotics or subjected to different processing methods to be safe for poultry birds.

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