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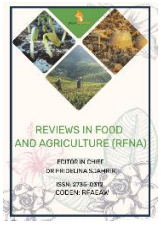

RESEARCH ARTICLE

EFFECT OF DIETS CONTAINING BIOCHAR AND CLAY ON THE SERUM BIOCHEMISTRY, HEMATOLOGICAL INDICES AND TOTAL BACTERIA VIABLE COUNT IN BROILER

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ABSTRACT

The study was conducted to determine the effect of diets containing biochar and clay on the hematological indices, serum biochemistry and total bacterial viable count of broiler. Ninety six broilers were allocated to four treatments diets having three replicates of eight broilers in a completely randomized design. Data were collected on red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), hematocrit (HCT), platelets count, lymphocytes, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) levels, creatinine, total protein, total cholesterol, albumin, globulin, urea, Alanine amino transferase (ALT), Aspartate Aminotransferase (AST) and triglyceride in broiler chickens. Results showed that there were significant ($p < 0.05$) differences in all the serum indices and hematological parameters determined in this study. Treatment 4 had the highest white blood cell count, lymphocytes, hematocrit but the lowest creatine and bacteria viable count while treatment 1 had the lowest platelets but the highest bacteria viable count. The T4 diets containing clay and biochar at 2.5% inclusion levels each improved the indices of broiler better than the other treatments used in this experiment. This implies that T4 diets had the greatest capacity to bind the toxins contained in the feed.

KEYWORDS

toxin binder, blood profile, birds, bacteria count

1. INTRODUCTION

The population of the world was projected to increase to 10 billion people by 2050, but there is the need for adequate and nutritious food supply for the teeming human population, through sustainable Agricultural production systems (FAO 2009). Poultry production is one sustainable means proposed for the attainment of this goal of global food sufficiency. Sustainable poultry production is also a key measure in alleviating poverty and addressing the menace of protein insufficiency among many households in sub-Saharan Africa, particularly, Nigeria (Ani, 2007). Poultry are fast growing birds that yield products containing essential amino acids which the body requires for normal metabolic activities (Ibe, 2004). Poultry have potential to give high turnover rate on investment and are also efficient converters of feed to animal protein (Oluyemi and Roberts, 2000). Large percentage of the world population depends on livestock for their livelihood. This has contributed to the improving human nutrition, provided food such as (eggs and meat) with better quality nutrients; generated revenue for women by improving their capacity to cope and reducing economic vulnerability. It also provides farm yard manure for vegetable garden and crop production. Poultry products like chicken and turkey are rich in essential nutrients and can help to reduce diseases associated with deficiencies in critical dietary minerals, vitamins and amino acids.

However, the presence of mycotoxins in feed ingredients and in the environment remains one of the greatest challenges that poultry farmers and the entire feed industry face (Lee et al., 2013). Mycotoxins can cause several toxic effects in poultry animals. Mycotoxins can suppress the immunity of poultry species with their action mechanism based on enzyme inhibition to reduce protein synthesis and consequently the immune response. The combined effect of several mycotoxins can be more severe than when the animal suffers from the effect of one mycotoxin. The mycotoxins that affect the immune system of poultry are mainly Aflatoxins. Over the last few decades, the use of dietary inclusions, majorly, toxin binders have been explored with the aim of increasing efficiency of feed utilization in poultry (Hamasalim, 2016). Notwithstanding, numerous health complications are often associated with the use of antibiotic growth promoters in poultry nutrition. Hence there is the need for natural and locally available feed additives that can bind toxins at a cheaper and more environmentally friendly manner.

