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Research Article

Haematological and Serum Biochemical Parameters of Broilers Slaughtered at Bukuru Live Bird Market of Jos South Local Government Plateau State Nigeria

Barde Israel Joshua[1*], Habila Stephen Luka[1], Ishaku Leo Elisha[1], Leo Shedua Nyam[1], Isa Suleiman[1], Rimfa Amos Gambo[1], Shekaro Audu[1], Ahmed James Saidu[1], Ugbe Aondona Dominic[1], Barde Dorcas Jummai[2], Dashe Yakubu Gunya[1] and Ngulukun Sati Samuel[1]

[1]Veterinary Pathology Division, National Veterinary Research Institute Vom Plateau, Nigeria

[2]Nassarawa State University Keffi Nigeria

***Corresponding Author:** Barde Israel Joshua, Veterinary Pathology Division, National Veterinary Research Institute Vom Plateau, Nigeria.

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Abstract

Broiler is any chicken (*Gallus gallus domesticus*) that is bred and raised specifically for meat production. Comparatively, they are characterised by fast growth rates, high feed conversion ratio and low levels of activity. They reach slaughter weights between 4 to 7 weeks. Commercial broilers are subjected to very strenuous conditions before slaughter which includes , overcrowding in crates during transportation, deprivation of feed and water. Haematology helps in examination of cellular and fluid protein of blood. However, the serum is the component that is neither a blood cell nor a clotting factor but a part of blood like water that contains substances called antibodies that fight disease. This study seeks to ascertain the health and physiological status of these birds at the time of slaughter by analysing their blood and serum biochemistry. Fifty commercial broiler chickens were randomly selected at the Bukuru live bird market of Jos south Local Government Area in Plateau State, Nigeria. The haematological parameters of the chickens were PCV 27-42%,RBC 2.2-4.0 × 106 µl, WBC 1.0-9.5 × 103µl L 20.50 and all the values were within normal range. Similarly, all the serum biochemical values in this study were wthin normal range for broiler chickens. This signifies that the broilers slaughtered in the study area are apparently healthy and fit for consumption. We recommend humane handling of these birds and improved sanitary conditions at the slaughter premises.

Keywords: Broiler; Haematology; Serum-Biochemistry; Jos; Nigeria

Introduction

A broiler is any chicken (*Gallus gallus domesticus*) that is bred and raised specifically for meat production [1]. Most commercial broilers birds attain table size between four and seven weeks of age, although slow growing breeds achieve this at approximately 14 weeks of age [2]. Due to extensive breeding selection for rapid early growth and the husbandry used to sustain this, broilers are susceptible to several welfare concerns, particularly skeletal malformation and dysfunction, skin and eye lesions and congestive heart conditions. Management of ventilation, housing, stocking density and in-house procedures must be evaluated regularly to support good welfare of the flock. The breeding stock (broiler-breeders) do grow to maturity but also have their own welfare concerns related to the frustration of a high feeding motivation and beak trimming. Broiler birds are usually reared as mixed-sex flocks in large pens under intensive condition. Before the development of modern commercial broiler meat breeds, broilers were mostly young male chickens culled from farm flocks for meat needs. Pedigree breeding began around 1916 [3]. Magazines for the poultry industry existed at this time a crossbred variety of chicken was produced from a male of a naturally double-breasted Cornish strain, and a female of a tall, large-boned strain of white Plymouth Rocks [4]. This first attempt at a meat crossbreed was introduced in the 1930s and became dominant in the 1960s. The original crossbreed broilers were plagued by problems of low fertility, slow growth and disease susceptibility. They were also noted for having very fast growth rates, a high feed conversion ratio, and low levels of activity. Modern commercial broilers are bred to reach a slaughter-weight of about 2 kg in only 35 to 49 days. As a consequence, the behavior and physiology of broilers reared for meat are those of immature birds, rather than adults. Slow growing free-range and organic strains have been developed which reach slaughter-weight at 12 to 16 weeks of age [5]. Typical broilers have white feathers and yellowish skin. Recent genetic analysis has revealed that the gene for yellow skin was incorporated into domestic birds through hybridization with the grey jungle fowl [6]. Modern crosses are also favorable for meat production because they lack the typical "hair" which many breeds have that must be removed by singeing after plucking the carcass. Both male and female broilers are reared for their meat. Broiler behavior is modified by the environment, and alters as the broilers' age and bodyweight rapidly increase. For example, the activity of broilers reared outdoors is initially greater than broilers reared indoors [7]. Once broilers have reached the target table size, they are caught, usually by hand, and packed live into crates for transport to the slaughterhouse for processing into meat. They are usually starved of food and water for several hours before slaughter; this is to reduce meat contamination from faecal materials in the processing hall. The process of catching, loading, and transport and unloading causes serious stress, injury and even death to a large number of broilers [5]. Slaughter is done by hanging the birds fully conscious by their feet upside-down in shackles on a moving chain, stunning them by automatically immersing them in an electrified water bath and exsanguinations by cutting their throats [8]. The poultry industry is facing some challenges in developing countries like Nigeria, one of which is increase in the cost of feed because of high prices of protein and energy sources [9]. Haematology helps in examination of cellular and fluid protein of blood, while the serum is the component that is neither a blood cell nor a clotting factor but a part of blood like fluid that contains substances called anti bodies which fight diseases.

