British Journal of Multidisciplinary and Advanced Studies:
Agriculture, 4(4),77-87, 2023
Print ISSN: 2517-276X
Online ISSN: 2517-2778
Website: https://bjmas.org/index.php/bjmas/index
Published by European Centre for Research Training and Development-UK

Hematology and Serum biochemistry of Broiler Chickens Fed Red Sorghum (*Sorghum bicolor* (L.) Moench) Based Diets supplemented with Complex Enzyme (Kingzyme®) in Girei, Adamawa State, Nigeria

***[1]Edeh, I. E., [1]Gworgwor, Z. A., [1]Yusuf, H. B. and [1]Soji, W. M.**
[1]Department of Animal Science and Range Management, Modibbo Adama University, Yola, PMB 2076, Adamawa State, Nigeria.
Corresponding Author: edehifeanyi605@gmail.com

doi: https://doi.org/10.37745/bjmas.2022.0284 Published: August 4 2023

Citation: Edeh, I. E., Gworgwor, Z. A., Yusuf, H. B. and Soji, W. M. (2023) Hematology and Serum biochemistry of Broiler Chickens Fed Red Sorghum (Sorghum bicolor (L.) Moench) Based Diets supplemented with Complex Enzyme (Kingzyme®) in Girei, Adamawa, *British Journal of Multidisciplinary and Advanced Studies*: Agriculture, 4(4),77-87

ABSTRACT: *The study was designed to study the Hematology and Serum biochemistry of Broiler Chickens Fed Red Sorghum (Sorghum bicolor (L.) Moench) Based Diets supplemented with Complex Enzyme (Kingzyme®). Two hundred (200) chicks (day-old) of mixed sex was randomly allotted to five dietary treatments (T_1, T_2, T_3, T_4 and T_5) supplemented with enzyme complex (Kingzyme®) at the levels of 0.0g, 0.2g, 0.5g, 0.8g and 1.1g kg per Kg of feed respectively in a Completely Randomized Design (CRD). Each treatment was replicated four (4) times to contain ten (10) birds each. Feed and water were provided ad libitum accompanied with standard management procedures for eight weeks. At end of the eight weeks' experimental period, blood samples were collected for hematological and serum chemistry analysis. Data were analyzed using Statitix Analytical Software, Version 10; and the treatment means were compared using Turkey HSD.Except the RBC, MCV, MCH and Eosinophil which showed significant ($P < 0.05$) difference across the treatment groups, all other hematological parameters were not significantly ($P > 0.05$) affected. The TP, Alb and Glb were also not significantly ($P > 0.05$) affected. The Urea and Glucose were not significantly ($P > 0.05$) affected. The Creatinine and Cholesterol were significantly ($P > 0.05$) affected across the dietary treatment. Based on the result of the study, it was concluded that Red Sorghum-based diet supplemented with enzyme complex (Kingzyme®) do not have any significant effect on hematological and serum biochemistry of broiler chickens.*

KEYWORDS: Hematology, Serum Chemistry, Broiler Chickens and Sorghum bicolor (L.) Moench

INTRODUCTION

The application of enzymes in poultry diets has been reported by many authors in various cereal based diets with little reference to Sorghum grains. The use of Sorghum in poultry feeding have not been fully exploited. Sorghum is least utilized as major energy source in poultry feed industry

Print ISSN: 2517-276X
Online ISSN: 2517-2778
Website: https://bjmas.org/index.php/bjmas/index
Published by European Centre for Research Training and Development-UK