Biochar, a carbonaceous material produced from the pyrolysis of organic biomass, has gained attention as a potential feed additive due to its reported benefits, including improved gut health, toxin adsorption, and reduced environmental impact (Yin et al., 2020). In pyrolysis, organic materials such as wood chips, leaf litter or wheat straw, rice husk, cow manure and other agricultural residue are burned in a container with very little oxygen. In the process of pyrolysis, the organic material changes into biochar, a carbonaceous material that remains in a stable form which cannot easily be released into the atmosphere. Clay minerals such as bentonite and kaolin have also been explored as feed additives due to their ability to bind toxins, enhance nutrient utilization, and improve the

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immune response in poultry (Wu et al., 2018). The inclusion of biochar in broiler diet improve growth performance, feed efficiency, carcass trait and meat quality (Zhang et al., 2019). Also the inclusion of clay to broiler diet positively influences body weight gain, feed conversion ratio and meat quality (Toghyani et al., 2015). Addition of biochar in poultry nutrition has been reported to rapidly decrease the incidence of diarrhoea, eliminate allergies and ameliorate the detrimental effect of mycotoxins in feeds (Marie, 2013).

Several studies have investigated the individual effects of biochar and clay on broiler performance and health parameters. However, limited research has focused on the combined effects of this mycotoxin binders on hematological indices, serum biochemistry and total bacterial viable count in broiler chickens. Understanding the hematological, serum biochemistry and total bacteria viable count in broiler responses to diets containing biochar and clay could provide valuable insights into their potential benefits for poultry health and performance. This knowledge will contribute to optimizing the use of broiler diets and promoting sustainable and efficient poultry production practices.

2. Materials and Methods

2.1 Location and duration of Study

The study was conducted at the poultry unit of the Department of Animal Science farm, Nnamdi Azikiwe University Awka, Ifite Ogwari campus, Anambra State. Ifite-Ogwari town is located 45 kilometres from Awka and is situated between latitude 6.6041° North and longitude 6.9507° East, at an elevation of 91 meters above sea level. The annual and monthly total rainfall averages are 5798.78 mm and 1739.62 mm, respectively (Ifeka and Akinbobola, 2015). The minimum and maximum temperatures are 25.4°C and 30.6°C respectively with a tropical vegetation forest (Nimet, 2014). The experiment lasted for eight weeks (56 days).

2.2 Experimental birds and management

Ninety-six-day-old broiler chicks (Ross 308) were purchased from a reputable hatchery in Ibadan, Oyo State, Nigeria. The birds purchased were randomly allotted to four dietary treatments groups of T1= 0% biochar and clay, T2= 5% biochar, T3= 5% clay, and T4= 2.5% biochar and 2.5% clay, with twenty-four (24) birds per treatment where each treatment was further replicated three (3) times with eight (8) birds per replicate in a Completely Randomized Design (CRD). The birds were raised on a deep litter system partitioned into four treatments of three replicates each. Feed and water were supplied *ad libitum*.

2.3 Experimental Diets

Four starter diets with biochar (BC) and clay (C) included at 0% (T1), 5% BC (T2), 5% C (T3) and 2.5% BC plus 2.5% C (T4), were used during the starter phase of the experiment. Four finisher diets having biochar (BC) and clay(C) inclusion at 0% (T1), 5% BC (T2), 5% C (T3) and 2.5% BC plus 2.5% C (T4), were also used during the finisher phase of the study.

The four finisher diets contained 20% crude protein. The ingredient compositions of the experimental diets are shown in Table 1.

Table 1: Composition of The Experimental Diet At Finisher Phase

Ingredients	**Treatments**			
	T1	**T2**	**T3**	**T4**
Maize	60.00	57.00	57.00	57.00
Soyabeans meal	18.00	18.00	18.00	18.00
Fish meal	3.00	3.00	3.00	3.00
Biochar	-	5.00	-	2.50
Clay	-	-	5.00	2.50
Wheat Bran	5.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00
Groundnut cake	10.00	10.00	10.00	10.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
V/M premix	0.25	0.25	0.25	0.25
TOTAL	**100**	**100**	**100**	**100**
ME (Kcal/kg)	2897.06	2768.98	2768.98	2768.98
CP (%)	20.43	19.85	19.85	19.85

2.4 Parameters Measured and Parameters Determined

The parameters measured include hematological indices such as haemoglobin concentration (hb), red blood cell count (rbc), white blood cell count (wbc), haematocrit (hct), mean corpuscular volume (mcv), mean corpuscular haemoglobin (mch), mean corpuscular haemoglobin concentration (mchc), lymphocyte, platelets, creatinine, total protein, total cholesterol, albumin, globulin, urea, alanine amino transferase(ALT), aspartate amino transferase(ast) and triglyceride.

