Effect of Dietary Inclusion of Agricultural Waste-Derived Activated Charcoal on Hematological and Serum Biochemical Indices of Layer Chickens

Abstract

The ban on sub-therapeutic dietary inclusion of antibiotics has necessitated the inclusion of natural additives such as activated charcoal (AC) produced from agricultural wastes. One hundred and twenty Isa Brown layer chickens aged 16 weeks with good management and vaccination history were used for this experiment. The birds were divided into four groups (G1-G4) of thirty birds each with each group further replicated three times comprising of 10 birds each. Chickens in G2-G4 were fed layer mash which contained 0.5g/kg,1.0g/kg and 1.5g/kg of AC, respectively with G1 as control groupand reared on deep litter. Blood samples were collected from the chickensat the end of 20th, 28th and 33rd week of the experimentand used for hematological and serum biochemical assays. Data collected were subjected to analysis using ANOVA. Hematological indices (RBC, Hb, PCV and lymphocytes) were significantly improved ($P < 0.05$) in the supplemented treatment group (G2, G3 and G4) at 28th and 33rd week than the control with G3 having the highest value. The MCV of G1 and G4 were both higher ($P < 0.05$) while the MCH, MCHC and neutrophil counts of G1 were significantly higher than other layer groups. The cholesterol level of G1 was significantly higher ($P < 0.05$) than the supplemented groups (G2-G4) whose total proteins were significantly higher ($P < 0.05$) than G1. AC is recommended as feed additive for improvement of hematological indices and for use in cases of hypercholesterolemia.

Keywords: Activated charcoal,agricultural wastes,palm kernel shell, palm fruit fibre and pig dung

Comment [HL1]: These key words are not in the Abstract. You can only include keywords that you have used in the abstract.

Introduction

Activated charcoal (AC) is a solid, porous, tasteless and black carbonaceous material (AAFCO, 2012) produced from a variety of carbon containing materials including agricultural residues and wastes. It is manufactured by a two-step process of carbonization followed by oxidation (activation) in a steam chamber (Schmidt *et al.*, 2019). Numerous studies have described the absorbance characteristics and potential clinical benefits of charcoal (Maklad*et al*. 2012) and its widely acceptance as an essential tool in disease management in human and veterinary medicines. Emerging reports have ~~however,~~ shown~~ed~~ that activated charcoal adsorbs more toxins than any other natural substance thus rendering them ineffective and harmless (Cooney, 1980; Maklad*et al.*, 2012). It has non-nutritive and non-digestible components and is effective in the elimination of mycotoxins, such as aflatoxins as well as pesticide residues that occasionally contaminate feed ingredients (Huwig*et al.*, 2001; Bhatti *et al.*, 2018; Schmidt *et al.*, 2019).

Comment [HL2]: "Emerging" reports would imply recent reports. I do not think the 1980 publication by Cooney qualifies as an emerging report. The authors should try and find another recent report in place of Cooney.

Research in 2005 showed that the quantity of agricultural wastes in Nigeria stood at 61 million tonnes per year of animal waste and 83 million tonnes of crop residues (Agba*et al.*, 2010; Akorede*et al.*, 2017) with

the major agricultural crops biomass feedstocks as millet, yam, cassava, sorghum, rice, groundnut, oil palm, sugar cane and soya-beans (Mohammed *et al*., 2013). Wastes from livestock activities include solid waste such as pig dung and organic materials in the slaughter houses and liquid waste such as urine which are capable of generating pollutants known for their characteristic offensive odour (Iregbu*et al*., 2014). Though endowed with these abundant agricultural wastes, Nigeria is yet to fully harness them to play a significant role in the production of value added products such as activated charcoal and renewable energy production (Oyedepo, 2012).

Comment [HL3]: The authors have referred to research in 2005 but none of the publications quoted was published in 2005. They need to rephrase this sentence.