in Nigeria. The limitation to use especially in poultry nutrition is due to its low starch and protein digestibility and tannin profile compared to maize. Sorghum grain can be an alternative energy source for poultry feed provided the factors limiting its digestibility is reduced or completely removed via processing and/or the use of additives (Khoddami *et al.* 2015). Exogenous enzymes - cellulase, β-glucanases, xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases - have been used in the feed industry in poultry diets to neutralize the effects of viscous, non-starch polysaccharides in cereals such as barley, wheat, rye, and triticale (Pan *et al.* 2017). Sorghum contains unique structure of protein which compromises nitrogen and energy utilization by physical and chemical interactions but this could be addressed by combined or individual action of exogenous enzymes addition in broilers diets and swine. In-vivo studies using pepsin and in-vitro studies show that the proteins of wet cooked sorghum are significantly less digestible than the proteins of other similarly cooked cereals (Pan *et al.* 2017). This is an implication that wet cooking may hamper the digestibility of Sorghum grain. Sorghum is second largest produced and abundant cereal grain after maize; fifth most widely grown cereal crop on global scale after rice, wheat and barley. Fifty-three percent (53%) of the world's sorghum production area is located in Sub-Saharan Africa where it covers the second largest cultivated land area after maize (USDA, 2019). In terms of nutritive value, cost and availability, sorghum grain is the next alternative to maize in poultry feed. Although Sorghum is considered the major source of energy for poultry feeds in some Asian and most African countries due to its high energy content. Rolled sorghum is a common practice in poultry feed formulation, and whole grain feeding is well known in rural areas (Liu *et al*., 2015); there are paucity of information on hematological values and biochemical indices of broiler fed on Red Sorghum (*Sorghum bicolor* (L.) Moench) based diet; since hematological values are index to predicting effect of rations (1). This research was therefore focused on Hematology and Serum biochemistry of Broiler Chickens Fed Red Sorghum (*Sorghum bicolor* (L.) Moench) Based Diets supplemented with Complex Enzyme (Kingzyme®).

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Poultry Unit of Teaching and Research Farm of the Department of Animal Science and Range Management, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria and it lasted for eight (8) weeks.

Experimental Animal, Management and Design

Two hundred (200) chicks (day-old) of mixed sex was subjected to standard management procedures. All the birds were housed in a deep litter system in an open-sided poultry house. Standard routine management activities were carried out. Water and feed were provided *ad-libitum.* The birds were monitored under strict hygienic conditions; and mortality was recorded following occurrence during the study. The birds were randomly selected, weighed to obtain the initial body weight; thereafter allotted to five (5) dietary treatments (T_1, T_2, T_3, T_4 and T_5). Each treatment was replicated four (4) times to contain ten (10) birds each in a Completely Randomized Design (CRD).

Experimental diet

The Enzyme blend (Kingzyme®) was purchased from Jos, Plataeu State, Nigeria. Red sorghum *(Sorghum bicolor* (L.) Moench*)* was purchased from Girei market, Girei Local Government area of Adamawa State, Nigeria. They were sorted to remove stones and other contaminants and then crushed. The crushed grain was analyzed for its proximate composition. The crushed red sorghum was used to formulate five dietary treatments containing the enzyme at different levels of inclusion (0.0g, 0.2g, 0.5g, 0.8g and 1.1g) of enzyme per kg of feed to represent the five (5) treatments (T_1 (control), T_2, T_3, T_4 and T_5) groups respectively.

Data collection

Two (2) birds were selected randomly from each replicate for hematological and biochemical analysis at the end of the feeding trial. Blood samples were collected into labeled sterile universal bottle with and without ethyl diamine tetracetic acid (EDTA) as anticoagulant to determine the hematological and biochemical components respectively according to the methods described by Association of Analytical Chemists (AOAC, 2010) to determine the Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC) and its differential counts (Monocyte, Lymphocyte, Eosinophil and Neutrophil). The Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated based on the data generated from Hb, RBC and PCV (Ritchie *et al.* 1994). The serum samples were separated using a centrifuge and used for the analysis of biochemical indices (Total Protein (TP), Cholesterol, Albumin, Globulin, Urea, Creatinine and Glucose).

$$\mathrm{MCV} = \frac{\text{Hematocrit value (PCV)} \times 10}{\text{Erythrocyte (RBC) count}}$$

$$\mathrm{MCH} = \frac{\text{Hemoglobin gL}^{-1} \times 10}{\text{Erythrocyte (RBC) count}}$$

and

$$\mathrm{MCHC} = \frac{\text{Hemoglobin (Hb) gL}^{-1} \times 100}{\text{Hematocrit (PCV) value}}$$

Statistical Analysis

The data collected was analyzed using Statitix Analytical Software, Version 10; and the treatment means were compared using Turkey HSD procedure as described by (Tukey, 1949).

