Asian Journal of Research in Animal and Veterinary Sciences

Volume 10, Issue 4, Page 1-11, 2022; Article no.AJRAVS.93399

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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/93399

Original Research Article

Received: 01/09/2022
Accepted: 02/11/2022
Published: 19/11/2022

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

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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 group and reared on deep litter. Blood samples were collected from the chickens at the end of 20th, 28th and 33rd week of the experiment and 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; hematology; serum biochemistry.

1. INTRODUCTION

Intensive poultry production has gained popularity in developing countries because of its ability to supply the much needed animal protein in diets at cheaper rates and the empowerment of resource poor segments of the society [1]. Poultry meat and eggs are the most consumed animal protein sources in Nigeria [2] and are unrestricted by any religion or taboos. The poultry industry is therefore a major source of animal protein supply in Nigeria. USDA [3] reported that "commercial poultry production in Nigeria is estimated at about USD 800 million and contributes about 25% of the agricultural gross domestic product (GDP) of the Nigerian economy". "Despite this, Nigeria is far from meeting her domestic demand for poultry products when compared with developed countries" [4] and this has been attributed to poultry input constraints [5,6].

Amongst all the inputs required for poultry production, feed makes up about 70% of the total cost of production [7] with availability of cheap and quality feeds being a major factor for poultry industry development. Production of cheap feeds from readily available local raw materials is however, a major challenge and this has engendered much research activities in the last few decades [8]. Nutritionists and experts in animal production have also advocated for inclusion of feed additives particularly natural growth promoters as a means of optimizing the uptake of nutrients from alternative feed raw materials to improve performance including hematological and serum biochemical indices [9]. The focus of research has therefore involved the development of novel feed additives such as probiotics, prebiotics, organic acids and phytogenics [10]. Adsorbents such as clay minerals and activated charcoal [11] have gained industrial recognition and are being promoted as products that have beneficial effects on animal production through modification of the intestinal ecology and growth promotion [12].

Activated charcoal (AC) is a solid, porous, tasteless and black carbonaceous material [13] produced from a variety of carbon containing materials including agricultural residues and wastes. "The expansion of agricultural production has naturally resulted in increased quantities of livestock wastes, agricultural crop residues and agro-industrial by-products. The palm oil industry generates a large quantity of wastes which makes their disposal a challenging task. Apart from few isolated cases where palm kernel shell (PKS) serve as source of fuel in cooking, they are usually dumped in the open field and water ponds which impact negatively on the environment" [14]. "The odor from pig dung is capable of diminishing air quality which brings about tension and complaints between pig farmers and their neighbors resulting to litigations and risk of possible closure of farms" [15]. "Therefore, the production of activated charcoal from palm fruit fibre, palm kernel shell and pig dung could be a value addition to palm oil processing and pig farming which are veritable economic activities in Nigeria and Malaysia" [16]. "Agricultural by-products are also being advocated for the production of adsorbents such as activated carbon (AC) due to their carbon content and the possibility of mitigating environmental pollution through such a process" [17].

Researchers have shown 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 [18,19] with the major

agricultural crops biomass feedstocks as millet, yam, cassava, sorghum, rice, groundnut, oil palm, sugar cane and soya-beans [20]. 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 [15]. Though endowed with these abundant agricultural wastes, Nigeria is yet to fully harness them to play a significant role in the production of renewable energy [21] and activated charcoal for application in poultry feeds. Feed remains the most important component of the cost of production in any poultry operations. Hence, the need to harness the potentials of numerous alternative ingredients such as activated charcoal (AC) produced from agricultural wastes for supplementation in layer chickens diets. The main objective of this study is to determine the value of agricultural wastes-derived activated charcoal in the improvement of hematological and serum biochemical indices of layer chickens. This becomes imperative since hematological and serum biochemical profile are important physiological indicators for evaluating the overall performances and health status of animals [22,23]. “This is why 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).

2. MATERIALS AND METHODS

2.1 Location of the Study

The study was carried out at the Teaching and Research Farm of Michael Okpara University of Agriculture Umudike, 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.