Dim *et al*. (2018) reported that the white blood cell (WBC) count and the packed cell volume (PCV) were not affected while the haemoglobin concentration (Hb) and the red blood cell (RBC) count and cholesterol level were significantly improved in broiler chickens whose diets were supplemented with activated charcoal.Abdel-Latif*et al*. (2021) also observed that the inclusion of activated charcoal did not significantly affect hematological indices of Nile tilapia fish except lymphocytes counts which were significantly increased in all AC supplemented groups compared with control. More so, Abdel-Latif *et al.* (2021) noted that there was a significant increase in serum albumin, globulin and total protein values and a significant decrease in ALP, AST and ALTactivities in all AC supplemented groups.Mabe*et al*. (2018) reported that the supplementation of juvenile common carp diet with 4% dietary bamboo charcoal improved serum indicators such as ALT, AST, TP, total cholesterol and high density lipoproteins (HDL). Transaminases are biomarkers of liver functions and the increase of these enzymes is regarded as an indicator of liver damage while a decrease in their values suggest a healthy status of the liver.Majewska *et al*. (2009) observed that dietary supplementation of charcoal at 0.3% did not have significant effect on the RBC, WBC, Hb and hematocrit values of broilers fed aflatoxin-B1 contaminated feeds. Boonanuntanasarn*et al.* (2014) also observed that the inclusion of activated charcoal did not significantly affect RBC, Hb or hematocrit values of Nile tilapia in a 28-days feeding trial whileBawala*et al*. (2007) and Kana *et al.* (2014) reported low levels for hematological parameters, especially the RBC values with the use of poor quality feed.

Comment [HL4]: The authors should first write these in full words and then show abbreviations in brackets. From there on, they can now use abbreviations only.

Hematological and serum biochemical profiles are important physiological indicators for evaluating the overall performances and health status of animals (Dawood*et al.*, 2020; Abdel-Hameed *et al.*, 2021).Hence, in veterinary and medical practice, a diagnosis is considered incomplete if information from history and clinical examination is not combined with laboratory test results, including results of assessment of hematological and serum biochemical indices (Ugochukwu, 2001). The increase leucocyte count reflects humoral and cellular immune response against pathogenic agent that causes diseases (Moye and Schute, 2008). It should be recalled that the animal's health could be measured from the total leucocyte count as increased leucocyte count represents the body's immune response while decreased

WHC may translate into non-existence of infection (Sufiriyanto and Emmy, 2008). Activated charcoal may contribute to enhancement of blood profiles and immune response since it has been reported to increase body weight in animals (Majewska *et al.*, 2009; Jiya*et al*., 2013). Hence, the need to determine the effects of its inclusion in layer feeds on hematology and serum biochemistry of layer chickens.

Location of the study

The study was carried out at the Teaching and Research Farm of Michael Okpara University of Agriculture Umudike, Umuahia, Abia State, located within the South East agro-ecological zone of Nigeria with geographical coordinates of 5.4801^{o} N and 7.5437^{o} E.

Materials and methods

One hundred and twenty Isa Brown layer chickens aged 16 weeks with good management and vaccination history were used for this experiment. The birds were divided into four groups (G1-G4) of thirty birds each with each group further replicated three times comprising of 10 birds each. Chickens in G2-G4 were fed layer mash which contained 0.5g/kg,1.0g/kg and 1.5g/kg of AC, respectively with G1 as control groupand reared on deep litter. Blood samples were collected at the end of 1st, 4th and 6th week of the experimentfrom the wing vein of the birds into EDTA (Ethylene-diamine tetra-acetic acid) and plain bottles for hematological and serum biochemical analysis respectively. The erythrocyte was counted using the haemocytometer method as describe by Schalm*et al*. (1975) while the hemoglobin concentration was determined according to the techniques described by Cole (1986). In determining the packed cell volume (PCV), the Wintrobmicroheamatocrit tube method was used while other hematological indices were calculated according to the formula reported by Schalm*et al*. (1975). Serum biochemical tests were carried out using Randox commercial test kit specific for each biochemical parameter in accordance with standard procedures prescribed by the producer Randox Laboratories (UK). The serum parameters analyzed include the following~~: included~~ total serum protein, serum albumin and globulin, urea, serum creatinine concentration, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), total bilirubin content and total serum cholesterol.

Production of activated charcoal

Palm kernel shell and palm fruit fibre were collected from a palm oil mill while freshly voided pig dung was collected from pig farms using plastic container. The materials were carefully collected to avoid contamination with sand or other objects. Each material was sun-dried to constant weight and crushed manually using a wooden pestle and mortar. The materials were then blended together at a ratio of 4:3:3 weight for weight for pig dung, palm kernel shell and palm fruit fibre, respectively and used to produce

Comment [HL5]: This part of the introduction does not flow with the first part of this section. It would appear that the authors are already discussing the results although they are not connecting the results from the literature with their own results. There is need to totally review this section. The purpose of an introduction in a scientific paper like this is to justify why the experiment or research was conducted. Thus, the literature that is quoted should directly be relevant to the research in question and should be used to justify the same. The authors are off tangent on this.
Further, an introduction is supposed to lead to the objective(s) of the research. The authors have not stated the objective of their research.