Materials and Method

Study area

The study was carried out in Bukuru market of Jos South Local Government Area, Plateau State, Nigeria located at latitude of 9.7051N and longitude of 8.8142E. The local government headquarter is Bukuru town, which is 15 kilometers south of the state capital.

Materials

Distilled water, universal bottle, polythene bags, labeling sheets, hand gloves, masking tape, spatula, microscope, refrigerator, flask or cooler, ice packs, 70% ethanol (alcohol), capillary tube, glass slide cover slips hematocrit centrifuge.

Study design

Sampling was done using simple random sampling method. A total of 50 broiler chickens were randomly selected from Bukuru market of Jos south Local Government Area of Plateau State.

Sample collection and handling

The blood samples were collected immediately after slaughtered of the live bird and the blood were transferred into EDTA bottles and plain bottles for haematological and serum biochemical analyses respectively. The samples were placed in a sample container labeled and carried in a flask (cooler) with ice packs and taken to haematology laboratory and biochemistry laboratory of National Veterinary Research Institute, VOM for analysis.

Biochemical parameter analysis

Preparation of the sera

Blood was collected in a plane red top venipuncture tubes in evacuate tube and the blood was allowed to clot for serum sample. The blood was centrifuge for five minutes to separate serum from plasma.

Test for total protein

Biuret method was used to determine the serum total protein, as described by Benjamin [10]. Twenty microliter of serum sample was dispense in the tube and 20 microliter of distilled and total protein standard was dispense into two test tube to serve as a blank and standard respectively and 1000 microliter of total protein reagent was added to all the test tube and was mixed and allowed to stand for 30 minutes at 25°C. The optical Density of test and standard was measure against reagent blank at 546nm.

Test for Aspartate amino transferase (AST)

Twenty microliter of the serum sample was dispensed in the test tube including blank (distilled water) and AST reagent 1. One hundred microliter was added and was incubated for 30 minute at 37 °C 100 microliter of AST reagent 2 was added, mixed and was incubated for 20 minutes at 25°C. 0.4 mole sodium hydroxide was added and was allowed for 5 minutes the optical density of test was measure against the reagent blank at 546 nm using spectrophotometer [11].

Test for alanine amino transferase (ALT)

This was done using the method of Buttery., *et al.* [12]. 20 microliter of the serum sample was dispense in the test tube including blank (distilled water) and (ALT) reagent 1. 100 microliter was added mixed and was incubated for 30 minute at 37°C. 100 microliter of (ALT) reagent 2 was added mixed and was incubated for 20 minutes at 25°C. 0.4 mole sodium hydroxide was added and was allowed for 5 minutes the optical density of test was measure against the reagent blank at 546 nm using spectrophotometer.

Test for urea concentration

Exactly, 100 µl of reagent 1 (sodium nitroprusside 6 mMol/l + urease 1 g/l) was dispensed into three separately labeled test tubes: reagent blank, standard and sample containing 10 µl of distilled water, 10 µl urea standard and 10 µl test serum respectively. The mixture was then incubated at 37°C for 10 minutes in a water bath. After the period of incubation 2.5 ml of reagent 2 (phenol 120 mMol/l) was added to the contents of each test tube followed by 2.5 ml of reagent 3 (Sodium hypochlorite 27 mMol/l). The mixture was then incubated for 15 minutes at 37°C in a water bath. The absorbance of the sample (Asample) and that of the standard (Astandard) were then read against the reagent blank using a colorimeter at wavelength of 546 nm and the readings obtained were used for the calculation of the serum urea concentration with the aid of the formula provided by the manufacturer viz: Urea concentration (mg/dl) = A sample × conc. of standard [13].

