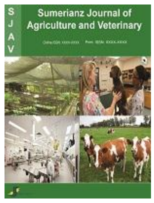
Sumerianz Journal of Agriculture and Veterinary, 2020, Vol. 3, No. 7, pp. 97-106
ISSN(e): 2617-3077, ISSN(p): 2617-3131
Website: https://www.sumerianz.com



Original Article Open Access

Enzyme Supplementation of Sorghum Diets Could Influence the Blood Profile and Histology of Digestive Organs in Broiler Chickens

Kennedy Oluwatosin Oluwamoroti Ohotuowo
Department of Animal Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, P.M.B 1115, Calabar, Nigeria

Izuki Esther Darlington
Department of Animal Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, P.M.B 1115, Calabar, Nigeria

Ozung Pascal Ogar*
Department of Animal Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, P.M.B 1115, Calabar, Nigeria
Email: pascalozung@yahoo.com

Article History
Received: June 7, 2020
Revised: June 30, 2020
Accepted: July 9, 2020
Published: July 12, 2020

Abstract
This study evaluated the effect of dietary enzyme supplementation (phytase - PHY, xylanase-amylase-protease mixture - XAP and xylanase-beta-glucanase - XBG) on the blood profile and histology of digestive organs of broiler chickens. Three hundred and thirty, day-old Hyline broiler chicks were assigned to 11 treatments. Two basal diets were formulated; maize-soybean (positive control) and sorghum- soybean (negative control) with the sorghum diet further subdivided into 9 diets containing PHY, XAP and XBG at three levels (250, 500 and 750 mg/kg) each. At the 8th week, the blood profile and histology of the organs were determined. Results indicated significant ($P < 0.05$) effect of treatments on haematological and serum biochemical indices. Histopathological examination of the gizzard, duodenum and liver revealed mild to no distortions. The study concluded that for optimum blood profile and histological integrity of digestive organs, 250 and 500 mg of phytase® enzyme should be supplemented to sorghum diets.
Keywords: Chicken; Haematology; Digestion; Enzymes; Supplementation.

1. Introduction
In Africa, there has been a declining growth rate due to low food resources including grains. According to FAO [1], report, Africa's population is about 870 million and over 750 million people are living in a state of malnutrition out of which 70% are in rural communities. This situation is further aggravated by the 2020 projection for increased global animal protein demand and population growth. The most wide spread domestic animals, with a population of about 19 billion are domestic chickens. They appear to be the easiest and fastest animal species that could alleviate this protein deficiency. Issa, *et al.* [2], noted that in West Africa, Nigeria plays a vital function in poultry feed production and supply. Nevertheless, the poultry industry is faced with several challenges especially that of high feed cost representing about 75 - 80% of total cost of production.

According to Oluyemi and Roberts [3], cereal grains like maize, wheat and rice constitute the major sources of energy in chicken diets in developing countries. Similarly, Ajaja, *et al.* [4] asserted that maize is the ultimate source of energy in formulated diets and composes of about 50% of the diets. In recent times there has been global pressure on maize production and emphasis is placed on its utilization for export, and biofuel production. Since broiler birds have similar gastro- intestinal tract with humans, this has created a great competition between humans and animals for food especially grains such as maize and wheat which are sources for both humans and livestock. This competition has translated to higher cost of production. Current researches are geared towards exploring alternative feed ingredients that could replace the conventional cereal grains in poultry production.

The largest producer of sorghum in West Africa, accounting for about 71 per cent of the total regional sorghum output is Nigeria [5]. Sorghum production in Nigeria also accounts for 35 per cent of the Africa's production in 2017 and is the third largest world producer after the United States and India [1]. An important third cereal in terms of capacity of production in Nigeria is Sorghum. Its production declined since 2009, due to the strong reduction of harvested areas and yields. The major reason for the decline in production level is because producers are shifting to more profitable crops, despite its high potential demand from the brewery industry. Also, marketing sorghum offers low financial returns and the market opportunities are limited between producers, industries and international marketers. Sorghum is also considered a key crop in Nigeria's Agriculture Transformation Action plan (ATAP). Farmers expand the planting area of maize and soybean in the planting region against sorghum and millet production [1]. There is an increase use of sorghum as alternative feed to maize in Nigeria. Recent studies have shown that, the use of sorghum in crop and livestock production is an important means of increasing sorghum utilization and consumption in Nigeria.

*Corresponding Author

Sorghum is one of the alternative cereals used in poultry production. Globally, sorghum is an important grain and ranks fifth behind corn, rice, wheat and barley. The crop is hardy and can be planted in areas of low rainfall, hot or dry climate around the world especially where maize and wheat cannot be planted. According to Gangaiah [6], sorghum grain has 11% crude protein, 3% crude fat and 70% carbohydrates. Therefore, it is as good as maize in feeding poultry [7]. Nevertheless, sorghum utilization in poultry feed is limited due to the high amount of tannins. Sorghum cultivars with lighter seed coat colour have been reported to contain low tannin with good nutritional values than the darker coat colour [7]. Etuk, *et al.* [8], outlined the different "anti- nutritional effects of tannins which include: reduction in voluntary feed intake due to reduced palatability, diminished digestibility and utilization of nutrients, adverse effect upon metabolism and toxicity. For effective utilization of sorghum by poultry, several strategies including enzymes supplementation have been adopted. The demand for microbial enzyme supplementation has expanded due to the increased use of unconventional feed ingredients.

Khattak, *et al.* [9], reported that low feed efficiency associated with sorghum diets is as a result of poultry not being able to produce enzymes for the hydrolysis of non- starch polysaccharides present in the cell wall of the grains and thus remain un-hydrolyzed. Enzymes currently incorporated into poultry diets include; β-glucanases, xylanases, phytases, proteases, lipases, and galactosidases [9]. These enzymes act by reducing digesta viscosity, enhancing digestion and absorption of nutrients (especially fat and protein), improving apparent metabolizable energy (AME) value of diet, increasing feed intake, weight gain, feed gain ratio, reducing beak implication and vent plugging, decreasing size of gastrointestinal tract, reducing water intake as well as reducing water content of excreta [10]. Enzyme combinations have further improved the availability of several nutrients and feed utilization with significant effects on reduced cost of production [10]. To improve sorghum utilization, exogenous enzyme supplementation is hereby advocated. However, there is paucity of reports on the application of phytases, xylanases-amylase-protease and B-glucanase mixtures in broiler diets comprising sorghum. The aim of this study was to determine the effect supplementing sorghum based – diets with exogenous enzymes on the blood profile and histology of digestive organs in broiler chickens.

2. Materials and Methods

2.1. Location of the Study

The study was carried out at the Teaching and Research Farm, University of Calabar, Calabar, Nigeria. The study site (Calabar) is located within latitudes 4^0 58^1N and 15^0 39^1 N of the equator and longitudes 8^0 17^1E and 10^0 43^1E of the Greenwich meridian with relative humidity of 55- 99% at an elevation of 99 meters above sea level; the average temperature ranges between 25^0 and 30^0 C while average rainfall is between 1,260 and 3,500 mm per annum.