Thus, the whole introduction needs to be reviewed.

Comment [HL6]: I suggest that this section should be broken down as follows:
1.Experimental design
2. The birds
3. Production of activated charcoal
4.Rations and feeding
5.Collection of blood samples
6.Analysis of blood samples

Comment [HL7]: In the abstract, the authors have indicated that blood samples were collected at the end of 20th, 28th and 33rd week of the experiment. This is very confusing. The main text should speak to the abstract.
Further, why did the authors collect blood samples at these times and not any other?

Comment [HL8]: Give a full description of how the blood samples were collected, how they were processed and stored before analysis.

Comment [HL9]: Indicate which hematological indices these were.

Comment [HL10]: Describe exactly how the collection was done in order to avoid contamination.
How much of each material was collected?

Comment [HL11]: How long did take to sun-dry the materials?
How, exactly was the sun-drying done? Were the materials dried on nets or some other materials? Was the drying done on the ground (floor) or on some raised tables?

the activated charcoal which was subsequently supplemented in layerfeeds. The physical method of activated charcoal production described by Gunamartha and Widana (2018) was employed in this study.

Comment [HL12]: Show, in a table, the actual amounts of these supplements added to the layers marsh. The readers should clearly see the composition of G1, G2, G3 and G4 rations.

Comment [HL13]: The authors need to briefly describe this procedure.

Results

Tables I, II and III presents the hematological indices of the experimental birds at twenty, twenty eight and thirty three weeks of age, respectively, while the serum biochemical indices of the experimental birds at week twenty, twenty eight and thirty three weeks of age are presented in Tables IV, V, and VI respectively.

Table I: Hematological indices of 20weeks of age layer chickens fed varying dietary levels of AC

Comment [HL14]: The Table should be able to "stand alone". In other words, it should contain all the necessary information such that it can be interpreted even without the main body of text. For example, if this table was pulled out of this paper, the reader would not know what Group 1 through to Group 4 are. No one would know what AC stands for, etc.
What do the super scripts mean?

All the above apply to the other tables that follow.

Parameters	Group 1	Group 2	Group 3	Group 4
RBC ($x10^6/mm^3$)	3.61±0.18	3.38±0.13	3.44±0.06	3.49±0.11
PCV (%)	31.67±1.53	30.00±1.00	30.33±1.53	30.67±1.16
HB (g/dl)	$11.03±0.25^b$	$10.13±0.23^a$	$10.17±0.15^a$	$10.63±0.32^b$
WBC ($X10^3/mm^3$)	$40.57±1.12^a$	$42.03±0.38^a$	$40.53±1.78^a$	$45.07±0.32^b$
Platelets ($x10^3/mm^3$)	113.67±8.96	126.00±5.29	122.33±13.50	119.67±6.51
MCV (f1)	90.44±8.82	91.91±6.30	90.86±5.43	90.81±5.57
MCH (pg)	30.56±1.00	30.02±0.65	29.53±0.93	30.52±1.27
MCHC (g/dl)	34.91±2.39	33.80±1.35	33.59±2.16	34.72±1.93
Neutrophils (%)	$51.67±1.53^a$	$54.00±1.00^{a,b}$	$54.68±2.31^{a,b}$	$56.66±1.54^b$
Lymphocytes (%)	34.67±5.13	37.00±1.00	36.33±0.58	35.33±1.53
Monocytes (%)	$6.67±0.58^b$	$6.00±0.00^{a,b}$	$6.33±0.58^b$	$5.33±0.58^a$

Eosinophils (%)	3.67±0.58	3.00±0.00	2.67±1.16	2.67±0.58

Table II: Hematological indices of 28weeks of age layer chickens fed varying dietary levels of AC