2.5 Experimental Design and Model

The experiments were carried out using completely randomized design (CRD). The experimental model of the design is as follows:

$Xij = \mu + Ti + \sum ij$

Where Xij = any observation or measurement taken

μ = population mean

Ti = Treatment effect

$\sum ij$ = Experimental error

i = number of treatments

j = number of replicates

2.6 Haematology analyses

At the 8th week, the birds were tested on hematology to see if the biochar and clay have any effect on them. Three birds per replicate were randomly selected and blood samples collected from the wing veins of each bird using sterilized syringe and emptied into sterilized bottles containing EDTA (Ethylene diaminetetracetic acid) for haematological analysis. Haematological parameters that were determined include haemoglobin concentration (Hbn), Haematocrit (Hct), white blood cell count, red blood cell (Rbc) count and the method used in determining them were:

2.6.1 Haematocrit Determination

The micro-haematocrit method was used according to (Cheesbrough, 2000). Capillary tubes were filled up to two-thirds to three-quarters full with well mixed oxalated venous blood. One end of the tube was sealed with clay and placed in a microhaematocrit centrifuge at 12,000rpm for 5min. The tubes were placed in microhaematocrit reader and were read following the manufacturer's instructions.

2.6.2 Hemoglobin Concentration Determination

This was determined using Sahli-Hellige method of (Cheesbrough, 2000). 20 µl of Blood was sucked up into the capillary pipette up to the mark and

the blood blown into the measuring tube containing 0.1N hydrochloric acid, a good mixture of the liquid was achieved by repeated sucking and blowing after about 1mins. Distilled water was added by water pipette and mixed with a glass stirrer until the color of the solution matched the color of the test rods. The result was read 3mins after adding the blood sample to the HCL.

2.6.3 Determination of Total white blood cell count and Differentials

The method of a study was used to determine the TWBC and WBC Differentials; the absolute number of each white cell was counted microscopically using an improved Neubauer ruled counting chamber (haemocytometer) (Cheesbrough, 2000). Diluting fluid of 0.38ml was dispensed into a small tube, Twenty microlitre (20ul) of well mixed EDTA anticoagulated venous blood was added in the tube and mixed, the counting chamber was assembled, the cover glass was slid into position over the grid areas and pressed down on each slide until rainbow colors (Newtons ring) were seen. The diluted blood sample was re-mixed using capillary tube held at an angle of about 45^{o}, one of the grids of the chamber was filled with the sample, and care was taken not to overfill the area. The chamber was left undisturbed for 2mins to allow the white cells time to settle. The underside of the chamber was dried and placed on the microscope stage. Using the 10x objective with the condenser iris close sufficiently to give good contrast, the ruling of the chamber and white cells was focused. The cells were focused until they appeared as small black dots. The cells in the four large corner squares of the chamber marked W1 W2 W3 W4 (Total area of 4mm^{2}) was counted. Cells lying on the lines of two sides of each large square were also counted, the number of white cells per liter of blood was calculated thus:

Divide the total number of cells counted by 2, divide the number obtained by 10 and then the number obtained x 10^{9} becomes the white cell count.

The absolute number of each white blood cells was calculated by multiplying the number of each cell counted (expressed as a decimal fraction) by the total WBC Count.

2.7 Serum Biochemistry analysis

At the 8th week, the birds were tested on serology to see if biochar and clay have any effect on them. Three birds per replicate were randomly selected and blood samples were collected from the brachial wing veins of each bird using sterilized syringe and emptied into plain sample bottles for serological analysis. The BCG method as described by a group was used for albumin determination (Dumas et al., 1971). Total protein determination was done using the Biuret method. Creatinine content was determined using commercial kits from Randox Laboratories using colometric method described (Bartels and Bohmer, 1972). The method of a group study was adopted for total cholesterol determination (Allain et al., 1974). The cholesterol content of the serum was measured at 546nm using a UV-Visible spectrophotometer. The method described by Fossati and Prencipe was used to determine the triglyceride content (Fossati and Prencipe, 1982). The triglyceride content of the serum was measured at 546nm using a UV-Visible spectrophotometer. Colorimetric method was used to determine the blood urea concentration. Randox kit was used for AST activity determination based on the modified method of (Reitman and Frankel, 1957). Randox kit was also used for ALT activity determination based on the modified method of (Reitman and Frankel, 1957).