Test for catalase

To a clean cuvette, one thousand micro litre (1000 µL) of Sample Dilution Buffer was added and placed in the reference cuvette holder. Wavelength was set to 240 nm and the instrument was set at zero. To a clean semi-micro UV cuvette, 950 µL of Working Assay Buffer was added. 50 µL of diluted standard or sample was pipette into the cuvette, mixed as quickly as possible by repeated pipetting (~10 times) with the same pipette tip, or by capping/inverting the cuvette. This was then followed by immediate recording of the absorbance at 240 nm, every 2 second or at the smallest time interval allowed for 0.25 minutes (15 seconds). Note: Because data must be recorded within 30 seconds, do not allow pipetting/mixing time to exceed 15 seconds. Keep the same pace of pipetting/mixing throughout the whole experiment. A practice run with a timer using Sample Dilution Buffer is recommended.

Test for Superoxide dismutase (SOD)

Sample/standard layout was recorded. To each well used for testing, 230 µL of Assay Buffer was added. Next 10 µL of Assay Buffer (for blank) or 10 µL Sample was added. Shake to mix and incubated for 2 minutes.10 µL of Hematoxylin Reagent was added to begin reaction. A multi-channel pipette was preferably used. The mixture was mixed quickly using the instrument's shaker function and immediately began recording the absorbance at 560 nm every 10 seconds or smaller time interval for at least 5 minutes. Note: The reaction rate should be linear for approximately 10 minutes. Undialyzed samples may need longer recording time.

Hematological analysis
Packed cell volume
Procedure: Micro-hematocrit method

Whole blood sample was mixed in EDTA anticoagulant gently by rocking or repeated inversion to ensure proper mixing of the sample and the anticoagulant. Using a clean micro-hematocrit capillary tube, the sample was allowed to flow by capillary attraction through negative gradient up to ¾ capacities. The lower end of the tube was sealed with plastacine or a burning flame. It was centrifuged at a predetermined speed for 5 minutes using the hematocrit centrifuge The PCV was read using the hematocrit Reader. The Result was read and reported in percentage (%).

Total white blood cell
Procedure: Hemo-cytometer method

Test tube for test(s) was labeled. The blood sample was mixed gently by repeated inversion.380µl of Turk's solution was Pipette into test tubes. 20µl of the sample was Pipette into the test tubes and mix gently. By means of a Pasteur pipette the counting chamber was filled and allows to settle for about 2 minutes. It was viewed

using the low power objective (× 10) of microscope in all the 16 small square boxes of the 4 large outer squares

Calculation: $N \times DF \times 10^6 / A \times D$ Where: N = Number of cells counted; DF = Dilution factor; A = Area of chamber; D = Depth of chamber; 10^6 = Conversion factor

Red blood cell count

Procedure: Hemocytometer method

Test tube(s) for the test sample(s) were labeled. The blood sample was mixed gently by repeated inversion. 4000µl of formal citrate solution was Pipette into the test tube(s). It was allowed to stand on the bench for about 5 minutes. The chamber was filled by means of a Pasteur pipette and allows to settle for 2 minutes. The number of cells in the central squares was counted using a low power objective, (5 squares).

Calculation: $N \times DF \times 10^9 / A \times D$

DF = Dilution factor; A = Area of chamber; D = Depth of chamber; 10^6 = Conversion factor

Method: Thin smear and Leishman's staining technique

Procedure for thin film making

Drop of well mixed blood about 1cm was placed away from the edge of a clean grease free slide. A spreader was placed in front of the blood at an angle of 45º and allows the blood spread to across the edge of the spreader. The spreader was moved forward, quickly and swiftly. The smear was allowed to air dry. It was labeled with the appropriate lab number and date and was stained.

Leishman's staining

Procedure: Leishman's staining technique

The air dried smear was placed on a staining rod. It was Flood with a volume of filtered Leishmann's stain. It was allowed to fix for 2 minutes. It was dilute with an equal amount of buffered distilled water (PH 6.8) and allow to stain for 8 minutes. Excess stain was washed off and differentiates until salmon-pink. It was allowed to air dry and viewed using the oil immersion objective of a microscope.