Parameters	Group 1	Group 2	Group 3	Group 4
RBC ($x10^6/mm^3$)	$2.59±0.04^a$	$3.23±0.01^b$	$3.72±0.08^c$	$3.32±0.06^b$
PCV (%)	$25.67±0.58^a$	$30.33±0.58^b$	$34.00±1.00^c$	$33.00±1.00^c$
Hb)g/dl)	11.40±0.20a	$12.20±0.20^b$	$12.97±0.20^c$	$12.73±0.15^c$
WBC ($X10^3/mm^3$)	31.70±0.98	35.07±1.21	36.30±1.28	35.10±0.70
Platelets ($x10^3/mm^3$)	143.67±3.89	161.67±14.15	134.00±1.36	149.33±6.11
MCV (f1)	$100.47±1.75^b$	$94.88±1.49^a$	$92.12±1.72^a$	$100.35±2.85^b$
Neutrophils (%)	$30.67±1.53^c$	$26.67±2.08^b$	$21.67±1.53^a$	$23.33±2.08^{a,b}$
Lymphocytes (%)	$60.00±1.00^a$	$63.67±1.53^b$	$70.00±2.00^c$	$66.33±2.08^b$
Monocytes (%)	$6.67±0.58^b$	$7.33±0.58^b$	$4.67±1.53^a$	$7.67±0.58^b$
Eosinophils (%)	2.67±0.58	2.33±0.58	2.67±0.58	2.67±0.58
MCH (pg)	$44.03±1.43^c$	$37.81±0.50^b$	$34.86±0.16^a$	$38.31±0.23^b$
MCHC (gldl)	$45.86±3.56^b$	$41.20±2.08^{a,b}$	$38.94±1.51^a$	$39.50±2.38^a$

Results are presented as mean ± standard deviation (n = 3). The results with different letter superscripts are significantly different ($P < 0.05$) from any paired mean across the row.

Table III:Hematological indices of 33weeks of age layer chickens fed varying dietary levels of AC

Parameters	Group 1	Group 2	Group 3	Group 4
RBC ($x10^6/mm^3$)	$2.73±0.05^a$	$3.31±0.08^b$	$3.88±0.02^d$	$3.75±0.05^c$
PCV	$26.67±0.58^a$	$33.00±1.00^b$	$36.67±1.16^c$	$35.33±0.58^c$
Hb	$12.10±0.10^a$	$12.47±0.49^a$	$13.47±0.06^b$	$13.50±0.10^b$
WBC ($x10^3/mm^3$)	$33.00±0.66^a$	$33.93±1.10^{a,b}$	$36.07±1.04^b$	$34.87±1.45^{a,b}$
Platelets ($x10^3/mm^3$)	131.67±18.77	139.67±8.51	$145.67±6.66^a$	146.00±5.20
MCV (fl)	$97.56±1.19^{b,c}$	$99.59±1.28^c$	$94.58±2.71^{a,b}$	$94.22±0.59^a$
MCH (pg)	$44.27±0.61^d$	$37.62±1.11^c$	$34.74±0.22^a$	$36.00±0.19^b$
MCHC (g/dl)	$45.39±1.06^b$	$37.77±0.65^a$	$36.75±1.07^a$	$37.33±1.16^a$
Neutrophils (%)	$31.33±1.53^c$	$30.33±0.58^{b,c}$	$28.33±1.53^{a,b}$	$27.33±1.16^a$
Lymphocytes (%)	60.67±1.53	61.67±1.53	63.33±1.53	63.00±2.00

Monocytes (%)	5.67±0.5	5.33±0.58	5.67±0.58	6.33±0.58
Eosinophils (%)	2.33±0.58	2.67±0.58	2.67±0.58	3.33±0.58

Table IV: Serum biochemical indices at 20 weeks of age for layer chicken fed varying dietary levels of activated charcoal