2.2. Experimental Diets

The three commercial enzymes were used as follows:

- Single enzyme: Phytase - Axtra® PHY
- Enzyme combination containing Xylanase-Amylase-Protease – Axtra® XAP
- Enzyme combination containing Xylanase-Beta-glucanase – Axtra® XBG.

Axtra® PHY, a phytase feed enzyme, was sourced from a *Buttiauxella* bacterium species and expressed in a *Trichodema reesei* fungus. Axtra® XAP had fixed ratios (10:1:25) of xylanase (2,000 xylanase units/kg), amylase (200 amylase units/kg), and protease (5,000 protease units/kg; XAP). While Axtra® XB was a preparation of endo-1, 4-β-xylanase and endo -1, 3 (4) – β - glucanase produced by *Trichodema reesei*. Furthermore, two basal diets were formulated (standard corn-soybean and sorghum- soybean diets) to meet the requirements for broilers as presented in Table 1. Corn-soybean and sorghum-soybean diets served as positive and negative control, respectively. In order to improve sorghum utilization by broilers, the single enzyme (PHY) and two enzyme mixtures (XAP and XB) were supplemented into the sorghum diet. The sorghum diets were formulated in one batch then further subdivided into 10 experimental diets, with nine diets containing PHY, XAP and XB at three levels (250, 500 and 750 mg/kg of sorghum diet) each.

The eleven dietary treatments were as follows:

T_1 Corn-soybean diet – positive control
T_2 Sorghum- soybean diet – negative control
T_3 Sorghum- soybean diet plus supplemental PHY at 250mg/kg
T_4 Sorghum- soybean diet plus supplemental PHY at 500mg/kg
T_5 Sorghum- soybean diet plus supplemental PHY at 750mg/kg
T_6 Sorghum- soybean diet plus supplemental XAP at 250mg/kg
T_7 Sorghum- soybean diet plus supplemental XAP at 500mg/kg
T_8 Sorghum- soybean diet plus supplemental XAP at 750mg/kg
T_9 Sorghum- soybean diet plus supplemental XBG at 250mg/kg
T_{10} Sorghum- soybean diet plus supplemental XBG at 500mg/kg
T_{11} Sorghum- soybean diet plus supplemental XBG at 750mg/kg

Table-1. Gross composition of basal diets (%)

Ingredient	Sorghum- soybean diet		Standard corn – soybean diet	
	Starter	**Finisher**	**Starter**	**Finisher**
Corn	0.00	0.00	50.00	55.00
Sorghum	50.00	55.00	0.00	0.00
Soybean meal	32.00	25.00	32.00	25.00
Palm kernel cake	4.45	4.45	4.45	4.45
Wheat offal	5.00	7.00	5.00	7.00
Crayfish dust	5.00	5.00	5.00	5.00
DCP	2.00	1.80	2.00	1.80
Methionine	0.30	0.20	0.30	0.20
Lysine	0.25	0.15	0.25	0.15
Vit. min. Premix	0.50	0.20	0.50	0.20
Palm oil	0.25	1.00	0.25	1.00
Salt	0.25	0.20	0.25	0.20
Total	100.00	100.00	100.00	100.00
Calculated nutrients:				
% Crude protein	23.00	20.80	23.30	21.00
% Crude fibre	3.93	3.95	3.96	3.99
ME (Kcal/kg)	2,840.29	2,920.57	2,800.00	2,920.14
Determined nutrients:				
% Crude protein	22.84	18.74	23.60	18.36
% Crude fibre	4.06	4.19	4.15	4.24

2.2. Experimental Animals and Management

A total of 330, day-old Hyline broiler chicks were used in this study. The chicks were weighed (40 $\pm$ 0.55g) and allocated to 33 pens which consisted of the 11 treatments and 3 replicates per treatment with 10 birds per replicate. All birds were managed under the deep litter system in line with good management practices throughout the 56 days feeding trial. Prior to the arrival of the birds, the pens were sanitized using disinfectants to get rid of pathogens. The litter materials used (wood shavings) were dried for two weeks to avoid moulding, while an anti-stress vitalyte (anidone- vitadox) at 0.2 g/4 litres of chlorine- free water was provided.

2.3. Blood Collection and Evaluation

Samples of blood were collected from twelve birds per treatment at day 57 of the study. Exactly 5ml of blood was collected by puncturing the brachial vein with a 5ml scalp needle and syringe and separated into two portions. One portion was placed into sterile plastic bottles containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for determination of haematological parameters; while the other blood sample was placed in un-heparinsed tubes for determination of serum biochemical indices.

2.4. Determination of Haematological Parameters

All haematological parameters were determined by standard laboratory methods.

2.5. Serum Biochemical Parameters

Serum total protein was determined by the Biuret method. Albumin was determined using Bromocresol green method. The albumin/ globulin ratio was obtained by dividing the albumin value by the globulin value. Alanine amino transferase (ALT) and Aspartate amino transferase (AST) activity was determined using spectrophotometric method.

2.6. Histopathological Examination

Samples of the liver, kidney, gizzard and duodenum where carefully harvested and preserved in 10% buffer formalin solution for histopathological examination using standard laboratory procedures described in Drury and Wallington [11].

2.7. Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) for a Completely Randomized Design (CRD) using the generalized model of the GENSTAT [12] software package. Respective significant means were separated using the Duncan multiple range test.

3. Results

3.1. Blood Chemistry

The results of haematological and serum biochemical parameters of broiler chickens fed dietary treatments are presented in Tables 2 and 3, respectively. Haematological values except for basophils showed significant ($P<0.05$) differences between groups fed maize, sorghum without enzyme and sorghum with various enzymes.

Supplementation of sorghum diet with 500 mg PHY increased WBC, RBC, PCV values in broilers while platelets, neutrophils, MCV, MCH and MCHC values were higher (P<0.05) following supplementation with 750mg XBG enzymes. Inclusion of 250mg XBG in sorghum diet significantly increased haemoglobin value. No pattern was observed in haematological parameters as level of enzyme supplementation increases. The results for the serum biochemical indices of broilers fed experimental diets were significantly (P<0.05) influenced by sorghum enzyme supplementations. Elevated levels (P<0.05) of cholesterol and AST concentrations were observed in birds fed sorghum than other diets. The addition of 250 mg PHY to sorghum diet led to significant increase in albumin, chloride and bilirubin concentrations with reduction (P<0.05) in the concentrations of sodium, potassium and AST/ALT ratio in the sera of the birds. At 500 mg PHY supplementation, serum concentrations of globulin, triglycerides and calcium were significantly (P<0.05) increased.

3.2. Histopathological Examination

Photomicrographs of the gizzard, liver and duodenum are shown in Plates 1 (a- k), 2 (a- k) and 3 (a- k), respectively. Mild or moderate variations were observed in the tissues.