Counting Technique

Examine the stained thin blood film using the oil immersion objective (× 100). Bring the cells to focus and report counts as percentage of the total White Blood Cell count as indicated below:

Neutrophils/Heterophils... %

Lymphocytes...

% Monocytes..

% Eosinophils...

% Basophils... %

The data analysis was done using Graphpad prism 6.0 as mean± SD. Statistical comparison between groups was made using one-way analysis of variance (ANOVA) with post hoc Bonferroni multiple comparison Test to identify differences in means where appropriate. $P < 0.05$ was taken as statistically significant.

Results

Age (weeks)	8weeks	9weeks	10weeks	11weeks	12weeks
PCV	42.20 ± 8.24^{a}	31.17 ± 9.29^{bc}	39.10 ± 12.33^{ab}	35.92 ± 8.07^{abc}	28.80 ± 7.16^{c}
HB	10.070 ± 2.745^{a}	10.32 ± 3.110^{ab}	10.03 ± 4.11^{abc}	10.923 ± 2.740^{c}	9.60 ± 2.38^{bc}
RBC	2.220 ± 0.698^{a}	2.533 ± 0.708^{a}	2.510 ± 0.808^{a}	2.492 ± 0.698^{a}	2.440 ± 0.451^{a}
WBC	4.750 ± 1.095^{a}	5.400 ± 0.951^{a}	5.190 ± 1.399^{a}	5.123 ± 1.178^{a}	5.760 ± 1.108^{a}
N/H	58.000 ± 2.906^{a}	56.00 ± 4.49^{a}	57.90 ± 3.35^{a}	57.77 ± 3.77^{a}	55.00 ± 5.24^{a}
L	21.100 ± 2.601^{a}	23.33 ± 4.74^{a}	20.900 ± 3.035^{a}	21.62 ± 3.95^{a}	24.80 ± 5.07^{a}

Table 1: Haematological profile.

Parameter Age in weeks (mean ± SD).

Mean along that do not share a superscript are significant ($p < 0.05$).

Normal range for avian

PCV = 27-42, RBC = 2.2-4.0, WBC = 1.0-9.5, HB = 7.0-11.0, N/H = 50-65, L = 20-50, E = 0-4, B = 0-2

Key

PCV = Pack Cell Volume, RBC = Red blood cell, WBC = white blood cell,

HB = Heamoglobin, N/H = Neutrophils/Heterophils, L = Lymphocytes, E = Eosinophils, B = Basophils

Age (weeks)	8weeks	9weeks	10weeks	11weeks	12weeks
AST	55.98±8.74^{a}	51.71±8.95^{a}	52.92±10.95^{a}	53.42±8.81^{a}	49.02±9.38^{a}
ALT	7.100±1.197^{a}	7.417±0.900^{a}	7.600±0.966^{a}	7.615±0.870^{a}	8.000±1.225^{a}
ALP	147.10±25.87^{a}	146.66±19.51^{a}	152.98±23.99^{a}	150.93±23.43^{a}	151.21±22.08^{a}
TP	26.168±2.701^{a}	26.87±5.58^{a}	29.29±7.37^{a}	29.53±7.25^{a}	23.98±3.41^{a}
UREA	0.3485±0.2428^{a}	0.5024±0.2586^{a}	0.3342±0.1764^{a}	0.4732±0.2904^{a}	0.3260±0.2125^{a}
SOD	36.36±4.51^{a}	36.68±4.42^{a}	36.13±3.62^{a}	37.39±4.08^{a}	37.957±1.986^{a}
CATALASE	26.46±8.48^{a}	21.77±4.68^{a}	21.01±5.48^{a}	23.03±8.54^{a}	22.15±3.32^{a}

Table 2: Biochemical parameter

Parameter age in weeks (mean ± SD).

Mean along that do not share a superscript are significant ($p < 0.05$)

Key: AST = aspartase amino transferase, ALT = alanine amino transferase, ALP = alkaline phosphatase, SOP = Superoxide dismutase, TP = Total protein

Discussion

The normal range of haematological parameters in chicken are PCV 27-42%, RBC 2.2-4.0 × 10^{6} µl, WBC 1.0-9.5 × 10^{3}µl L 20.50. All the values obtained for the haematological parameters in this study were within their normal range (Table 1). This implies normal physiological status of the birds as concur by Etim, (2010) who reported that haematological parameters are good indicators of the physiological status of animals [14,15].