Parameters	Group 1	Group 2	Group 3	Group 4
TP (g/dl)	4.87 ± 0.05^{a}	5.23 ± 0.08^{b}	$5.57\pm0.13^{b,c}$	5.38 ± 0.22^{c}
Albumin (g/dl)	2.68 ± 0.12^{a}	3.20 ± 0.08^{b}	3.31 ± 0.17^{b}	3.24 ± 0.11^{b}
Globulin (g/dl)	2.19±0.09	2.01±0.13	2.25±0.32	2.14±0.11
ALT (µ/l)	27.67±1.53	25.00±2.65	26.33±1.53	27.33±2.52
AST (µ/l)	43.00±9.85	38.00±2.00	39.67±2.08	38.33±7.37
ALP (µ/l)	101.33±3.06	101.00±2.00	103.33±1.53	103.00±2.65
Cholesterol (mg/dl)	95.45 ± 2.43^{a}	112.72 ± 2.23^{b}	109.01 ± 390^{b}	110.87 ± 1.60^{b}
Urea (mg/dl)	10.01 ± 0.28^{a}	10.01 ± 0.22^{a}	11.11 ± 0.19^{b}	10.08 ± 0.22^{a}
Creatinine (%)	1.01 ± 0.04^{b}	$0.94\pm0.04^{a,b}$	$0.97\pm0.05^{a,b}$	0.90 ± 0.04^{a}
Bilirubin (mg/dl)	$0.54\pm0.03^{a,b}$	0.60 ± 0.03^{b}	0.61 ± 0.04^{b}	0.51 ± 0.07^{a}

Results are presented as mean ± standard deviation (n = 3). The results with different letter superscripts are significantly different ($P < 0.05$) from any paired mean across the row.

Table V: Serum biochemical indices at 28weeks of age for layer chicken fed varying dietary levels of activated charcoal

Parameters	Group 1	Group 2	Group 3	Group 4
TP (g/dl)	3.53 ± 0.06^{a}	3.91 ± 0.03^{a}	3.88 ± 0.58^{a}	4.07 ± 0.06^{a}
AST (µ/l)	35.33 ± 2.52^{b}	30.67 ± 1.16^{a}	31.00 ± 1.00^{a}	30.33 ± 0.58^{a}
ALT (µ/l)	21.67 ± 1.53^{a}	20.67 ± 1.16^{a}	19.67 ± 1.53^{a}	20.33 ± 2.08^{a}
ALP (µ/l)	82.00 ± 3.00^{b}	$79.67\pm1.53^{a,b}$	75.33 ± 3.06^{a}	$78.33\pm2.52^{a,b}$
Albumin (g/dl)	2.30 ± 0.14^{a}	2.44 ± 0.13^{a}	2.99 ± 0.07^{b}	2.87 ± 0.04^{b}
Globulin (g/dl)	1.24 ± 0.15^{a}	1.48 ± 0.16^{b}	1.22 ± 0.04^{a}	1.20 ± 0.05^{a}
Cholesterol (mg/dl)	104.50 ± 3.20^{b}	$99.10\pm0.84^{a,b}$	92.47 ± 2.25^{a}	94.66 ± 4.62^{a}
Bilirubin (mg/dl)	0.61 ± 0.02^{c}	0.51 ± 0.02^{b}	0.41 ± 0.01^{a}	0.49 ± 0.03^{b}

Urea (mg/dl)	12.88±0.60[b]	11.75±0.43[a]	12.38±0.28[a,b]	12.11±0.12[a,b]
Creatinine (mg/dl)	0.77±0.03[a,b]	0.72±0.03[a]	0.79±0.03[b]	0.78±0.03[b]

Table VI: Serum biochemical indices at 33weeks of age for layer chicken fed varying dietary levels of activated charcoal

Parameters	Group 1	Group 2	Group 3	Group 4
TP (g/dl)	3.63±0.06[a]	3.94±0.05[b]	4.19±0.11[c]	4.26±0.04[c]
AST (µ/l)	36.67±2.08[a]	35.00±1.00[a]	33.33±2.31[a]	32.67±2.52[a]
ALT (µ/l)	22.33±2.52[a]	24.67±1.53[a]	23.67±1.53[a]	22.33±3.22[a]
ALP (µ/l)	80.67±2.08[a]	86.00±2.65[b]	79.00±1.73[a]	79.33±3.06[a]
Albumin (g/dl)	2.24±0.07[a]	2.71±0.09[b]	2.93±0.04[c]	3.04±0.08[c]
Globulin (g/dl)	1.38±0.02[a]	1.23±0.13[a]	1.26±0.14[a]	1.22±0.08[a]
Cholesterol (mg/dl)	108.00±5.92[b]	101.80±3.92[a,b]	97.88±1.33[a]	98.27±1.31[a]
Bilirubin (mg/dl)	0.64±0.18[a]	0.60±0.01[a]	0.49±0.02[a]	0.53±0.01[a]
Urea (mg/dl)	11.07±0.13[b]	10.05±0.22[a]	10.07±0.16[a]	11.40±0.30[b]
Creatinine (mg/dl)	0.70±0.02[a,b]	0.71±0.02[a,b]	0.69±0.01[a]	0.73±0.02[b]

Results are presented as mean ± standard deviation (n = 3). The results with different letter superscripts are significantly different ($P < 0.05$) from any paired mean across the row.