2.8 Collection and processing of test ingredients

Biochar was bought at Onitsha market while clay was got from Ifite-ogwari river bank and incorporated into the feed. The clay was air dried. The dried biochar and clay were crushed using mortar and pestle to 2mm sieve particle size to enable birds pick feed easily while feeding.

2.9 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using statistical package for social science (SPSS) and the significant means were separated using Duncans New Multiple Range Test as described (Steel and Torrie, 1980).

3. RESULTS

3.1 Hematological Indices of Broilers Fed Diets Containing Biochar and Clay at Finisher Stage

The hematological indices of broilers fed diets containing biochar and clay at finisher stage are presented in Table 2.

Table 2: Hematological indices of broiler chickens fed varying dietary levels of Biochar and clay at finisher stage

Parameters			**Treatments**		
	T1	**T2**	**T3**	**T4**	**P values**
White blood cell count	101.77^{c}	105.33^{b}	92.33^{d}	116.33^{a}	0.000
Lymphocytes	81.67^{bc}	80.67^{c}	81.97^{b}	83.47^{a}	0.004
Hemoglobin	10.97^{a}	10.80^{b}	9.87^{c}	10.77^{b}	0.000
Red blood cell count (x10^{-3})	2.06^{b}	2.13^{a}	1.54^{d}	1.84^{c}	0.000
Hematocrit	27.33^{c}	26.67^{d}	30.57^{b}	32.37^{a}	0.000
Mean corpuscular volume	133.60^{c}	125.50^{d}	199.23^{a}	177.23^{b}	0.000
Mean corpuscular hemoglobin	53.80^{c}	51.07^{d}	64.33^{a}	58.77^{b}	0.000
Mean corpuscular hemoglobin concentration	40.27^{b}	40.47^{a}	32.30^{d}	33.20^{c}	0.000
Platelets	36.33^{d}	43.00^{c}	338.00^{a}	196.33^{b}	0.000

Means along the same row with different superscripts are significantly different (p < 0.05). T1 - Control Diet, T2 - Treatment with biochar (5%), T3 - treatment with clay (5%), T4 - treatment with clay (2.5%) and biochar (2.5%).

The table shows that there were significant (P<0.05) differences among the treatment means of the broilers in all the haematological parameters determined.

There were significant (P<0.05) differences in the white blood cell count in treatment 1, 2,3 and 4, with treatment 4 having the highest white blood cell count compared to the values from the birds on treatment 1, 2 and 3. The white blood cell count of birds in treatment 3 were significantly (P<0.05) lower than the white blood cell count in treatment 1, 2 and 4.

There were significant (P<0.05) differences in the lymphocyte value in treatment 1, 2, 3 and 4. Broilers fed with treatment 4 had the highest lymphocytes value. The lymphocyte value of birds in treatment 2 were significantly (P<0.05) lower than treatment 1, 3 and 4.

There were significant (P<0.05) differences in the hemoglobin level between treatment 2 and 4, with treatment 1 having the highest hemoglobin level than the values of the hemoglobin levels of the birds on treatment 2, 3 and 4. The hemoglobin level of birds in treatment 3 were significantly (P<0.05) lower than the hemoglobin levels in treatment 1, 2 and 4.

Broilers fed with treatment 2 diet had the highest red blood cell count value, which was significantly (P<0.05) different from birds on treatment 1, 3 and 4. The red blood cell of birds in treatment 3 were significantly (P<0.05) lower than the red blood cell count of broilers in treatment 1, 2 and 4.

There were significant (P<0.05) differences in the hematocrit value in treatment 1, 2, 3 and 4. Birds on treatment 2 had low hematocrit value which was significantly (P<0.05) lower than the hematocrit values of birds on treatment 1, 3 and 4. Birds on treatment 4 had the highest hematocrit value which was significantly (P<0.05) higher than the hematocrit value of birds on other treatments groups.