The function of RBC is to transport oxygen from the lung to tissue and remove carbon dioxide from the tissue to lung in the body via haemoglobin. The test for RBC can help diagnose anemia, and other conditions affecting red blood cells [16,17]. Red blood cell indices are blood tests that provide information about the haemoglobin content and size of red blood cells. Abnormal values indicate the presence of anemia and which type of anemia it is [16]. The RBC range of chickens can be affected by sex and diet of birds. All the Heamoglobin levels (HB/Hbg) Hb levels are within the normal range of 7.0-11.0g/dl except for those in the age bracket of 8 weeks and 10 weeks which appear a bid higher than normal indicating an above normal level of the iron containing protein in red blood cells. According to Merck Manual [18], higher than normal numbers of RBC may be due to congenital heart disease, dehydration (such as from severe diarrhea), low blood oxygen levels (hypoxia), polycythemia vera among other. Lower than normal numbers of RBCs may be due to anemia, bone marrow failure (for example, from radiation, toxins or tumour), erythropoietin deficiency (secondary to kidney disease), haemolysis (RBC destruction due to blood vessel injury or other causes) hemorrhage (bleeding), malnutrition, nutritional deficiencies or iron, copper, folate, vitamin B12, vitamin B6, over dehydration, pregnancy among others. Some drugs also decrease the RBC count [16,17]. Similarly high concentration of haemoglobin may be caused by polycythemia vera, lung disease, emphysema and heart disease [19]. Haemoglobin deficiency decreases blood oxygen carrying capacity. Haemoglobin deficiency is, in general, strictly distinguished from hypoxemia, defined as decreased partial pressure of oxygen in the blood. Other common causes of low haemoglobin include loss of blood, nutritional deficiency, and bone marrow problems among others. High haemoglobin levels may be caused by exposure to high attitudes, dehydrations and tumours [19].

The white blood cell count (RBC) also with normal range of 1.0- 9.5 100µl. the WBC aids the body from pathogen and to protect and caratenoids build up immunity other report also indicate that vitamin A and carotanoid increase the immune response in chicken and also reduce common avian infections b such as coccidiosis and lesion that cause significant losses to poultry farmers [20]. All the birds slaughtered were within the normal range of total protein values. A serum protein is synthesized in the liver. It is

responsible for transporting insoluble substance in the blood and aids to maintain oncontic pressure [21]. A higher concentration of albumin usually donates dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection [22]. Total protein values in the female usually higher than the male birds. This could be attributed to oestrogen induced in globulin in preparation of the female birds' body for egg laying. Liver enzymes High total protein level could indicate dehydration or a certain type of cancer, such as multiple myeloma that causes protein to accumulate abnormally. In table 2 above showed low total protein it may be that protein was not being digested or absorbed properly. Liver enzymes, namely the alanine transaminase albumin (ALT). Alkaline phorphatase (ALP) and aspartate transaminase (AST) are important in the determination of the proper functioning of the liver [23] these enzymes are present in negligible concentration. An increase in the concentration of these enzymes may be because of damaged or diseased cells which demote the status of the liver function. The high concentration of AST in the table 1 at 8weeks 12weeks of age could be on indication of damage to the liver [24] .Vitamin A deficiency increases the level of AST and ALT [25]. Creatinine is used to determine the status of the kidney. The functions of the kidney include excretion of waste products resulting from protein metabolism and muscles contraction [26].

Conclusion

The findings from this study indicate that the haematological and biochemical parameters broilers chicken slaughtered in the study area are within normal values, signifying the broiler birds are healthy. Therefore it is important to know the blood and serum profile of the broilers chicken slaughtered in the market for exact exposition of the health status thus information relating to the blood profile of broiler chicken will not only be useful for diagnosis and well-being of broiler chickens but also can help to improve several new strains of broiler which are genetically resistance to poultry diseases. That can also be used for genetic improvement of industrial and indigenous poultry birds. The results on the biochemical and haematological profile indicate that there were no significant difference found in the broilers examined. The overall results of tested broilers showed that all the mean values for each haematological and serum biochemical profiles possessed normal values for normal growth of the broiler chicken.

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