Table-2. Haematological indices of broiler chickens fed sorghum-soybean-based diets

Dietary treatments												
Parameter	Maize-Soybean	Sorghum-Soybean (SS)	SS250mg PHY(R)	SS 500mg PHY(R)	SS 750mg PHY(R)	SS 250mg XAP(R)	SS 500mg XAP(R)	SS 750mg XAP(R)	SS 250mg XBG(R)	SS 500mg XBG(R)	SS 750mg XBG(R)	SEM
White Blood Cell ($\times10^3\mu/L$)	241.30^{de}	242.40^{cd}	238.60^{fg}	255.50^{a}	244.20^{bc}	238.70^{g}	236.40^{g}	232.70^{h}	245.90^{b}	240.00^{ef}	230.70^{h}	1.15
Red Blood Cell ($\times10^6\mu/L$)	2.15^{cd}	2.25b^{cd}	2.03^{d}	2.60^{a}	2.34^{bc}	2.18^{cd}	2.12^{cd}	2.12^{cd}	2.47^{ab}	2.22^{ab}	1.98^{d}	0.04
Haemoglobin (g/dl)	9.50^{ab}	9.60^{ab}	8.70^{bc}	9.70^{b}	9.50^{ab}	9.10^{ab}	8.60^{c}	8.60^{c}	10.00^{a}	9.10^{ab}	8.80^{bc}	0.21
Pack cell volume (%)	30.10^{de}	30.80^{cd}	29.30^{ef}	35.70^{a}	31.80^{c}	30.20^{de}	28.60^{f}	28.60^{f}	33.20^{b}	30.10^{de}	29.20^{ef}	0.38
Mean Corpuscular Volume(fl)	135.60^{de}	136.90^{cd}	144.30^{b}	137.30^{cd}	135.90^{de}	138.50^{c}	134.90^{ef}	134.90^{ef}	134.40^{ef}	135.60^{de}	147.50^{a}	0.74
Mean Corpuscular Haemoglobin (pg)	42.80^{b}	42.70^{b}	42.90^{b}	41.20^{c}	40.60^{cd}	41.70^{bc}	40.60^{cd}	40.60^{cd}	40.50^{cd}	41.00^{cd}	44.40^{a}	0.26
Mean Corpuscular Haemoglobin concentration (g/dl)	31.60^{a}	31.20^{a}	29.70^{b}	30.00^{b}	29.90^{b}	30.10^{b}	30.10^{b}	30.10^{b}	3.10^{b}	30.20^{b}	30.10^{b}	0.12
Platelet($\times10^3\mu/L$)	0.00^{d}	2.00b^{c}	1.00^{c}	3.00^{b}	1.00^{c}	0.00^{d}	0.00^{d}	0.00^{d}	1.00^{c}	0.00^{d}	40.00^{a}	2.00
Neutrophils (%)	46.00^{bc}	26.00^{g}	36.00^{e}	38.00^{e}	44.00^{cd}	40.00^{de}	50.00^{b}	50.00^{b}	38.00^{e}	31.00^{f}	60.00^{a}	1.86
Lymphocytes (%)	42.00^{ef}	70.00^{a}	60.00^{b}	48.00^{cd}	44.00^{de}	60.00^{b}	50.00^{c}	50.00^{c}	56.00^{b}	58.00^{b}	0.00^{f}	3.13
Eosinophils (%)	8.00^{b}	4.00^{c}	2.00^{d}	10.00^{a}	8.00^{b}	0.00^{e}	0.00^{e}	0.00^{e}	4.00^{c}	7.00^{b}	0.00^{e}	0.63
Mesophils (%)	4.00^{a}	0.00^{c}	2.00^{b}	4.00^{a}	4.00^{a}	0.00^{c}	0.00^{c}	0.00^{c}	2.00^{b}	4.00^{a}	0.00^{c}	0.32
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

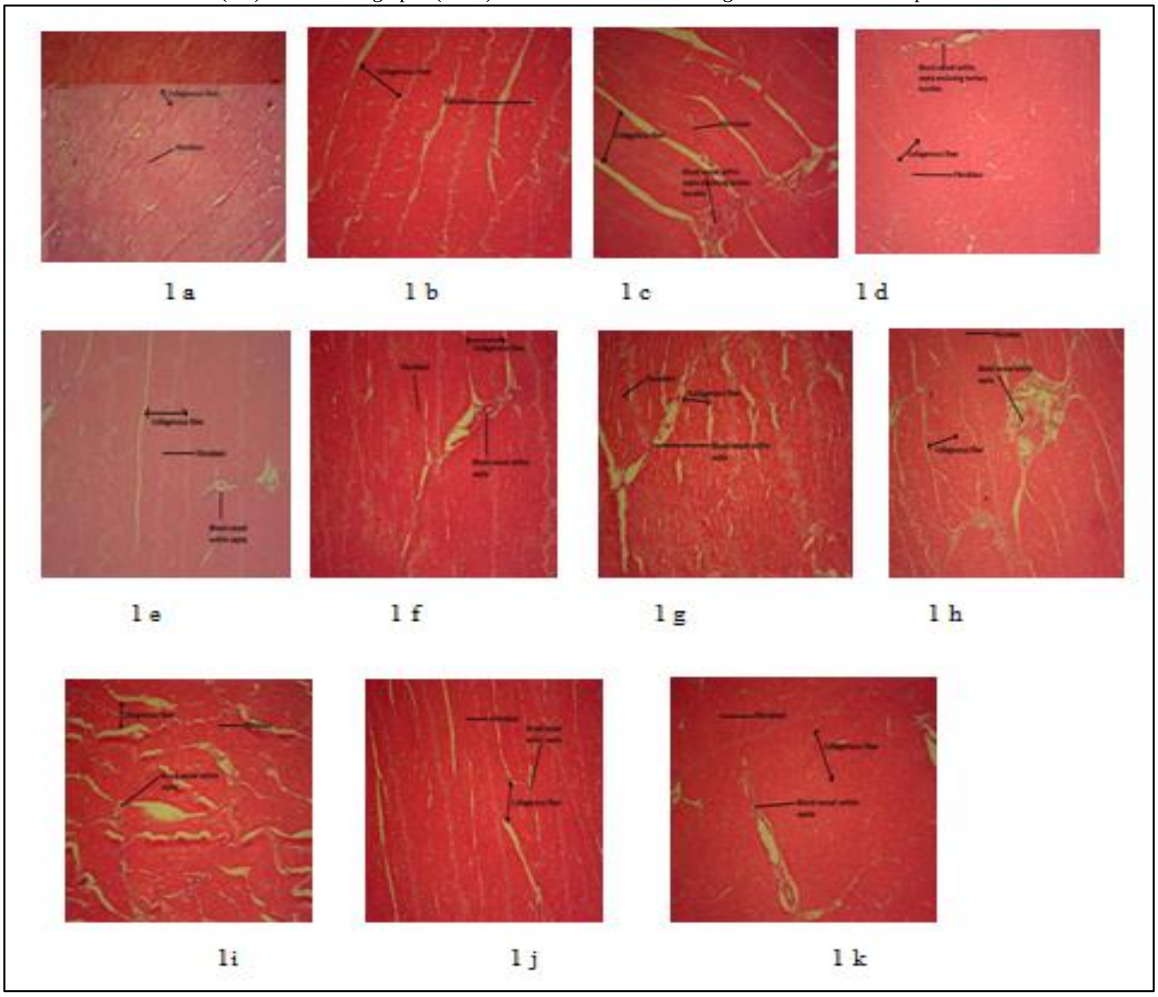
^{abc} Means with different superscripts on the same row differ significantly (P< 0.05)
SEM = Standard error of means
SS = sorghum-soybean base diet
PHY = Phytase $^{(R)}$ Enzymes
XAP = xylanase - amylase - protease $^{(R)}$ Enzymes
XBG = xylanase - B-glucanases$^{(R)}$ Enzymes