There were significant (P<0.05) differences in the mean corpuscular volume value in treatment 1, 2, 3 and 4. The mean corpuscular volume

value of birds in treatment 2 were significantly (P<0.05) lower than treatment 1, 3 and 4. Birds on treatment 3 had the highest mean corpuscular volume value which was significantly (P<0.05) higher than the mean corpuscular volume value of birds on other treatment groups.

There were significant (P<0.05) differences in the mean corpuscular hemoglobin value in treatment 1, 2, 3 and 4. The mean corpuscular hemoglobin value of birds in treatment 2 were significantly (P<0.05) lower than treatment 1, 3 and 4. Birds on treatment 3 had the highest mean corpuscular hemoglobin value which was significantly (P<0.05) higher than the mean corpuscular hemoglobin value of birds on other treatments groups.

There were significant (P<0.05) differences in the mean corpuscular hemoglobin concentration value in treatment 1, 2, 3 and 4. The mean corpuscular hemoglobin concentration value of birds in treatment 3 were significantly (P<0.05) lower than treatment 1, 2 and 4. Birds on treatment 2 had the highest mean corpuscular hemoglobin concentration value which was significantly (P<0.05) higher than the mean corpuscular hemoglobin concentration value of birds on other treatments groups.

There were significant (P<0.05) differences in the platelets value between treatment 1, 2, 3 and 4, with treatment 3 and 4 having the highest value, followed by treatment 2 which was significantly (P<0.05) higher than treatment 1.

3.2 Serum biochemistry of broilers fed diets containing biochar and clay

The results of the serum biochemistry of broilers fed diets containing biochar and clay is presented in table 3. The results show that there were significant differences (p<0.05) in all the serum biochemical parameters.

Table 3: Serum biochemistry of broilers fed diets containing biochar and clay

Variables	T1	T2	T3	T4	P value
Creatine	0.57^{c}	0.83^{b}	0.93^{a}	0.48^{d}	0.000
Total protein	3.64^{d}	17.66^{b}	18.15^{a}	10.09^{c}	0.000
Total cholesterol	325.34^{a}	128.33^{d}	182.26^{c}	250.67^{b}	0.000
Albumin	1.72^{a}	0.69^{c}	1.32^{b}	1.33^{b}	0.000
Globulin	1.91^{c}	16.97^{a}	0.51^{d}	8.76^{b}	0.000
Urea	11.86^{d}	29.58^{a}	15.77^{c}	19.83^{b}	0.000
AST	157.60^{b}	165.10^{a}	108.40^{d}	122.17^{c}	0.000
ALT	40.70^{c}	66.17^{a}	36.50^{d}	61.30^{b}	0.000
Triglyceride	198.24^{c}	203.85^{a}	144.35^{d}	198.88^{b}	0.000

Means with same superscript are not significantly (p>0.05) different (AST- Aspartate Aminotransferase, ALT- Alanine Aminotransferase)

Broilers fed with treatment 3 diet had the highest creatinine value of 0.93 while those fed with treatment 4 diet had the lowest creatinine value of 0.48. The highest value of total protein of 18.15 was recorded in broilers fed with treatment 3 diet while the lowest value of 3.64 was recorded in broilers fed with treatment 1 diet. Total cholesterol was highest in broilers fed with treatment 1 diet with a value of 325.34 and lowest in broilers fed with treatment 2 diet with a value of 128.33. Broilers fed with treatment 1 diet had the highest value of 1.72 of albumin while those fed with treatment 2 diet had the lowest value of 0.69. Globulin was highest in broilers fed with treatment 2 diet with a value of 16.97 and lowest in those fed with treatment 3 diet with a value of 0.51. Broilers fed with treatment 2 diet had the highest urea concentration of 29.58 while the lowest urea concentration was from those fed with treatment 1 diet with a value of 11.86. Broilers fed with treatment 2 diet was highest in Aspartate Aminotransferase with a value of 165.10 while those fed with treatment 3 diet had the lowest value of 108.40. Broilers fed with treatment 2 diet was highest in alanine amino transferase with a value of 66.17 while those fed with treatment 3 diet had the lowest value of 36.50. Broilers fed with treatment 2 diet had the highest triglyceride value of 203.85 while those fed with treatment 3 diet had the lowest triglyceride value of 144.35.