Discussion

Hematological Parameters:

More effects of the activated charcoal were noticed on the hematological indices at twenty eight and thirty three weeksof age than at twenty weekswith RBC, Hb and PCVand lymphocytes significantly higher in the supplemented layer groups (G2, G3 and G4) when compared with the control with G3 having the highest value. The MCV of G1 and G4 were both higher while the neutrophil counts of G1 was higher than other groups and closely followed by G2. The monocytes counts of G2, G4 and G1 were significantly higher than G3 while the MCH and MCHC of G1 were greater than other layer groups with no significant differences in other hematological parameters determined.

Notwithstanding, in all the weeks, the results of the PCV, Hb and RBC values were in good agreement with normal ranges of hematological parameters in chicken with only the MCHC, total WBC, lymphocytes and platelets exceeding their normal ranges. The normal ranges of the hematological indices in chickens are RBC 2.5-3.5x10^6µl, PCV 22-35%, Hb 7-13g/dl, WBC 12-30x10^3µl, MCV 90-140fL, MCH 33-47 Pg/cell and MCHC 26-35g/dl (Bounous& Stedman, 2000). The mean corpuscular hemoglobin concentration (MCHC) measures the concentration of hemoglobin in a given volume of packed red blood cells and is useful in the characterization of erythrocytes, especially in the evaluation of anemia (Igwe*et al.,* 2017). The significant higher MCHC value across the groups (especially in group 1) at week 28 and 33 could be associated with the presence of immature erythrocytes in circulation (Polychromasia) which are produced during erythrocyte regeneration (Irrizarry-Rovira, 2004; Samour, 2009).

Leukocytosis most especially could be due to heterophilia, lymphocytosis and monocytosis which can be caused by inflammation, infections or heat stress (Irrizary-Rovira, 2004; Igwe*et al.,* 2017). These must have stimulated the hematopoietic production of the granulocyte (lymphocytes and platelets) precursors which occur when there is increased demand for them in the peripheral tissue (Campbell & Cole, 1986; Irrizary-Rovira, 2004; Juul-Madsen *et al.,* 2008). Hence, the most cause of an increase in the WBC count is the normal response of the body to an infection, certain drugs and release of immature or abnormal WBC from bone marrow into the blood.

Laying hens have been reported to have predominant lymphocytes in good health. The lymphocytes are found in significant number in the ovaries and oviduct where they are indicators of stress (Latimer and Bienzle, 2000). This is true considering the fact that the point of lay birds were brought in November and raised till March when heat stress was at its peak in this location. Lymphopoesis or enhanced lymphocytes from lymphomyeloid tissue act as a defense mechanism to tolerate infections or environmental insults such as heat stress (Irriary-Rovira, 2004).

Comment [HL15]: It is very difficult to follow this discussion when the results in each table are not being referred to. Results of each table need to be discussed adequately.
I propose that the authors combine Results and Discussion so that when they present tables on hematological parameters, they also discuss the same at the same time. The authors should systematically discuss and compare the parameters at each point when blood samples were collected.

Serum Biochemical Parameters:

Serum parameters just like hematological indices are used to assess the clinical and physiological responsiveness and well-being of chicken (Sharma *et al.,* 2015) which can be influenced by feed, medication, toxic compounds, infections, age and sex of the birds (Huff *et al.,* 2008). Total protein is made up of albumin and globulin and the normal ranges of total protein, albumin and globulin in chicken's blood are 3.0-4.9mg/dl, 1.17-2.74mg/dl (Meluzzi*et al.,* 1992) and 2.33-3.33 (Makama*et al.,* 2021) respectively. The results of the total protein, albumin and globulin obtained were in good agreement with this range. Albumin is a serum protein synthesized in the liver and is responsible for

transporting insoluble substance in the blood and aids in maintaining oncotic pressure (Fischbatch& Dunning, 2009). Total protein and albumin are indicators of protein reserve in the body (Makama*et al.*, 2021). A higher concentration of albumin usually denotes dehydration while lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection (Esubonteng, 2011).