RESULT AND DISCUSSION

Hematological

Blood represents a means of assessing clinical and nutritional health status of animals on feed trial. The hematological parameters most commonly used for assessment in nutritional studies include the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Lymphocyte, Monocyte, Eosinophil, Neutrophil and Basophil (Adeyemi *et al.* 2000). The normal ranges for hematological parameters in chickens have been stated as PCV (25 – 45%), RBC ($2 - 4 \times 10^6$/mm^3), Hb (7 – 13g/dl) (Benerjee, 2008); WBC ($3.7 - 11.9 \times 10^9$/L), Monocyte ($0.0 - 1.2 \times 10^9$/L), Neutrophil ($0.8 - 7.4 \times 10^9$/L) (8); Lymphocyte ($1.2 - 4.2 \times 10^9$/L) and Eosinophil ($0.0 - 1.8 \times 10^9$/L) (15). The MCV is used to calculate the average erythrocyte size; MCH to measure hemoglobin amount per blood cell and MCHC to know the amount of hemoglobin relative to the size of the cell per red blood cell. Their normal ranges are MCV (104 – 135 fL), MCH (32.0 – 43.9 pg/cell) and MCHC (30.2 – 36.2 g/dl) (Guland and Hawkey, 1990).

The hematological parameters recorded in the study are presented in Table 1. Except the RBC, MCV, MCH and Eosinophil which showed significant ($P < 0.05$) difference across the treatment groups, all other hematological parameters – PCV, Hb, MCHC, WBC, Monocyte, Lymphocyte, Eosinophil, and neutrophil - were not significantly ($P > 0.05$) affected. The PCV (hematocrit) is the measurement of the percentage of RBCs in the circulatory system. It plays a role in determining the state of health. Higher or lower values of PCV than normal indicate dehydration or anemia (Peter, 2002). The PCV recorded in the current study was not significant ($P > 0.05$) across the treatments; and however, within the normal range. This is an indication that the energy sources in the diets were effectively utilized and the health of the birds was not compromised. It also indicates that erythropoiesis was not affected in the internal system of the birds. The finding is in agreement with (Afolayan *et al*, 2014) who reported no significant difference in the PCV of local grower chickens fed palm kernel cake meal. Similar report has been made by (Kaminski *et al.* 2014) and (Daramola, 2017) for broiler chickens fed enzyme (Maxigrain®) and Phytase treated diets respectively.

Hemoglobin (Hb) is the iron-containing oxygen-transport metalloprotein in the red blood cells (Erythrocyte) of almost all vertebrates. It decreases due to anemia and/or inadequate production of the RBCs (Maton *et al*, 1993). The Hb values recorded in the current study were not significantly ($P > 0.05$) affected by the dietary treatments; and however, within the normal range and consistent with (11) who reported similar result for broiler chicken fed phytase treated diets. Contrary to the current Hb result, (Maton *et al.* 1993) reported significant differences in Hb for broiler chickens fed enzyme (Maxigrain ®) and Pro-Vitamin A Bio-fortified diets respectively. This variation could be due to diet composition, type and inclusion level of the additives.

The function of the RBC is to transport oxygen from the lungs to tissues and remove carbon dioxide from the tissue to lungs in the body via hemoglobin. Higher or lower values of RBCs than normal indicate Polythemia and anemia (Guyton *et al.* 1976) The values recorded for RBC was significantly different across the treatment groups and however within the normal range. The RBC ($P < 0.05$) recorded in the current study is consistent with (Ehebha and Eniola, 2018) for broiler chicken fed enzyme (Maxigrain ®) treated diet; and (Kaminski *et al.* 2014) who reported that RBC range of broiler chickens is affected by sex and diet. A similar finding has been reported (Ripon *et al.* 2017) for indigenous, exotic and hybrid chickens. In the current study, the effect of the diet on RBC was more evident in birds fed diet containing 0.8g of enzyme (KINGZYME®) per kilogram of feed. This better RBCs value may be due to better performance of birds on that treatment group as evidenced by their final body weight and FCR at finisher phase.