3.3 Bacterial Load in the Blood of Broiler Chickens fed diets containing biochar and clay

The impact of diet containing biochar and clay on the bacterial load in the bloodstream of broiler chickens was investigated. The findings from this investigation are presented in Table 4.

Table 4: Bacterial Load in the Bloodstream of Broiler Chickens

Parameters	T1	T2	T3	T4	P-value
Bacteria colony	151.33^{a} ± 0.88	55.00^{c} ± 0.88	67.00^{b} ± 0.88	41.00^{d} ± 0.58	0.000
Bacteria count	1510.33^{a} ± 0.33	557.00 ^{c}± 0.58	680.00 ^{b}± 0.58	407.00 ^{d}± 0.58	0.000

^{a,b,c} Means bearing different letters of superscript within the same row differ significantly (P<0.05). key: T1= biochar and clay 0%, T2= biochar 5%, T3= clay 5% and T4= biochar and clay 2.5%+2.5%

There were significant differences (p<0.05) on the bacterial colony and bacterial count in the blood of broiler fed diets containing biochar and clay presented in Table 4. The highest bacterial colony count of 151.33 was observed in the control group (T1) with no biochar or clay while the group treated with a combination of 2.5% biochar and 2.5% clay (T4) had the lowest colony count of 41.00. This trend was similarly reflected in bacteria count with the control group (T1) showing the highest count of 1510.33, and T4 having the lowest of 407.00. The control group (T1) exhibited the highest colony count, serving as a baseline, while treatments with biochar at 5% (T2) and clay at 5% (T3) demonstrated a substantial reduction, indicating the individual toxin binding effects of these additives.

4. Discussion

4.1 Hematological indices of broiler chickens fed varying dietary levels of Biochar and clay at finisher stage

The results showed that there were significant (P<0.05) differences between the treatment means in all hematological parameters measured. Values for each parameter ranged as follows: Hbn (9.87- 10.97g/dl), Rbc (1.54- 2.13×10^{12}/L), wbccount (92.33 - 116.33×10^{9}/L), Hct (26.67 - 32.37%), Mcv (125.50- 199.23FL), McH (51.07 - 64.33Pg/cell), McHc (32.30 - 40.47g/dl), Lympho (80.67- 83.47%), Platelet (36.33 - 338.00×10^{9}/L). The normal range for hemoglobin and red blood cell counts as reported by Bounous and Stedman were 7-13 g dl–1 and 2.5-3.5× 106 µl, respectively. The range of hemoglobin and red blood cell determined from the experiment were within the normal range of a healthy bird as reported by (Bounous and Stedman, 2000). Hemoglobin is the oxygen carrying protein in the red blood cell. Hemoglobin levels on the other hand are a direct reflection of the amount of oxygen in the blood. The rise in hemoglobin level is observed in conditions of dehydration, chronic obstructive pulmonary disease while a decrease results in anemia, blood loss, liver disease etc. Red blood cell transports oxygen to animal tissues for the oxidation process to release energy and transport carbon-dioxide out of the tissues and the manufacture of haemoglobin. The values of hemoglobin and red blood cell count show that the broilers were not anemic during their growth period. The normal range of lymphocyte in broiler is (45-70%), as reported by a group researcher which is lower than the lymphocyte values as determined in this experiment (Aeangwanich et al., 2004). It has been established that an animal's health can be measured from the total lymphocyte count because increased lymphocyte count represents the body's immune response while decreased lymphocyte count may mean non-existence of infection.

White blood cell count normal range in broilers is between 12 and 30 × 10^{3} µl which were lower than the white blood cell count obtained in this experiment (Bounous and Stedman, 2000). White blood cell defends the body against invasion by foreign organisms and to supply antibodies for immune response. The major cause of an increase in the white blood cell count is the normal response of the body to an infection, certain drugs and release of immature or abnormal white blood cell from bone marrow into the blood. Animals with high white blood cell values can generate antibodies and a high degree of disease resistance. The higher white blood cell value in the study is an indicator of stress which could be nutritional stress as reported (Minka et al., 2007).

