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Growth promoting and health enhancing effects of aged palm sap-enriched activated biochar in broiler nutrition

Albert Uzochukwu Chinenye Ohanaka [a,*], Judith Nkechinyere Ohanaka [b], Chinwe Mary Nwogu [c], Ifeanyi Princewill Ogbuewu [a], Idorenyin Friday Etuk [a], Ifeanyi Charles Okoli [a]

[a] *Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria*
[b] *Department of Biochemistry, Nile University of Nigeria, Abuja, Nigeria*
[c] *Department of Animal Production and Health Technology, University of Agriculture and Environmental Science, Umuagwo, Nigeria*

Abstract

This study aimed to assess the impact of aged palm sap-enriched activated biochar (AC+APS) supplementation on the growth performance, carcass yield, haematological parameters and biochemical indices of broilers. Two hundred and forty (240) male day-old Arbor Acres broiler chicks were assigned to five dietary treatment groups with six (6) replicates in a completely randomized design (CRD). The control group was fed a basal diet (A0) containing no additives. Groups A1 and A2 were fed a basal diet containing activated biochar (AC) at 0.50 and 1.00 %, respectively, while groups A3 and A4 were fed a basal diet containing 0.50 and 1.00 % activated biochar enriched with aged palm sap (AC+APS), respectively during an experimental period of 6 weeks. The results revealed significant ($p < 0.05$) improvements in growth performance in 0.50 % AC+APS-supplemented broilers compared to those in the AC-supplemented or control group during the finisher and overall production stages. The effect of AC and AC+APS inclusion on average daily feed intake was pronounced ($p < 0.05$) only during the finisher (22–42 days) period. All the test groups exhibited similar ($p > 0.05$) carcass traits to those of the control group and the treatments had no deleterious effects on organ development. Moreover, the AC+APS additive improved ($p < 0.05$) haematopoietic processes / parameters, serum protein profiles, glucose synthesis, and decreased serum enzyme activities while increasing mineral retention in broilers compared to those in the control or AC-supplemented broilers. The 0.50 % AC+APS therefore proved to be the most beneficial additive for improving productive and physiological functions in broilers and could be a useful substitute for antibiotic growth-promoting additives in poultry diets.

Keywords: adsorbent, carcass quality, charcoal, organic acid, haematological-biochemical profiles, mineral retention

1 Introduction

The sustainability of any livestock enterprise lies in the availability of cheap and quality animal feeds. However, this singular objective is becoming increasingly difficult to sustain owing to the scarcity and increasing cost of feedstuffs and finished feeds. Therefore, to increase the utilisation of available scarce resources while reducing the cost incurred in poultry diet formulations, the inclusion of non-antibiotic feed additives becomes imperative. The use of feed additives in poultry production has been popular over the years for enhancing productive functions in animals. Additives are preparations intentionally incorporated into feeds to improve productivity and health functions in animals (Ohanaka *et al.*, 2023). They are widely available on the market as enzymes, probiotics, adsorbents, organic minerals, prebiotics, vitamin preparations, etc. Oftentimes, the inclusion of feed adsorbents and acidifier products is used to immobilise toxins in poultry diets and improve nutrient availability and gut health (Khan & Iqbal, 2016; Prasai *et al.*, 2016). Activated biochar (AC) or charcoal has gained popularity as a universal adsorbent material with the capacity to effectively adsorb and neutralise ingested toxins and other contaminants in feed while supplying some essential minerals to animals (Schmidt *et al.*, 2019). It is a porous carbon-rich product from the pyrolysis of organic or agricultural biomass under

[*] Corresponding author: ohanakaalbert@gmail.com

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limited oxygen conditions and is activated using chemicals, carbon dioxide, or water vapour. Reports have shown the performance-enhancing functions of AC supplementation in poultry diets (Dim *et al.*, 2018; Kalus *et al.*, 2020). Attributes that are even more beneficial were reported with dietary supplementation of poultry diets containing AC and organic acid blends (Rattanawut *et al.*, 2021; Sittiya *et al.*, 2021). Dietary supplementation could improve air and litter quality in poultry pen houses through decreased volatilization of nutrients/gases in faecal droppings (Sarker *et al.*, 2016; Sha *et al.*, 2019; Kalus *et al.*, 2020). The sap from tropical palm trees and associated products has shown great promise as a growth-promoting additive for livestock improvement (Ohanaka *et al.*, 2023) due to its ability to produce rich suspensions of nutrients and microbial products, including yeast, lactic acid-producing bacteria (LAB), and acetic acid-producing bacteria (AAB) (Djeni *et al.*, 2020). When allowed to age, the sap undergoes spontaneous fermentation, which increases its concentrations of organic acids, fatty acids, and antimicrobial substances, which reportedly improved performance and health-promoting functions in poultry and albino rats (Fossi *et al.*, 2017; Erukainure *et al.*, 2019; Ohanaka *et al.*, 2023). The valorisation of pig manure through its co-pyrolysis with fire accelerants (palm kernel shell and bamboo biomass) into biochar and enriched with fermented palm wine will promote sustainable livestock production while addressing the disposal and pollution challenges that threatens its sustainable operations. In this study, we hypothesized that the dietary supplementation of activated charcoal and activated charcoal-aged palm wine coproducts, were similar to antibiotic growth promoting and probiotic additives, and would enhance productive and physiological performance in broilers. However, no reports are available on the effects of pig manure-palm kernel shell-bamboo waste-derived activated charcoal or aged palm sap on broiler chickens. The objectives of the present study were therefore to determine which group is better at promoting growth performance, carcass traits, or haemato-biochemical functions in treated broilers.