There was significant ($P < 0.05$) in the MCV and MCH values across the treatment group; but the MCHC was however not affected. The MCH and MCHC values recorded were within their normal ranges. The results regarding MCV ($P < 0.05$) and MCHC ($P > 0.05$) was in agreement with (13) who reported same for broiler chicken fed enzyme (Maxigrain ®) treated diets. The MCH ($P < 0.05$) recorded in this study however negates (Daramola, 2017) who reported no significant difference in MCH values of broiler chickens fed phytase enzyme treated diets. The MCV was significantly affected across the treatment and however above the normal ranges. Higher significant ($P < 0.05$) MCV was recorded in birds on the control and 0.5g/kg treated diets respectively. Increase MCV, MCH, or MCHC has been reported to be due to anemia (Sievered, 1972). The cause of higher MCV values in the current study was unknown. According to (Maner and Moosavi, 2019), A high MCV (Macrocytosis) indicates that the RBCs are larger than average. This condition is usually known as Macrocytic anemia and associated with deficiency factor such as Vitamin B12 deficiency anemia. Higher values of RBCs than normal range indicate Polythemia – a condition in which the number of RBC increases and eventually thickens the blood – and lower RBC than normal indicates anemia. MCV is used to calculate the average erythrocyte (RBC) size (Bounous and Stedman, 2000). Based on these statements by (Maner and Moosavi, 2019) and (Bounous and Stedman, 2000), it thus implies that any factor that would influence MCV absolutely depends on the value of RBCs. The RBC recorded in the current study was within the normal range.

The WBC and its components aid to protect the body against pathogen and infectious agents. The WBCs values recorded in the current study was not significant and however within the normal range. This is in agreement with (Ehebha and Eguaoje, 2018). Birds fed diets containing 0.8g and 1.1g of enzyme per kilogram of feed showed higher WBC values compared to other treatment

British Journal of Multidisciplinary and Advanced Studies:
Agriculture, 4(4),77-87, 2023
Print ISSN: 2517-276X
Online ISSN: 2517-2778
Website: https://bjmas.org/index.php/bjmas/index
Published by European Centre for Research Training and Development-UK

group. This is an indication of better body immune system as evidenced by their better performance. In response to infection and in some cancer conditions such as leukemia, the WBCs count increases. Reduced number of WBCs can occur due to bone marrow disease, severe acute disease and other conditions (Peter, 2002). The component of WBCs includes neutrophil, lymphocyte, monocyte, eosinophil and basophil. Stress induced factors can trigger lymphocytosis (increased in lymphocyte) and/or neutrophilia while allergic or parasitic conditions may induce eosinophilia. Chronic disease condition may display increased number of monocytes (monocytosis) (Peter, 2002). All the WBCs components recorded in the current study were within their normal range (Christine *et al.* 1990) and (Gulland and Hawkey, 1990).

Table 1. Hematological parameters of Broiler Chickens Fed Red Sorghum-based Diets Supplemented with Enzymes Complex

	Dietary Treatments					
Parameters	**T_1(0.0g/kg)**	**T_2(0.2g/kg)**	**T_3(0.5g/kg)**	**T_4(0.8g/kg)**	**T_5(1.10g/kg)**	**SEM**
Hematology						
PCV (%)	38.46	34.33	39.16	37.05	39.57	1.48 ^{ns}
Hemoglobin (g/100mg)	9.12	8.71	9.17	9.13	9.26	0.30 ^{ns}
RBC (x10^6/mm^3)	2.32^b	2.96^b	2.50^b	3.12^a	2.99^b	0.19*
MCV (μm^3)	167.29^a	118.06^c	157.72^{ab}	118.91^{bc}	134.84^{abc}	8.97*
MCH (Pg)	39.69^a	30.18^b	37.00^b	29.26^c	31.86^b	2.55*
MCHC (%)	23.72	25.66	23.68	24.62	23.56	1.43 ^{ns}
WBC (x10^6/mm^3)	9.99	9.77	9.41	10.58	10.25	0.58 ^{ns}
Monocyte (%)	1.12	1.19	1.22	1.18	1.19	0.04 ^{ns}
Lymphocyte (%)	1.65	1.28	1.54	1.40	1.66	0.15 ^{ns}
Eosinophil (%)	1.41^b	1.76^{ab}	1.72^{ab}	1.82^a	1.61^{ab}	0.08*
Neutrophil (%)	1.51	1.72	1.68	1.70	1.69	0.05 ^{ns}