2.2 Collection, Drying and Blending of Agricultural Wastes

Palm kernel shell and palm fruit fibre were collected from a palm oil mill while freshly voided pig dung was collected from pig farm using plastic container and a parker. Each material was sun-dried for 2weeks on a concrete floor to a constant weight and crushed manually to reduce the particle size before being blended at the ratio of 4:3:3 weights for weight for pig dung, palm kernel shell and palm fruit fibre respectively and used to produce activated charcoal.

2.3 Activated Charcoal Preparation

The physical method of preparation employed which involved thermal decomposition or carbonization of the precursors followed by steam activation was employed in this study as described by Gunamantha and Widana [24]. The blended biomass materials were weighed using HN 289 digital scale (Omron Co., Ltd, Japan) and transferred to a clay pot of about 30 litres volume for carbonization. The pot containing the precursors was tightly covered except for a small vent that allowed limited entry of oxygen into the mixture. The pot was placed on open fire for a combustion period of five (5) hours till no more smoke was produced. At this point, water was introduced quickly to stop the carbonization of the biomass and also to achieve activation of the carbon. Thereafter, the pot was tightly closed and brought down from the fire. The clay pot was left to cool completely after which the charcoal product was taken out, rinsed with cold water to remove ash and any other debris and air dried. The dried activated charcoal was transferred to a wooden mortar and ground with pestle into fine powder and stored in an air tight plastic container and was used subsequently for dietary formulation and supplementation. Flow chart representation of the production process is as shown below in Fig. 1.

2.4 Experimental Design and Management of Birds

This study was carried out using point of lay birds of 16 weeks of age. A total of 120 Isa Brown layer chickens with good management and vaccination history were used for the experiment. The birds were shared randomly into four groups (G1- G4) of 30 birds each. Each group was further divided into 3 replicates of 10 birds each. Layers in control group were fed only layer diet while those in G2, G3 and G4 were fed layer mash supplemented with activated charcoal at inclusion levels of 0.5, 1.0 and 1.5kg per 100kg, respectively. The birds were maintained on deep litter and were fed 125g of feed per bird per day and fresh water made available to them at all times. They were given appropriate vaccinations and preventive medications. The care and management of the birds followed accepted guidelines for layers as recommended by FASS [25].

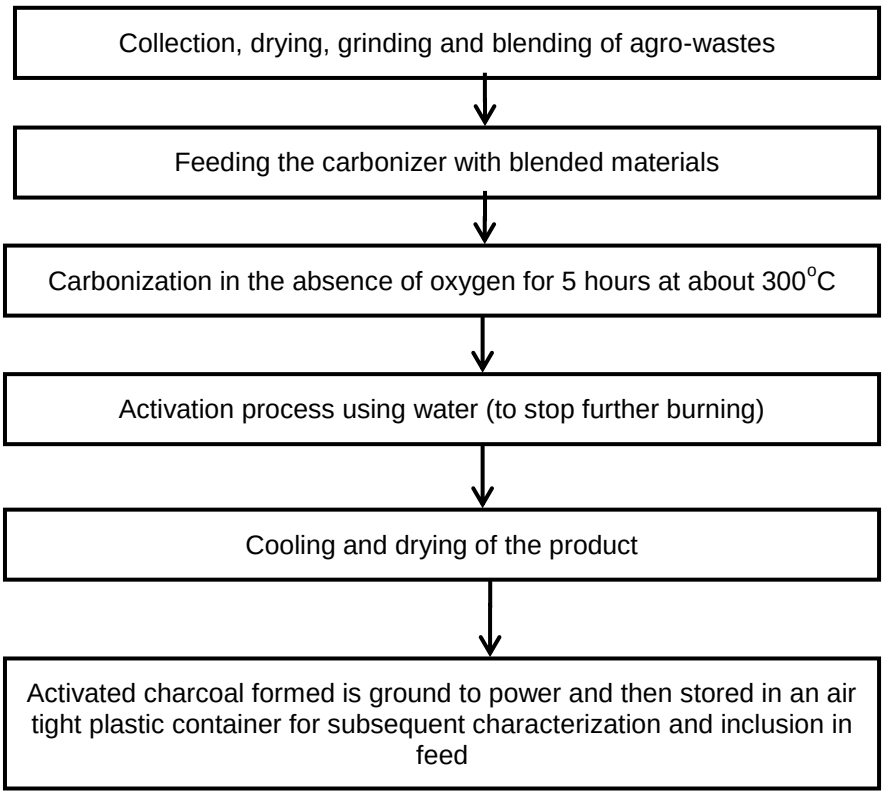

Fig. 1. Flow chat for producing activated charcoal.