The mean corpuscular volume was above the normal level of 90-140 fL, as reported by (Bounous and Stedman, 2000). The mean corpuscular volume was used to calculate the average erythrocyte size, high mean corpuscular volume indicate various health conditions such as nutritional deficiencies, certain diseases, or issues with red blood cell production. The mean

corpuscular hemoglobin was above the normal level of 33-47 pg/cell as reported by (Bounous and Stedman, 2000). High mean corpuscular hemoglobin indicates an increased average amount of hemoglobin within each red blood cell. This can be influenced by various factors, including nutritional imbalances, certain diseases, or genetic factors.

The mean corpuscular hemoglobin concentration in treatment 1 and 2 were above the normal level of 26-35 g/dl as reported by (Bounous and Stedman, 2000). High mean corpuscular hemoglobin concentration indicates an increased average amount of hemoglobin within each red blood cell. This can be influenced by various factors, including dehydration, certain diseases, or genetic factors. The high values of mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration may be due to the environment which includes the test ingredients. The values for haematocrit falls within the normal range of 35.90 - 41.00% as reported and between 22 and 35% as reported by (Wikivet, 2013; Bounous and Stedman, 2000). The platelet value of treatment 3 falls within the normal range of 300 - 800 as reported (Merck, 2012).

According to a study, the fall in platelet count was associated with high values of bleeding and clothing times with bleeding problems as consequences (Ijioma et al., 2014). Low numbers of circulating platelets as well as platelet dysfunction increase the risk of bleeding. The values of hemoglobin, red blood cell count and heamatocrit were within the normal range, while white blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration were higher than the normal range as reported by (Bounous and Stedman, 2000). Results show that T4 hematological indices were within the normal range of healthy chickens followed by T3, T2 and then T1. This is in accordance with the report of a group researchers that the inclusion of clay (0.05%) and charcoal (3%) bind and immobilize the mycotoxins in the gastrointestinal tract, thereby reducing the absorption of toxins into the gastrointestinal tract in poultry (Lee et al., 2023).

4.2 Serum biochemistry of broilers fed diets containing biochar and clay

The results from the experiment indicated that feeding broilers with diets containing biochar and clay had significant ($p<0.05$) difference on the serum biochemical parameters of the broilers. It was observed that the total protein level in the broilers fed T2 diets, T3 diets and T4 diets were significantly ($p<0.05$) higher than that of the control group(T1) and also higher than the standard range of total protein (3.5 - 5.5g/dL) in broilers serum biochemistry. The control group was within the standard range and this difference in protein level could be attributed to the dietary inclusion of the test ingredients (biochar and clay) as they constitute different nutrient elements which could have had an effect on the serum biochemistry of broilers. The result got disagrees with who reported that the total protein of broiler fed cooked kenaf seed meal was between the range of 2.20 - 2.93 g/Dl (Odetola et al., 2012).

It was also evident that dietary inclusion of biochar in treatment 2 significantly ($p<0.05$) increased the level of urea when compared with other treatments. The urea values in T1, T3 and T4 diets were in line with the standard range for urea (10 - 25mg/dL) while T2 diets was higher (29.58mg/dL) than the standard range which may be an indication of dehydration, gastrointestinal bleeding or primary kidney disease. The presence of urea in the body of birds can be because of imbalance in amino acid in diets, which elevated blood urea concentration in blood. Blood urea is also an indication of protein quality. Blood urea level in monogastric animals is influenced by quantity and quality as well as proximity of glycoproteins and other anti-nutritional factors which might adversely affect the regulation of insulin from islet of langerhans. Broilers fed T4 (2.5% biochar and 2.5% clay) diets gave the best urea result.