Liver enzymes namely the alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) are important in determining the proper functioning of the liver (Ambrosy*et al.*, 2015). These enzymes are present in negligible concentration and an increase in the concentration of these enzymes may be suggestive of damaged liver cells which denote the status of the liver function. The normal ranges of the concentration of liver enzymes are AST 70-220 µ/L, ALP 40-129 µ/L and ALT 7-55 µ/L in poultry (Makama*et al.*, 2021) and the results obtained were within these reference values. Increased AST is related with the necrosis of the liver cells that can lead to the escape of this enzyme into the blood while ALT is generally increased in situation where there is damage to the liver membranes. The increased blood total bilirubin concentration observed was compatible with other reported results.

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 muscle contraction (Ilek*et al.*, 2014). Creatinine is excreted by the kidney as a by-product of creatinine phosphate metabolism which is produced as a result of energy production by the skeletal muscles (Esubonteng, 2011). The creatinine level in this study did not fall within the normal reference value of between 0.88-0.95 mg/dl as established by Wikivet (An online Veterinary encyclopedia). The slight increment observed in the level of creatinine is expected in female birds in view of the metabolic changes as a result of sexual maturity and their involvement in egg lay. High Protein intake increases metabolism, stress and dehydration which influence the concentration of uric acid in the blood (Chernecky& Berger, 2008). The urea concentrations obtained were within the normal range of urea/uric acid of 1.9-125mg/dl in poultry (Clinical diagnostic division, 1990). Age, Sex and diets of birds influence the amount of uric acid. A high level of uric acid (hyperuricemia) is usually evident in female birds due to ovulatory activities (Ibrahim *et al.*, 2012).

Cholesterol is synthesized from fats consumed and could also be synthesized endogenously within the cells. A high level of cholesterol is an indication of high risk to cardiovascular disease (Ugbogo*et al.*, 2017). The standard range of values of cholesterol in domestic fowl is between 87-192mmol/L (Meluzzi*et al.*, 1992). The cholesterol level may increase significantly during vitellogenesis and egg formation in birds (Harr, 2002). The increase in the cholesterol level may be due to increased biosynthesis and accumulation in the egg yolk. Notwithstanding, the supplemented layer groups witnessed lower levels of

cholesterol which seemed to be dose-dependent as against G1 that witnessed higher level of cholesterols at 28 and 33weeks of age. This result was in good agreement with the results of previous researchers that observed that serum cholesterol levels were reduced in birds whose diets were supplemented with activated charcoal (Neuvoneu*et al.*, 1989; Shabani*et al.*, 2010; Dim *et al.*, 2018).

These reductions in the serum cholesterol level could make activated charcoal a product of choice in the management of hypercholesterolemia (Joseph *et al.*, 2015; Roosdiana*et al.*, 2019). It becomes imperative to explore AC as an alternative medication for the treatment and management of hypercholesterolemia in both man and animals. The high availability and low price of AC produced in this study makes it affordable and within the reach of patients with this medical conditions. This coupled with the fact that activated charcoal is non-toxic and safe to be used in oral administration since it is neither absorbed nor metabolized in the GIT (Davis, 2005) becomes an added advantage. Therefore, the activated charcoal produced from co-pyrolysis of precursors (pig dung, oil palm fiber and palm kernel shell) in this study with high surface area and adsorption capacity is suitable for use in cases of hypercholesterolemia.

Comment [HL16]: See my comments above (on hematological parameters).

Conclusion and recommendations

Most of the hematological and biochemical parameters examined were within the patterns often found in avian species meaning that AC is non-toxic and safe to be used in oral administration. Its availability and low price makes it affordable to be used as feed additive for improvement of hematological indices and for patients with hypercholesterolemia to use and keep their serum cholesterol level under control at best inclusion level of 1.0kg/100kg of feeds. Therefore, agricultural waste-derived activated charcoal used in this study is suitable for use in cases of hypercholesterolemia to bind cholesterol and cholesterol-containing bile acids in the gut. It could also serve as a replacement for the synthetic drug used for this condition which is currently very expensive coupled with their long standing side effects which have generated a lot of complaints from patients.

Comment [HL17]: What does "most" mean? It means that there is one or more parameters that were not within the normal parameters. This needs to be explained.

Comment [HL18]: What is this drug? This statement should be removed as it is tantamount to speculation.
The authors should restrict their conclusion to the layers experiment only.

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