Means on the same row with different superscripts are significantly different (P > 0.05). SEM: Standard error of mean; PCV: Packed cell volume; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell; TP; Total protein

Serum Chemistry

Serum chemistry is routinely used for detection of organ diseases in domestic animals, and the amount of available protein in the diets (Iyayi and Twew, 1998). The Total Protein (TP) is made up of Albumin (Alb) and Globulin (Glb). Globulin is calculated as the difference between TP and Alb. Their normal ranges in birds are TP (3.0 – 4.9 mg/l), Alb (1.17 – 2.74 g/dl) and Glb (1.83 –

2.1 g/dl) (Meluzzi *et al*, 1992). The values of Serum Chemistry recorded in the current study are presented in Table 2. The TP, Alb and Glb were not significantly (P > 0.05) affected. The results regarding TP, Alb and Glb (P > 0.05) was consistent with (Ehebha and Eguaoje, 2018) and (Daramola, 2017) who reported similar findings for broiler chickens fed enzyme (Maxigrain ®) and phytase treated diets respectively. However total serum protein, albumin and globulin synthesis are not affected by sources of dietary proteins (Iyayi and Tewe, 1998). Enzymes are protein and based on this study has shown no significant effect on TP, Alb and Glb levels.

Though TP, Alb and Glb were not significant, the TP of birds fed the control diet were within the normal range (3.0 – 4.0mg/L) (Meluzzi *et al.* 1998). However, other treatment groups showed slight increase in TP. The albumin levels of birds fed the control and diet containing 1.10g of enzyme (KINGZYME®) per kilogram of feed were within the normal range (1.17 – 2.74 g/dL) for domestic chickens (Meluzzi *et al*, 1992). Slight increase in Alb was observed in other treatment groups. The Globulin levels recorded in all the treatments groups were slightly higher than the normal range (1.83 – 2.1 g/dL) (22). The slight increase in TP, Alb and Glb recorded in some treatment groups could be due to dehydration and/or reduced water intake or inflammation; although water was provided throughout the experimental period and no inflammation was observed during the experiment. According to (Esubonteng, 2011), albumin is the most abundant protein in blood plasma (Deldar, 1994). Globulin carries essential metals through the bloodstream to various parts of the body and helps the body to fight infections. Elevated globulin levels are often associated with serious infection due to abnormal increased production of antibodies. However, the WBC recorded in this study was within the normal range. A higher concentration of Alb. usually denotes dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection (Esubonteng, 2011). Serum protein, albumin and globulin are relative to availability of protein and micro nutrients. Albumin is a serum protein synthesized in the liver. It is responsible for transporting insoluble substances in the blood and aids to maintain oncotic pressure (Fischbach and Dunning, 2009).

High protein intake, age and sex, increased protein metabolism; stress and dehydration influence the concentration of Uric acid in blood as it is produced as a result of protein metabolism. Increase value of Urea indicates renal disease and severe dehydration and decrease infers liver failure and/or starvation (Chernecky and Serger, 2008). The normal range for Uric acid is 1.9 – 12.5 Mg/dl (CDD, 1990). The value for Urea recorded in the current study was not significantly (P > 0.05) affected and however within the normal range. The result is consistent with (Ehenha and Eguaoje, 2018) and (Daramola, 2017) who respectively used enzyme (Maxigrain ®) and Phytase on broiler chickens and reported no significant effect for Urea; and (Polat *et al*, 2019) reported that Serum Urea did not vary in broiler chickens due to diet.