Table-3. Serum biochemical indices of broiler chickens fed sorghum-soybean based diets

Dietary treatments												
Parameter	Maize-Soybean	Sorghum-Soybean(SS)	SS 250mg PHY(R)	SS 500mg PHY(R)	SS 750mg PHY(R)	SS 250mg XAP(R)	SS 500mg XAP(R)	SS 750mg XAP(R)	SS 250mg XBG(R)	SS 500mg XBG(R)	SS 750mg XBG(R)	SEM
Glucose	5.40^{bc}	5.26^{cd}	5.50^{b}	5.46^{b}	3.80^{e}	6.06^{a}	5.29^{cd}	5.48^{b}	5.20^{d}	5.12^{b}	5.58^{b}	0.09
Total protein	4.84^{f}	4.86^{f}	4.60^{h}	5.21^{d}	5.42^{b}	4.85^{f}	5.11^{e}	4.76^{g}	4.87^{f}	6.80^{a}	5.35^{c}	0.10
Albumin	2.24^{i}	2.43^{e}	3.08^{a}	1.83^{j}	2.33^{g}	2.39^{f}	2.30^{h}	2.58^{d}	2.67^{b}	2.63^{c}	2.63^{c}	0.05
Globulin	3.16^{b}	2.82^{c}	2.42^{d}	3.64^{a}	1.47^{e}	3.67^{a}	2.90^{c}	2.91^{c}	2.52^{d}	2.49^{d}	2.95^{c}	0.10
Cholesterol	5.74^{h}	6.10^{a}	5.58^{d}	5.80^{d}	5.94^{b}	5.90^{c}	5.80^{f}	5.86^{de}	5.78^{g}	5.94^{b}	5.85^{e}	0.02
Triglycerides	0.98^{g}	1.19^{f}	1.40^{c}	3.64^{a}	0.87^{h}	0.81^{i}	1.28^{e}	0.95^{g}	1.34^{d}	1.75^{b}	3.84^{a}	0.06
HDL-c	4.21^{e}	3.33^{g}	4.58^{c}	5.80^{a}	3.14^{i}	3.62^{f}	2.93^{j}	5.43^{b}	4.38^{d}	3.16^{h}	2.72^{k}	0.17
LDL-c	1.33^{f}	2.54^{c}	1.01^{h}	0.87^{h}	2.62^{b}	2.13^{e}	2.62^{b}	0.24^{i}	1.13^{g}	2.43^{d}	2.76^{a}	0.17
VLDL-c	0.20^{g}	0.24^{f}	0.28^{c}	0.38^{a}	0.17^{h}	0.16^{j}	0.26^{e}	0.19^{g}	0.27^{d}	0.35^{b}	0.37^{a}	0.01
HDL-c/TC (%)	73.35^{e}	54.59^{g}	82.08^{c}	100.00^{a}	52.86^{h}	61.36^{f}	50.52^{i}	92.66^{b}	75.78^{d}	53.20^{h}	46.50^{j}	2.98
LDL/HDL-c (%)	31.59^{f}	76.28^{d}	22.05^{h}	15.00^{j}	83.44^{c}	58.84^{e}	89.42^{b}	4.42^{i}	25.80^{g}	76.90^{d}	101.47^{a}	6.11
AST (IU/L)	13.00^{e}	36.00^{a}	13.00^{e}	13.00^{e}	36.00^{a}	13.00^{e}	16.00^{d}	36.00^{a}	36.00^{a}	19.00^{c}	23.00^{b}	1.75
ALT (IU/L)	8.00^{c}	12.00^{b}	12.00^{b}	8.00^{c}	12.00^{b}	8.00^{d}	12.00^{b}	12.00^{b}	12.00^{b}	12.00^{b}	17.00^{a}	0.56
AST/ALT	1.63^{b}	3.00^{a}	1.08^{e}	1.63^{b}	3.00^{a}	1.63^{b}	1.33^{d}	3.00^{a}	3.00^{a}	1.58^{c}	1.35^{d}	0.28
Chloride	97.30^{f}	88.93^{c}	101.03^{a}	97.63^{e}	95.33^{i}	100.83^{ab}	100.73^{b}	96.13^{g}	100.83^{ab}	97.83^{d}	95.90^{h}	0.37
Urea	29.69^{e}	15.30^{f}	31.70^{b}	13.19^{h}	33.37^{a}	31.53^{b}	13.67^{g}	30.33^{d}	12.87^{i}	12.83^{i}	30.67^{c}	1.56
Calcium	7.23^{h}	7.39^{e}	5.31^{k}	7.89^{a}	7.46^{d}	7.83^{b}	7.62^{g}	7.31^{g}	7.37^{f}	6.82^{j}	6.96^{i}	0.12
Sodium	111.53^{ab}	105.57^{c}	103.97^{c}	111.57^{ab}	109.50^{b}	108.90^{b}	103.33^{c}	111.07^{ab}	112.90^{a}	109.90^{b}	109.17^{b}	0.58
Potassium	2.19^{ab}	1.61^{e}	1.71^{de}	2.02^{bcd}	2.38^{a}	2.11^{abc}	1.99^{bcd}	1.78^{de}	1.84^{cde}	2.18^{ab}	2.16^{abc}	0.05
Bilirubin	1.11^{de}	1.24^{bc}	1.92^{a}	0.82^{f}	1.07^{e}	1.10^{de}	1.19^{bcd}	1.13^{cde}	1.29^{b}	1.12^{de}	1.17^{ade}	0.05
CO_3^{2-}	42.00^{ab}	34.50^{cd}	40.33^{abc}	39.33^{bc}	32.33^{e}	38.67^{bc}	38.33^{bc}	37.67^{bcd}	39.00^{bc}	41.67^{ab}	45.33^{a}	0.75