2.5 Experimental Diet for Layers

The feed ingredients used in the diet formulation were purchased from a reliable distributor mill in Umuahia, Abia State, Nigeria. Maize-soya based layer diet was formulated to conform to the nutrient requirements of layer chickens according to NRC [26]. Four experimental diets were formulated such that the control (G_1) had no activated charcoal supplementation. The other three diets had graded levels of activated charcoal at inclusion levels of 0.5, 1.0 and 1.5kg per 100kg and were regarded as G2, G3 and G4, respectively. The inclusion levels were based on recommendations by FFTC [27] that suggested 1-1.5kg per 100kg as the best inclusion of activated charcoal in layer chicken diets.

2.6 Determination of Hematological and Serum Biochemical Indices

2.6.1 Hematology

The blood samples were collected at the end of 20^{th} , 28^{th} and 33^{rd} week of the experiment from the wing vein of the birds into K3 EDTA (Ethylene-diamine tetra-acetic acid) and plain bottles for hematological and serum biochemical analyses respectively. The erythrocyte was counted using the haemocytometer method as describe by Schalm et al. [28] while the hemoglobin concentration was determined according to the techniques described by Cole [29]. In determining the packed cell volume (PCV), the Wintrob microheamatocrit tube was filled with blood by capillary action up to two thirds (2/3). The samples were spurn for 5 minutes at 10,000 rpm and the PCV was read as a percentage using the designed scale reader. Other hematological indices were calculated according to the formula reported by Schalm et al. [28]. The Mean cell Hemoglobin was determined as MCH (pg) = Hb x 10/RBC, the Mean Cell Volume MCV, (fl) = PCV x 10/RBC and Mean Cell Hemoglobin Concentration was determined as MCHC (g/dl) =Hbx100/PCV. The leukocyte or white blood count was obtained using a haemocytomer with Natt and Hendricks diluent to obtain a 1:200 blood dilution. The number of leukocytes was estimated in accordance with method of Schalm et al. [28] while the white blood cell was differentiated into granulocytes (heterophils), lymphocytes, monocytes, eosinophil and basophils with the aid of automated WBC differential machine.

2.7 Serum Biochemistry

The blood samples contained in plain bottles were allowed to cloth and retract within 2 hours of collection before being centrifuged at 3000 rpm for 10 minutes to obtain clear sera which were transferred into fresh plain bottles and labeled appropriately. The sera so collected were preserved in the refrigerator at low temperature for serum biochemical tests. 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.

3. RESULTS AND DISCUSSION

Tables 1, 2 and 3 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 4, 5, and 6 respectively.

3.1 Hematological Parameters

More effects of the activated charcoal were noticed on the hematological indices at twenty eight and thirty three weeks of age than at twenty weeks with RBC, Hb and PCV and lymphocytes significantly higher in the supplemented layer groups (G2, G3 and G4) when compared with the control with G3 having the highest value. Hematological indices which ranged from

Table 1. Hematological indices of 20weeks of age layer chickens fed varying dietary levels of AC

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

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 2. 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

Parameters	Group 1	Group 2	Group 3	Group 4
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 3. 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

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 4. 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±0.13^{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±0.04^{a,b}	0.97±0.05^{a,b}	0.90±0.04^{a}
Bilirubin (mg/dl)	0.54±0.03^{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 5. 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±1.53^{a,b}	75.33±3.06^{a}	78.33±2.52^{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±0.84^{a,b}	92.47±2.25^{a}	94.66±4.62^{a}

Parameters	Group 1	Group 2	Group 3	Group 4
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}

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 6. 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