Creatinine is used to determine the status of the kidney. The function of the kidney includes excretion of waste products resulting from protein metabolism and nucleic muscle contraction (Ileke *et al*, 2014). Creatinine is excreted by the kidney as by-product of creatine-phosphate metabolism which is produced as a result of energy production by the skeletal muscle (Esubonteng, 2011). The normal range is 0.5 – 2.0 mg/dL for domestic chickens (CDD, 1990). The Creatinine level recorded in the current study was within the normal range; and however significantly ($P < 0.05$) different across the treatment group. This result is in agreement with the report of (Esobonteng, 2011) that Creatinine levels vary significantly in broiler chicken due to diet.

The normal range of Cholesterol in broiler chicken is 87 – 192 mg/ml (Meluzzi *et al*, 1992). Cholesterol value recorded in the current study was significantly ($P < 0.05$) affected across the treatment groups and however lower than the normal range. This is indication that the birds were not at risk of cardiovascular disorder. The higher value for cholesterol and glucose in birds on diet supplemented 0.5g enzyme per kilogram of feed compared to others is an indication of tendencies for fat accumulation as evidenced in the abdominal fat weight of that same group. The result is consistent with (Ranyhon 2001) who reported significant variation in cholesterol levels of weaner pigs fed varying levels of cassava peel meal supplemented with enzyme (Maxigrain®). Cholesterol is synthesized from fats consumed and endogenously synthesized within the cells. High level of cholesterol is an indication of a high risk of cardiovascular disease. The absence or presence of cholesterolaemic effects of dietary components depends on various factors such as breed, sex and age, and also the composition of diet (Toghyani *et al*, 2010). The Glucose value was not significantly ($P > 0.05$) affected across the dietary treatment and however, lower than the normal range (200 – 500 mg/dL) reported by (Thrall, 2007) for broiler chickens for ringed-neck Pheasant as well as for domestic chickens (Coles, 1977).

Table 2. Serum Chemistry of Broiler Chickens Fed Red Sorghum-based Diets Supplemented with Enzymes Complex

	Dietary Treatments					
Parameters	**T_1(0.0g/kg)**	**T_2(0.2g/kg)**	**T_3(0.5g/kg)**	**T_4(0.8g/kg)**	**T_5(1.10g/kg)**	**SEM**
Cholesterol (mg/100ml)	84.71^a	84.59^a	84.93^a	81.21^b	74.26^c	0.59*
TP (g/100ml)	4.75	5.80	5.06	5.70	4.97	0.38^{ns}
Albumin (g/100ml)	2.44	2.95	2.77	3.14	2.69	0.28^{ns}
Globulin (g/dl)	2.31	2.85	2.29	2.56	2.27	0.26^{ns}
Urea (g/dl)	1.74	3.22	2.21	2.01	2.91	0.59^{ns}
Creatinine (Mmol/L)	1.68^a	1.60^a	1.16^b	1.72^a	1.34^{ab}	0.10^{ns}
Glucose (mg/100ml)	109.52	108.93	119.57	100.23	113.17	6.20^{ns}

Means on the same row with different superscripts are significantly different ($P > 0.05$). SEM: Standard error of mean; PCV: Packed cell volume; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell; TP; Total protein

REFERENCES

Adeyemi, O.A., Fashina O.E. and Balogun, M.O. (2000). Utilization of full-fat jatropha seed in broiler diet: Effect on hematological parameters and blood chemistry. *In: Preceding of 25th Annual Conference of Nigerian Society of Animal Production (NSAP,* Umudike, pp: 108-109.

Afolayan, M., Bawa, G.S., Sekoni, A.A., Abeke, F.O. and Inekwe, V.O. (2014). Nutritional evaluation of locust bean (*Parkia biglobosa*) fruit pulp. *Nigerian Society for Animal Production.* March 16-19, 2014. Babcock University, Ilishan-Remo, Ogun State, Nigeria.