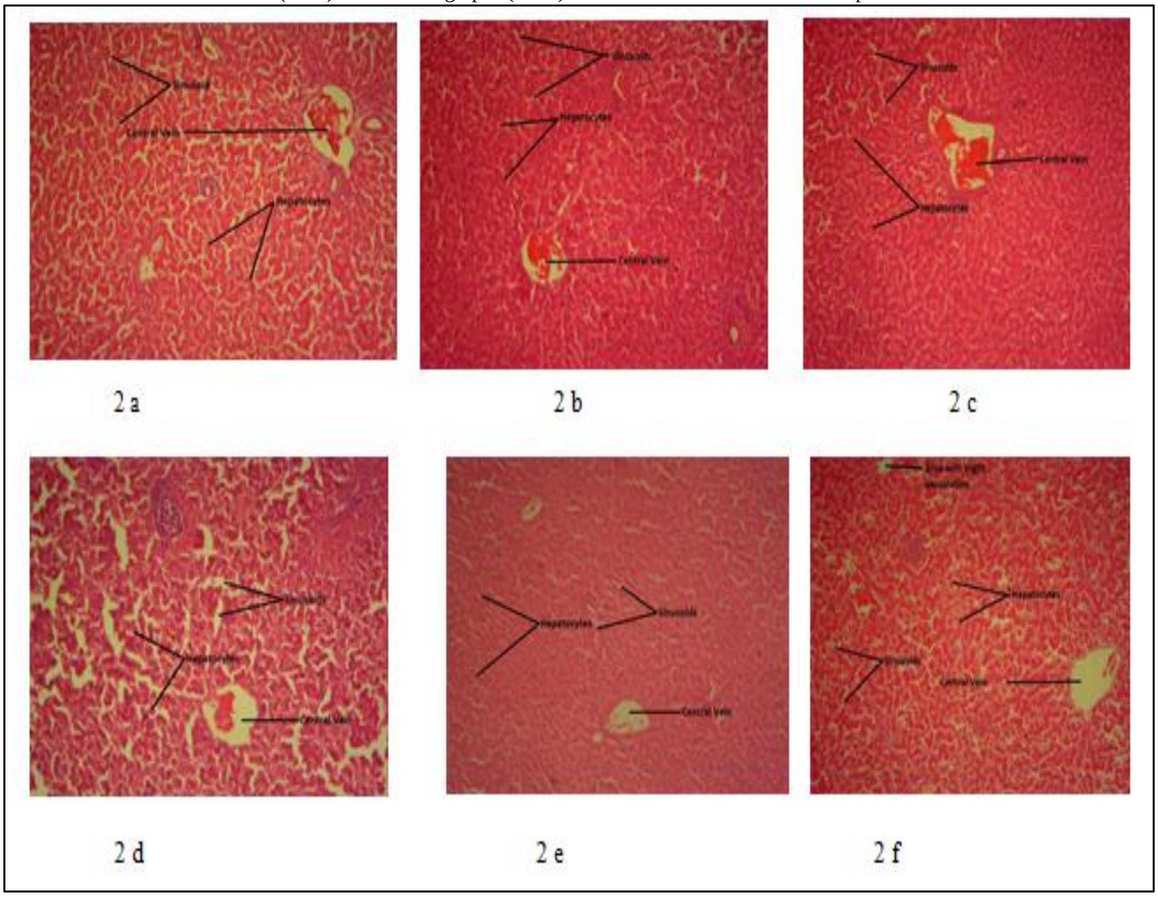
^{abc}Means with different superscripts on the same row differ significantly (P<0.05), SEM = Standard error of means
SS = sorghum-soybean base diet, PHY = Phytase $^{(R)}$ Enzymes, XAP = xylanase amylase and protease$^{(R)}$ Enzymes, XB = xylanase and B-glucanases$^{(R)}$ Enzymes
ALT = alanine aminotransferase, HDL-c= High density lipoprotein cholesterol, LDL-c = Low density lipoprotein cholesterol, VLDL-c = Very low density lipoprotein cholesterol
AST = aspartate aminotransferase, HDL-c:TC= High density lipoprotein cholesterol: Total cholesterol, LDL:HDL-c= low density lipoprotein: High density lipoprotein cholesterol, AST:ALT = Aspartate amino transferase: alanine amino transferase

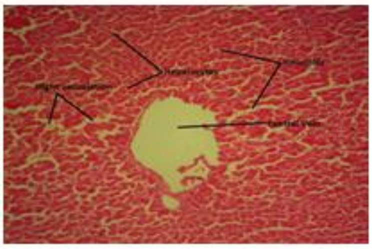
PLATES-1. (a-k): Photomicrographs (×100) of the cross section of the gizzard of birds fed experimental diets



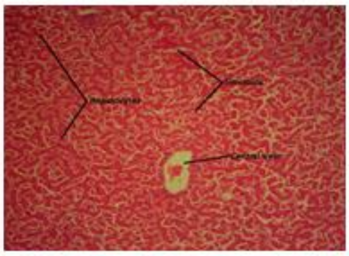
1a (maize group), 1b (sorghum group), 1c (250 PHY), 1d (500 PHY), 1e (750 PHY), 1f (250 XAP), 1g (500 XAP), 1h (750 XAP), 1i (250 XB), 1j (500 XB), 1k (750 XB)

PLATES-2. (a – k): Photomicrographs (×100) of the liver tissues of birds fed experimental diets

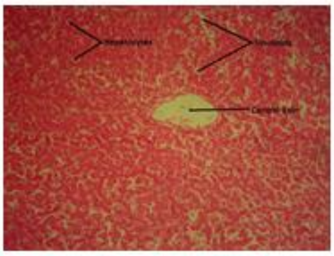




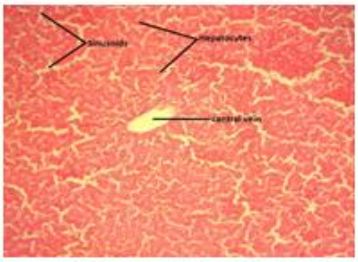
2 g



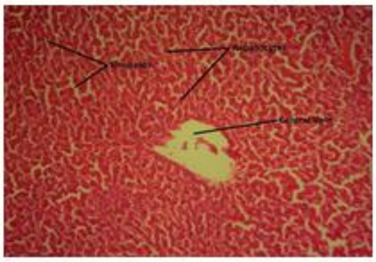
2 h



2 i



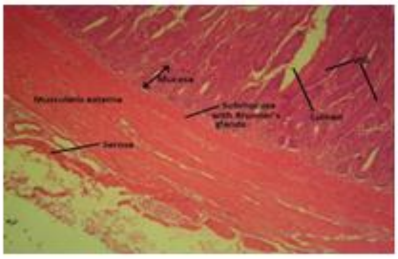
2 j



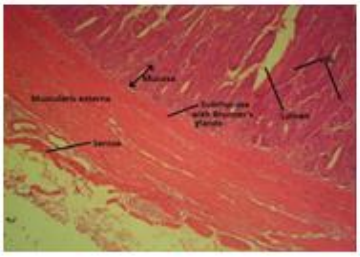
2 k

2a (maize group), 2b (sorghum group), 2c (250PHY), 2d (500PHY), 2e (750PHY), 2f (250XAP), 3g (500xap), 2h (750XAP), 2i(250XB), 2j (500XB), 2k (750XB)

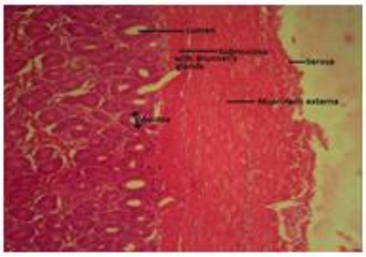
PLATES-3. (a – k): Photomicrographs (×100) of cross sections of the duodenum of birds fed experimental diets