The total cholesterol level in the broilers fed with the test ingredients were significantly ($p<0.05$) lower than that of the control group but in tandem with the standard range of cholesterol (100 - 250mg/dL) in broilers. The control group (T1) had a level of cholesterol (325.34mg/dL) which was higher than that of the standard range and this can be associated with hormonal and metabolic disease, liver disease and serious kidney disease. This implies that feeding the broilers diets that contained biochar and clay kept the cholesterol level of the broiler within the normal range. The result is in agreement with whose study showed that dietary supplementation of 20g/kg of activated charcoal had significant effect on blood cholesterol level in Nile tilapia which appeared to have a decreased level of cholesterol (Boonanuntanasarn et al., 2014). The result of this study however disagrees with the reports of whose study showed no significant difference in triglyceride and total cholesterol level and other biochemical indices of 20-weeks old turkeys fed diets containing charcoal, silica grit and hardwood ash (Majewska et al., 2009). Broilers fed T2 diets (5% biochar) gave the best result.

Triglyceride level was higher than the normal range of 30-100mg/dL and this indicates a defect in the kidney. Broilers fed with T3 diet had the highest level of triglyceride (203.85mg/dL) compared to other diets. This result also disagrees with whose study showed no significant difference in triglyceride and total cholesterol level and other biochemical indices of 20-weeks old turkeys fed diets containing charcoal, silica grit and hardwood ash and who reported that dietary supplementation of 0.6% bamboo charcoal decreased the concentration of LDH, triglyceride and bilirubin levels in fattening pigs (Majewska et al., 2009; Chu et al., 2013). Globulin level in broiler fed T1 and T3 diets were in line with the standard range of 1.5-3.0g/dL but broilers fed T2 and T4 diets produced significantly ($p<0.05$) higher globulin level than the standard range and this occurrence might be due to several reasons such as immune response as biochar and clay might stimulate the immune system in broilers, leading to an increase in globulin production as part of the immune response.

This could be beneficial in enhancing the birds' ability to combat pathogens and infection. Another possible reason is toxin binding as biochar and clay have adsorbent properties, meaning they can bind toxins and prevent their absorption in the gut. This detoxification process may trigger an immune response, resulting in elevated globulin levels. Gut health modulation can also be another probable cause as biochar and clay can improve gut health by promoting beneficial microbial populations and reducing pathogenic bacteria. This modulation of the gut microbiota could stimulate the immune system, leading to increased globulin levels. Broilers in the control group (T1) had the best globulin value. However, Creatinine, albumin and Aspartate Aminotransferase (AST) level were within the range of the standard values of 0.5 - 1.5mg/dL, 1.8 - 3.0g/dL and 100 - 300U/L respectively in broiler. Broilers fed T3 diet had the best result in terms of creatinine level.

For Abulmin level, the control group (T1) had the best result while T2 had the best result in terms of AST level. Alanine Aminotransferase (ALT) level in T1 and T3 were in tandem with the standard range of 20 - 60 U/L but broiler fed T2 and T4 diets were higher in AST than the standard range and this may be as a result of contaminants or toxins present in biochar or clay, such as heavy metals or mycotoxins which could contribute to liver damage and elevated ALT levels in broilers, or as a result of gut inflammation as biochar and clay may cause gut inflammation or irritation in some birds, leading to the release of inflammatory mediators that can affect liver function and increased ALT. Other probably causes of this increase in ALT may include impaired liver metabolism, microbial contamination, dietary imbalances and hepatoxic effects to the liver leading to increased ALT levels.

4.3 Bacterial Load in the Blood of Broiler Chickens fed diets containing biochar and clay

The current study reveals that the combination of 2.5% biochar and 2.5% clay (T4) led to the lowest bacterial colony count and bacteria count in the bloodstream, indicating a potential synergistic effect of these additives. This suggests that the dietary inclusion of biochar and clay not only positively influences broiler growth but also has antimicrobial effects, reducing bacterial load in the bloodstream.

5. Conclusion

The hematological indices, serum biochemistry and total bacterial viable count of broiler fed diets containing biochar and clay were highly affected. Treatment three containing clay (5%) improved the hematological indices and serum biochemistry but total bacterial viable count of broiler was reduced to the lowest level in treatment four containing 2.5% biochar and 2.5% clay. This implies that T4 had the greatest capacity to bind toxins contained in the feed due to its mineral content and synergistic effect.

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