Agbede, J.O. and Aletor, V.O. (2003). Evaluation of fish meal replaced with leaf protein concentrate from glyricedia in diets for broiler chicks: Effect on performance, muscle growth, hematology and serum metabolites. *International Journal of Poultry Science.* **2:** 242-250.

Akinola, L. A. F. and Egwuanumku, J. O. (2017). Hematology and Serum Biochemistry of Laying Hens fed Red Pepper *(Capsicum annum* L.*)* as feed additive. *Nigerian Journal of Animal Science.* 2017 (1): 127 – 134.

Association of Analytical Chemist (AOAC), (2010). Association of Analytical Chemist. Official Methods of Analysis 17th Edition AOAC Ic, Arlington, Virginia, USA.

Benerjee, G.C. (2008). "*Textbook of Animal Husbandry.* Bihdhan Chandra Krishi Viswadyala. Oxford and IBH publishing company. PVT Ltd.

Bounous, D. and Stedman, N. (2000). Normal avian hematology: chicken and turkey. *In: Feldman, B.F., Zinki, J.G., and Jain, N.C.* editors. Schalm's veterinary hematology. New York: Willey; 2000. p.1147-1154.

Chernecky, C. and Serger, B. (2008). Laboratory tests and diagnostic procedures. *St. Louis, Saunder Elsvier, 2008.*

Christine, H., Kock, R.A., Henderson, G.M. and Cindery, R.N. (1990). Hematological changes in domestic fowl *(Gallus gallus)* and cranes *(Gruiformes)* with *Mycobacterium avium* infection. *Avian Pathology,* **19**(2), 223-234, DOI:10.1080/0307945900418675.

Clinical Diagnostic Division (CDD), (1990). Veterinary reference guide. *Rochester Eastman Kodak Company, New York, USA 1990.*

Coles, R.H. (1977). *Avian Medicine and Surgery,* Blackwell, Oxford, UK, 2nd edition.

Daramola, O.T. (2017). Hematological Parameters, Serum Metabolites and Enzyme Activities of Broiler Chickens Fed with or without Phytase. *Asian Journal of Advances in Agricultural Research,* **2(4):** 1-7.

Deldar, A. (1994). Blood and Bone Marrow. *In: Vetrinary Histology. 5th Edition. Delman, H.D., Eurel, J. (eds), Williams and Wilkens. A Wavely Company,* **4:** 62-79.

Ehebha, E.T. and Eguaoje, A.S. (2018). Haematological and Serum Biochemical Indices of Broiler Chickens fed Varrying Dietary Levels of Sundried Cassava (*Manihot esculenta*) Peel Meal Supplemented with Enzyme (Maxigrain®).

Esubonteng, P.K.A. (2011). An assessment of the effect of *Moringa oleifera* leaf powder as a nutritional supplement in diet. *Kwame Nkruma University of Science and Technology, Kumasi. Ghana.*

Fischbach, F.T. and Dunning M.B. (2009). A manual of laboratory diagnostic tests. *Philadelphia: Lippincott Williams and Wikkins, 2009.*

Gulland, F.M.D. and Hawkey, C.M. (1990). Avian hematology. *Vet Annual*, **30**: 126-136

Guyton, C. and Arthur, M. D. (1976). Transport of Oxygen and Carbon Dioxide in the Blood and Body Fluid. *Textbook of Medical Physiology* (Fifth ed.). Philadephia, PA: W. B. Saunders. p.556. ISBND- 7216-4393-D. The Respiratory Exchange Ratio is 1:1 when carbohydrate is consumed, it is as low as 0.7 when fat is consumed.

Ileke, K.D., Odeyemi, O.O., Ashamo, M.O. and Oboh, G. (2014). Toxicological and histopathological effect of cheese wood (*Alstonia boonei de wild*) stem bark powder used as protectant against cowpea bruchid (*Callosobruchus maculatus (fab);* Coleoptera: Chrysomelidae)] on albino rats. *Ife Journal of Science.* **16 (1)**: 23-33.

Iyayi, E.A. and Tewe, O.O. (1998). Protein, Urea and Creatinine Levels as indices of Quality of Cassava Diets for Pigs. *Tropical Veterinary.***16:** 59-67.