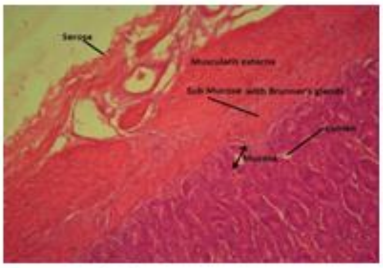
3 a



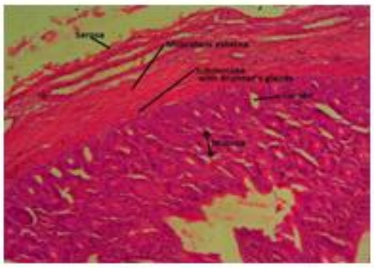
3 b



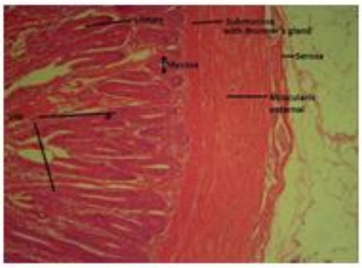
3 c



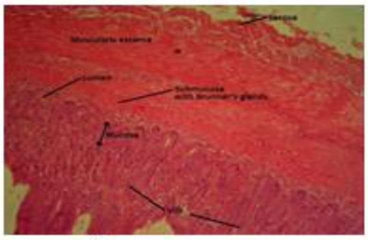
3 d



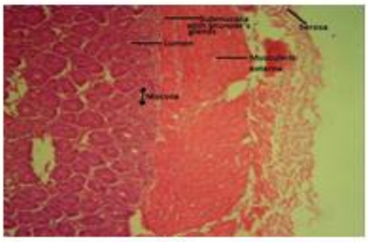
3 e



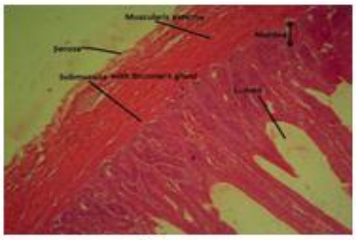
3 f



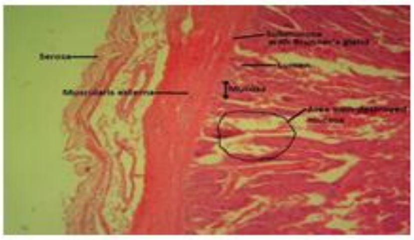
3 g



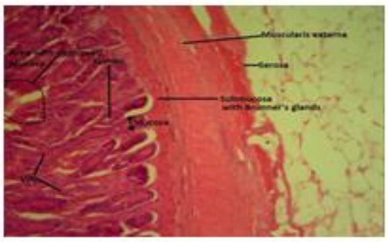
3 h



3 i



3 j



3 k

3a (maize group), 3b (sorghum group), 3c (250PHY), 3d (500PHY), 3e (750PHY), 3f (250XAP), 3g (500xap), 3h (750XAP), 3i(250XB), 3j (500XB), 3k (750XB)

4. Discussion

4.1. Haematological Parameters

Variation in all haematological parameters (Table 2) indicated that the diets affected the blood profile of the broilers in line with observations of Trîncă, *et al.* [13]; Adeyemi and Sani [14]. The absence of basophils in all groups suggested that the birds were healthy without any disease condition, as basophils are rare in circulating blood except in cases of severe infections [15]. Comparable haematological values were obtained between birds fed maize and sorghum diets, except for platelets and white blood differential counts. Birds on sorghum diet had higher platelets and lymphocytes with lower neutrophils, eosinophils, and mesophils than those on maize diet. Similarities in haematological parameters (within normal ranges) between maize and sorghum diets implied that both grains maintain the health status of birds as reported by Kwari, *et al.* [16]. Nevertheless, the higher lymphocytes recorded in sorghum fed birds was indicative of the physiological adjustment against negative antigens as a result of anti-nutrients present in the sorghum grains as observed by Adeyemi and Sani [14] in broilers fed cassava peel diets. Differential leucocytes are used as indicators of stress response and as sensitive biomarkers crucial to immune functions. Previous findings [14] have shown that variations exist in the number of white corpuscles and their ratios in health animals but could be greatly modified in sick animals. Values were similar to those reported by Adeyemi and Sani [14] in broilers fed cassava peel diets. Increased WBC at 500 mg PHY suggested acute or chronic inflammation in the tissues without severe necrosis Trîncă, *et al.* [13].

The packed cell volume (PCV) greater than 56 % is an indicator of polycythemia caused by dehydration [13]. PCV values in this study were lower than 56% suggesting that all birds were normally hydrated. MCH is an indicator of the blood carrying capacity of the RBCs; this could suggest that birds on 250 mg PHY and 750 mg XB sorghum diets were more efficient in performing respiratory functions as observed by Soetan, *et al.* [17] in birds fed raw and processed sorghum diets. MCHC value of less than 29 g/d is indicative of iron or mineral deficiencies [13]. Values obtained for MCHC in this study implied that no iron or mineral deficiencies were observed in all birds. White blood cells (WBCs) are known to fight infections/ diseases, thus animals with low WBCs are exposed to high risk of disease infections while those with higher counts are capable of generating sufficient antibodies required for phagocytosis with higher disease resistance. Reduction in the WBC, PCV and haemoglobin (Hb) and higher differential counts could imply adverse immune response to 750 mg XB diet causing decreased red blood cell production due to the presence of anti-nutrients and consequent sub-clinical mild bacterial infections in the birds.

Furthermore, the values for all the haematological parameters measured were within the normal ranges for apparently healthy broiler chickens as reported by previous workers [18]. These results indicated that despite the variation in haematological values probably due to sub-clinical infections, all birds were healthy without any clinical symptom of anaemia, dehydration, allergic conditions or bacterial infections. Maize diet did not have any superiority over sorghum on the health status of broilers and enzyme supplementations especially at 500 mg PHY provided additional immunity to the birds.

Although, there were significant differences in Hb and MCH, the values of Hb obtained were within the normal range for chickens (7-13g/l) Nkwocha, *et al.* [19]. The significant ($P<0.05$) value of MCH was within the normal range since the Hb and RBC obtained were within normal range for chickens. This result indicated that the nutrients were adequately utilized by the broiler chickens and posed no problem to the birds. This explains why the birds were healthy, not anaemic and were capable of withstanding stress. The values of PCV, RBC, WBC, platelets, MCV and MCHC obtained in this study were also within the normal ranges for chickens. This showed that the bone marrows of the birds were functioning normally. It revealed the absence of macrocytic and hypochronic anaemia. The normal values of PCV obtained indicated that enzyme supplementation increased the availability of protein, energy and the degradation of anti-nutritional factors thus improving broiler performance [20]. Haematological traits, especially PCV and Hb have been reported to be correlated with the nutritional status of the animal [21] and PCV is an index of toxicity in the blood and high levels usually suggest the presence of toxic factors which has adverse effect on blood formation [22]. The normal values of MCV, MCHC and MCH obtained in this study indicated that there was no negative interaction between the energy and protein levels in the diets. The leucocyte differential counts indicated that the birds were not under different stressed conditions since leucocyte responses are considered as better indicators of chronic stress [23].