2.59 – 3.72 x 10^{6}/mm for RBC, Hb (11.40 – 12.97 g/dl), PCV (25.67 –34.00%), and lymphocytes (60.00 – 70.00%) were significantly increased and improved (P < 0.05) in hens fed diets with AC (G2-G4) than those without AC (control-G1). This increase in these hematological indices in the supplemented layer groups could be a pointer to their immune response against pathogenic agent that causes diseases [30]. It is an established fact that the animal's health could be measured from the total lymphocyte count since increased leucocyte count represents the body's immune response while decreased leucocyte count may translate into non-existence of infection [31]. The neutrophils (21.67 – 30.67%) and MCH (34.86 – 44.03pg) decreased significantly with inclusion of AC in hens' diet while the WBC (31.70 – 36.30 x 10^{3}/mm^{3}), Platelets (134.00– 161.67 x 10^{3}/mm^{3}) and Eosinophils (2.33 – 2.67%) were similar. 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^{6}µl, PCV 22-35%, Hb 7-13g/dl, WBC 12-30x10^{3}µl, MCV 90-140fL, MCH 33-47 Pg/cell and MCHC 26-35g/dl [32]. 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 [33]. 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 [34,35].

Leukocytosis as a result of increased values of WBC, lymphocytes and platelets across the groups could be due to heterophilia and lymphocytosis which can be caused by inflammation, infections or heat stress [34,33]. Stress in this case could be responsible considering that the birds were raised during the hot season which 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 [36,34,37]. Though, 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.

More so, 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 [38]. 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 [34].

3.2 Serum Biochemical Parameters

Serum parameters just like hematological indices are used to assess the clinical and physiological responsiveness and well-being of chicken [39] which can be influenced by feed, medication, toxic compounds, infections, age and sex of the birds [40]. The addition of AC elicited a significant decrease in blood cholesterol level from 104.5 mg/dl in G1 to 99.1, 92.47 and 94.66mg/dl among hens in G2, G3 and G4 respectively. TP (3.53 – 4.07 g/dl) and ALT (19.67 – 21.67 µ/l) were similar while Albumin significantly increased beyond 0.5g/kg AC levels. 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 [41] and 2.33-3.33 [42] respectively. "The results of the total protein, albumin and globulin obtained in this study 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" [43]. Total protein and albumin are indicators of protein reserve in the body [42]. "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" [44].

"Liver enzymes namely the alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) are important in determining the proper functioning of the liver" [45]. The normal ranges of the concentration of these enzymes are AST 70-220 µ/L, ALP 40-129 µ/L and ALT 7-55 µ/L in poultry [42] and the results obtained in this study were within these reference values. The increased blood total bilirubin concentrations observed across the group were 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" (Ileke 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" [44]. 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 [46] which is 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" [47]. "The urea concentrations obtained were within the normal range of urea/uric acid of 1.9-125mg/dl in poultry" [48]. "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" [49].

"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" [50]. "The standard range of values of cholesterol in domestic fowl is between 87-192mmol/L" [41]. "The cholesterol level may increase significantly during vitellogenesis and egg formation in birds" [51]. "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" [52,53]. Dim et al. [54] in an experiment he carried out in broilers noted that "the hemoglobin concentration (Hb) and the red blood cell (RBC) count were significantly improved, while the cholesterol was significantly reduced at both starter and finisher phases. This was attributed to the ability of the birds fed activated charcoal to maximally utilize the vitamin-mineral premix in the diet, especially iron and B-complex vitamins probably due to the binding of activated charcoal with toxins and anti-nutritional factors in the gut of chickens".

These reductions in the serum cholesterol level could make activated charcoal a product of choice in the management of hypercholesterolemia [55] (Joseph et al., 2015). 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 [56] 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. All hematological and serum biochemical indices were within the normal range of values reported for healthy hens.

4. CONCLUSION AND RECOMMENDATIONS

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 in cases of hypercholesterolemia to bind cholesterol and cholesterol-containing bile acids in the gut at the best inclusion of 1.0kg per 100kg feed.

ETHICAL APPROVAL

The Animal Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike approved this experiment before commencement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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