Kaminski, P., Sparks, T.H., Johnston, A., Bocheminski, M., Kasprzak M. *et al*. (2014). Sex and other sources of variation in hematological parameters of White Stork *(Ciconia ciconia)* chicks. *Journal of Ornithology.* **155(1):** 307-314.

Khoddami, A., H. H. Truong, S. Y. Liu, T. H. Roberts, and P. H. Selle. 2015. Concentrations of specific phenolic compounds in six red sorghums influence nutrient utilisation in broiler chickens. Anim. Feed Sci. Technol. 210:190–199. doi: 10.1016/j. anifeedsci. 2015.09.029.

Liu, H.-X., J.-S. Gong, H. Li, Z.-M. Lu, H. Li, J.-Y. Qian, Z.-H. Xu, and J.-S. Shi. 2015. Biochemical characterization and cloning of an endo β-1,4-β-mannanase from Bacillus subtilis YH12 with unusually broad substrate profile. Process Biochem. 50: 712-721.

Maner, B.S. and Moosavi, L. (2019). Mean Corpuscular Volume (MCV). *In: StatPearls, Treasure Island FL: StatPearls Publishing Company.*

Maton, A., Jean, H., Charles, W.M., Susan, J., Maryanna, Q.W., Daud, L. and Jill, D.W. (1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA. Prentice Hall. ISBN:970-0139811760.

Meluzzi, A., Primiceri, G., Giodani, R. and Fabris, G. (1992). Determination of blood constituents reference values in broilers. *Poultry Science.* **71(2):** 337-345.

Pan, L., Q. H. Shang, X. K. Ma, Y. Wu, S. F. Long, Q. Q. Wang, and X. S. Piao. (2017). Coated compound proteases improve nitrogen utilization by decreasing manure nitrogen output for growing pigs fed sorghum soybean meal-based diets. *Animal Feed Science Technology*. 230:136-142. doi: 10.1016/j.anifeedsci.2017.05.014

Peter, S. (2002). Essentials of Avian Medicine: A practitioner's Guide, 2nd Edition. AAHA Press.

Polat, U., Yesilbag, D. and Eren, M. (2011). Serum biochemical profile of broiler chickens fed diets containing Rosemary and Rosemary volatile oil. *Journal of Biology and Environmental Science.* **5(13):** 23-30.

Ranyhon, S.K. (2001). Animal nutrition in the tropics. 5th edition, Vilas Publishing House PVT, New Delhi, India. 2001; 576.

Ripon, K.D., Saiful, M. and Ashraful, K. (2013). Hematological and Biochemical Profile of *Gallus* Indigenous, Exotic and Hybrid Breeds (*Gallus domesticlus L.*) from Rasjshahi, Bangladesh. *Bangladesh Journal of Zoology*. **41(12):** 135-2013.

Ritchie, B.W., G.J. Harrison and L.R. Harrison, (1994). Avian Medicine: Principles and Application. *Wingers Publishing Inc*., Lake Worth, Florida, USA

Sievered, C.E. (1972). Hematology for Medical Technologist. Lea and Febger, Philadelphia, 4th edition.

Thrall, M.A. (2007). *Hematologiae bioquimica clinica veterinaria*. Philadelphia, Lippincott, Williams & Wilkins, Sao Paulo: Roca, p:582.

Toghyani, M., Ghesari, A., Ghalamkari, G. and Mohammadrezaei, M. (2010). Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed *(Nigella sativa)* and peppermint *(Mentha piperita). Livestock Science,* **129(1):** 173-178.

Tukey, John (1949). "Comparing Individual Means in the Analysis of Variance". *Biometrics*. **5** (2): 99–114. *Journal Storage (JSTOR)*, 3001913

United State Department of Agriculture (USDA). (2019). *National nutrient database for standard reference legacy release: Fullreport (all nutrients) 20067, sorghum grain*. Retrievedfromhttps://ndb.nal.usda.gov/ndb/foods/show/20067?n1=%7BQv%3D1%7D