4.2. Serum Biochemical Indices

The total protein is a reflection of protein quality and high value indicates sufficient levels in the diet to sustain normal protein level in the blood of the birds. Similar protein values recorded in birds fed maize and sorghum diets implied that both diets can sustain normal protein synthesis in the blood and enzyme supplementation tends to improve the protein quality. Kwari, *et al.* [18], however reported higher protein in the blood of birds fed sorghum compared to maize diet. The present study was in line with the findings of Satish and Asit [24] as there was no significant difference in blood glucose in broilers fed 100% sorghum to replace maize but contrary to the report of Kumar, *et al.* [7] who indicated no significant differences on blood albumin, globulin, total protein, glucose, calcium, phosphorus and uric acid levels after replacing corn by reconstituted or un-reconstituted grain sorghum in poultry diets. Findings of Kwari, *et al.* [16] however indicated different effects of sorghum varieties on the blood glucose of birds and low levels of tannin in birds fed sorghum diets or enzyme supplemented diets will lead to decrease low density lipoprotein oxidation in the body.

Globulin level is commonly used as an indicator of immune responses and source of antibody production [25]. Higher globulin level and low A/G ratio is known to reflect the increased immunoglobin synthesis in response to variety of pathogens [26]. Values for albumin and total protein were significantly ($P<0.05$) influenced by dietary treatments in line with observation of Raji, *et al.* [27] when broilers were fed African yam been seed meal supplemented with enzymes. Increased serum ALT, AST and ALP activity as well as AST: ALT ratio were suggestive of liver and intestinal damages indicating decreased absorption and digestion of protein as observed in birds fed sorghum and XB diets. Present results have demonstrated mild to severe distortion in the internal organs of birds fed in all dietary treatments. The ratio increased significantly as level of PHY increased whereas decreased as XB increased. The addition of 750 mg PHY and XAP resulted in organ damages similar to those observed in birds fed sorghum diets as well as those fed 250mg XB. There was no relationship between liver enzymes and glucose concentrations in this study as Jansman [28] had suggested that differences in blood glucose level in chicks were due to elevated levels of the liver enzyme UDP-glucuronosyl transferase following high-tannin sorghum consumption. Reduction in total lipid profile following enzyme supplementation could be due to their influence in decreasing the microbial intracellular pH, forcing the bacterial cell to use energy to release the acid. Enzyme supplementation led to significant reduction in the serum cholesterol concentration, especially in birds fed XB supplemented sorghum diets. Serum concentrations of potassium, urea and AST were elevated following 750 mg PHY supplementation; whereas at 750 mg XB supplementation, serum LDL-c, CO_3^{2-}, ALT and LDL/HDL-c ratio were significantly elevated, LDL/HDL-c ratio dropped drastically in birds fed 750 mg XB sorghum diet. Elevated total cholesterol level was observed in birds fed sorghum compared to those fed maize diet, but this level reduced significantly ($P<0.05$) following 250 mg PHY supplementation. According to the report of Kumar, *et al.* [7], the effect of sorghum consumption on blood lipids levels of birds may be explained by the role of tannins as antioxidants which inhibit lipid peroxidation. The significant increase of serum urea in birds fed 750 mg PHY suggested that there was greater catabolic activity in their muscle tissues and birds survive at the expense of their body reserve [14] as observed in the lower breast yield. The significant reduction in some serum metabolites following enzyme supplementations especially with PHY and XAP suggested that nutritional safety was enhanced. Significant improvement had earlier been reported in the biochemical parameters of broiler following the addition of enzyme or yeast in broiler diet [29]. Variation in blood indices in birds fed sorghum diets are related to the sorghum variety, strain of birds and experimental diets. Emami and Maheri-Sis [30], also noted that blood parameters directly affect the intestinal absorption of the nutrients thus affecting digestion.

4.3. Histopathological Evaluation

The histopathological examination of the gizzard, liver and duodenum (Plates 1(a – k), 2(a-k) and 3(a-k) showed that the gizzard had fine structure and the organization of muscles and connective tissues in the middle portion of the chicken gizzard (muscular stomach) (plates 1a – k) was perfect. The musculature shows long well defined bundles, arranged circularly and concentrically. The musculature contains fibroblast and interstitial cells and a small number of nerve bundles and blood vessels. There was no abnormality as all sections of the gizzard, showed dense regular connective tissue fibrocytic nuclei (basophilic and collagen fibres (eosinophilic) in a parallel orientation with normal bands of collagen fibers. The liver showed

mild alterations in the hepatocytes of birds fed dietary treatments. Plate 2a (Maize-fed birds) showed normal appearance of liver section with no visible distortion of the liver cells. There was neither enlargement of hepatocytes nor visible inflammation of the component of the liver. In Plate 2b (Sorghum-fed bird), normal liver architecture with no visible inflammation of the hepatocytes and vacuolation were seen. Liver section of bird on 250mg PHY sorghum diet (Plate 2c) showed congested blood vessel in the central vein however without inflammation in the hepatocytes. At 500mg PHY (Plate 2d), normal hepatocytes were seen but with blood congestions in the central veins with enlarged sinusoids. Plate 2e (750mg PHY) shows central vein with normal appearance of the various components of the architecture structure of the liver. The addition of 250 mg XAP (Plate 2f) led to slight vacuolation in the liver cells, though normal appearance was maintained. Normal appearance of the liver was seen (Plate 2g) birds fed 500 mg XAP. The cord of the hepatocytes was radiating from the central vein with no visible sign of fibrosis or necrosis. Plate 2h (750mg XAP) had similar appearance as observed in Plate 2g, though congested blood vessels were seen in the central vein. The addition of XB at 250, 500 and 750 mg also led to normal architecture of the liver cells with slight vacoulations observed. This result implied that sorghum diet with or without enzyme had no adverse effects on the histology of the chicken liver consistent with the findings of Kumar, *et al.* [7], but contradicted the report of Karimi, *et al.* [31] that high levels of sorghum grains in diet induce histological changes in kidney and liver of animals. The duodenum (plates 3 a – k) showed normal tissues with no visible corrosion. The mucosa, villi and *Muscularis externa* were intact across dietary treatments.

5. Conclusion

The study therefore concluded that:

i Sorghum can conveniently replace maize in broiler diets with enzyme levels of 250 and 500 mg/kg PHY as optimum for improved haematology and tissue integrity of digestive organs.

ii Phytase enzyme was more effective than XAP or XBG in sorghum based diet.

iii. Further research is recommended with highest levels of dietary supplementation, preferably 75 and 100 mg/kg in sorghum based diet.

Statement of Competing Interest

The authors have no competing interests.

References

[1] FAO, 2012. "Food and Agriculture Organization. Use of lesser- known plants and parts as animal feed resources in tropical regions. E. S. Quansah, H. P. S. Makkar (Eds.). Animal Production and Health working paper, No. 8, FAO, Rome."

[2] Issa, S., Hancock, J. D., Tuinstra, M. R., Kapran, I., and Kaka, S., 2007. "Effects of sorghum variety on growth and carcass characteristics in broiler chicks reared in West Africa." *Poultry Science,* vol. 86, pp. 69-76.

[3] Oluyemi, J. A. and Roberts, F. A., 2000. *Poultry production in warm wet climate*. 2nd ed. Ibadan, Nigeria: Spectrum Books Ltd., p. 210.

[4] Ajaja, K., O., A. J., and A., A. V., 2000/2002. "Replacement value of sorghum dust for maize in diets for broiler chicks." In *Proceedings of the 27th Annual Conference of the Nigerian Society for Animal Production, Federal University of Technology Akure, Nigeria.* pp. 109-112.

[5] Ogbonna, A. C., 2011. "Current developments in malting and brewing trials with sorghum in Nigeria: A Review." *Journal of Institute of Brewing,* vol. 117, pp. 394-400.

[6] Gangaiah, B., 2007. *Kharif crops*. New Delhi: NISCAIR (CSIR) Web publication.

[7] Kumar, V., Elangovan, A. V., Mandal, A. B., Tyagi, P. K., Bhanja, S. K., and Dash, B. B., 2007. "Effects of feeding high tannin red sorghum on nutrient utilization and certain welfare parameters of broiler chickens." *British Poultry Science,* vol. 48, pp. 198-204.

[8] Etuk, E. B., Okeudu, N. J., and Udedibe, A. B. I., 2012. "Anti-nutritional factors in sorghum: chemistry, mode of action and effects on livestock and poultry." *Online Journal of Animal and Feed Research,* vol. 2, pp. 113-119.

[9] Khattak, F. M., N., P. T., and Mahmud, A., 2006. "Enzymes in poultry production." *Journal of Animal and Applied Science,* vol. 16, pp. 60-75.

[10] Odetallah, N. H., Wang, J. J., Garlicch, J. D., and Shih, J. C. H., 2005. "Versazyme supplementation of broiler diets improves market growth performance." *Poultry Science,* vol. 84, pp. 858-864.

[11] Drury, K. A. S. and Wallington, A., 1976. *Histopathological Techniques*. 4th ed. London: Oxford University press. pp. 48-58.

[12] GENSTAT, 2012. *Release 3.2 version 5, Payne, R. W., Lane, P., Harding, S., Murray, D., Thompson, R., Todd, A. and Welham, S. J.* Numerical Algorithms Group, Oxford.

[13] Trîncă, S., Cernea, C., Arion, A., and Ognea, L., 2012. "The relevance of mean blood samples in haematological investigations of broiler chickens." *Veterinary Medicine,* vol. 69, pp. 209-214.

[14] Adeyemi, I. A. and Sani, A., 2013. "Haematological parameters and serum biochemical indices of broiler chickens fed Aspergillus niger hydrolyzed cassava peel meal based diets." *International Journal of Recent Research and Applied Studies,* vol. 15, pp. 410-415.

[15] Ozung, P. O., Oko, O. O. K., Henry, A. J., Jimmy, N. P., Eburu, P. O., and Etim, C. D., 2019. "Influence of dietary lanthanum oxide (La2O3) on the blood characteristics and ovarian morphometry of broiler chickens." *Nigerian Journal of Animal Production,* vol. 46, pp. 40-46.

[16] Kwari, I. D., Diarra, S. S., Saleh, B., Bovoa, P. R., Ramat, O. A., and Tochukwu, D., 2011. "Growth, hematology and serology of broiler chickens fed different cultivars of sorghum as replacement for maize in the semi-arid zone of Nigeria." *International Journal of Poultry Science,* vol. 10, pp. 608-612.

[17] Soetan, K. O., Akinrinde, A. S., and Ajibade, T. O., 2013. "Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor)." In *Proceedings of the 38th Annual Conference of Nigerian Society for Animal Production, Makurdi, Nigeria.* pp. 49-52.

[18] Kwari, I. D., Igwebuike, J. U., Shuaibu, H., Titimaand, S. I., and Raji, A. O., 2014. "Growth and carcass characteristics of broiler chickens fed maize, sorghum, millet and their combinations in the semi-arid zone of Nigeria." *International Journal of Science and Nature,* vol. 5, pp. 240-245.

[19] Nkwocha, G. A., Agbabiaka, L. A., Anukam, K. U., and Beketin, T. O., 2014. "Growth responses, carcass and blood characteristics of finisher broilers fed sorghum offal meal as dietary supplement." *International Journal of Agricultural Science,* vol. 4, pp. 392-398.

[20] Cary, N. C., Williams, P. E. V., Geraet, P. A., Uzu, G., and Annison, G., 2002. *Factors affecting non-starch polysaccharide digestibility in poultry. Rhone Poulen. Animal Nutrition, 42 Ar. Aristide Briand BP 10092 164*. France: Antony Cedex.

[21] Ubua, J. A., Ozung, P. O., Inagu, P. G., and Aboluja, B. A., 2018. "Blood characteristics of broiler chickens as influenced by dietary inclusion of Neem (Azadirachta indica) leaf meal." *Canadian Journal of Agriculture and Crops,* vol. 3, pp. 72-80.

[22] Oyawoye, E. O. and Ogunkunle, M., 1998. "Chemical analysis and biochemical effects of raw jack beans on broiler chickens." *Proceedings of Nigerian Society for Animal Production,* vol. 23, pp. 141-142.

[23] Siegel, H. S., 1998. "Stress, strain and resistance." *British Poultry Science,* vol. 36, pp. 3-22.

[24] Satish, J. M. and Asit, B. M., 2009. "Effect of reconstitution of sorghum with or without enzymes on production performance and immunocompetence in broiler chicken." *Journal of the Science of Food and Agriculture,* vol. 86, pp. 998-1005. Available: https://doi.org/10.1002/jsfa.3546

[25] Kamal, A. Z. and Ragaa, N. M., 2014. "Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken." *Nature and Science,* vol. 12, pp. 38-45.

[26] El-Kardawy, E. R., El-Ashmawy, M. B., Barghash, A. M., Shehata, I. A., and El-Kerdawy, M. M., 1996. "Synthesis of some new quinazolines and quinoxalines of potential anti-inflammatory activity." *Bollettino Chimico Farmaceutico,* vol. 135, pp. 617-620.
[27] Raji, M. O., Adeleye, O. O., Mosobalaje, M. A., Ogunjimi, J. T., and Tewe, O. O., 2016. "Growth response and serum biochemical parameters of starter broiler chickens fed toasted African yam bean (Sphenostylis stenocarpa) seed meal with enzyme supplementation." *Archivos De Zootecnia,* vol. 65, pp. 139-143.
[28] Jansman, A. J. M., 1993. "Tannins in feedstuffs for simple stomached animals." *Nutrition Research Reviews,* vol. 6, pp. 209-236.
[29] Basmacioglu, H., Oguz, H., Ergul, M., Col, R., and Birdane, Y. O., 2005. "Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology of broilers exposed to aflatoxin." *Czech Journal of Animal Science,* vol. 50, pp. 31-39.
[30] Emami, F. and Maheri-Sis, N., 2015. "Effects of reconstitution of high tannin sorghum grain on small intestine morphometry of Japanese quails." *Trakia Journal of Sciences,* vol. 13, pp. 77-82.
[31] Karimi, H., Kia, H. D., and Hosseinkhani, A., 2014. "Histological effects of different levels of sorghum grain on the liver and kidney of Ghezel×Arkhar-Merino crossbred lambs." *Animal and Veterinary Sciences,* vol. 2, pp. 130-134.