2 Materials and methods

2.1 Experimental location, animals, diets and design

The experimental procedures used in this study were approved by the Teaching and Research Committee, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Nigeria and in compliance with laws and institutional guidelines for animal research. The Teaching and Research farm is situated within Owerri West which lies between latitude 5°18'– 5°39' N and longitude 6°51'– 7°08' E, with an elevation of 300 m. It has a 1500–2200 mm annual rainfall (April to October) and a short dry season (November to March) with a relative humidity of approximately 50–90 % (Obi *et al.*, 2023). Two hundred and forty (240) male Abor Acres day-old broiler chicks were assigned to five (5) dietary treatments with six (6) replicates per treatment using a completely randomised design. The experimental groups were as follows: Basal diet containing zero feed additive (A0) or control; basal diet + 0.50 % activated charcoal or biochar (AC; A1); basal diet + 1.00 % AC (A2); basal diet + 0.50 % aged palm sap enriched AC (AC+APS) (A3); and basal diet + 1.00 % AC+APS (A4).

The AC additive used for the experiment was produced according to the modified pyrolysis procedure described by Ohanaka *et al.* (2021). The resultant powder had the following physicochemical components: pH, total carbon content, total ash content, specific gravity, oil adsorption capacity, bulk density, and water holding capacity, with values of 8.49, 75.35 %, 13.13 %, 0.87, 1.25 g g-1, 0.87, and 0.88 g cm^{-3}, respectively. The mineral concentrations in the derived ACs included calcium (5.56 g kg^{-1}), phosphorus (25.10 g kg^{-1}), potassium (9.30 g kg^{-1}), sodium (1.41 g kg^{-1}), manganese (0.67 g kg^{-1}), iron (1.30 g kg^{-1}), zinc (0.10 g kg^{-1}) and copper (0.04 g kg^{-1}).

A known weight (1000 g) of AC was placed into a plastic bowl, and a 72-hours fermented palm sap from *Raphia hookeri* palm tree that was harvested from the university community was gradually added while stirring intermittent, until a paste was formed to enrich the biochar. The paste was loosely covered and subsequently air-dried on a plastic tray at room temperature to produce the AC+APS additive.

The treatment feeds for each group were prepared weekly in a 20-litre plastic container to ensure even distribution of the investigated additives. The experimental diets were provided in two stages, starter and finisher, as shown in Table 1. The starter diets were offered during the first half of the experiment (0–21 days), while the finisher diets were offered in the second half (22–42 days). The basal diets were formulated to meet or exceed the NRC (1994) standards for broilers and were presented to the birds in a mash form. The birds were managed on a bedded concrete floor (deep litter) with fresh wood shavings throughout the experimental period (November–December) with ad lib feed and water provided. Each replicated groups were placed in separate pens in an open-sided, well-ventilated pen house.

2.2 Productive performance parameters

Live body weight (BW), body weight gain (BWG), feed intake (FI), and the feed conversion ratio (FCR) were measured and calculated weekly during the starter and finisher stages. These measurements were also taken throughout the production period (0-42 days). All animals were weighed initially (day 0) and then weekly using digital balance with an accuracy of 0.1 g.

Table 1: *Nutrient composition of the experimental starter and finisher broiler diet.*

	Feed components (%)	
Ingredients	*Starter*	*Finisher*
Maize	52.00	62.00
Soya bean meal	17.00	22.00
Groundnut cake	16.00	5.00
Palm kernel cake	6.00	2.00
Fishmeal	4.00	2.00
Wheat offal	0.00	2.00
Bone meal	2.00	2.00
Oyster shell	1.00	1.00
Limestone	1.00	1.00
Salt	0.30	0.30
Vitamin/mineral premix*	0.30	0.30
Lysine	0.30	0.30
Methionine	0.10	0.10
Calculated ingredients		
Crude protein	23.53	20.13
Crude fibre	4.02	3.83
Ash	3.39	2.90
Calcium	1.23	1.07
Phosphorus	0.59	0.51
ME	2943.3	3005.1

ME: Metabolizable energy (kcal kg^{-1}); * vitamin premix contained the following per kg of feed: Vit A = 12,500,000 IU, Vit D3 = 2,500,000 IU, Vit E = 40,000 mg, Vit K3 = 2,000 mg, and thiamine (B1) = 3,000 mg. B2 5,500 mg, niacin = 55,000 mg, calcium pantothenate = 11,500 mg, Vit. B6 5,000 mg, Vit B12 = 25 mg, folic acid = 1,000 mg, Biotin = 80 mg, choline chloride = 500,000 mg, manganese = 120,000 mg, iron = 100,000 mg, zinc = 80,000 mg, copper = 8,500 mg, iodine = 1,500 mg, cobalt = 300 mg, selenium = 120 mg, and antioxidant = 120 mg.

2.3 Carcass traits

At the end of the experiment (42 days), six birds were randomly selected from each treatment group (one per replicate), making a total of 30 birds for carcass evaluation. Their live weights were determined before slaughtering after an overnight fasting from feed (with free access to drinking water) and processed for the determination of carcass and organ weight characteristics. The percentages of dressed weight; thigh, drumstick, breast muscle, and wing weight, as well as organ (liver, gizzard, heart, and intestines) weight were expressed relative to the slaughter weight.

2.4 Blood samples

At the end of the experiment (42 days), blood samples were collected from thirty birds (one bird/replicate/group) through the wing vein and into heparinized and non-heparinized tubes to determine haematological and biochemical indices, respectively. The following haematological parameters were measured: packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin concentration and were estimated using the Wintrobe microhaematocrit tubes, improved Neubauer haemocytometer and cyanmethaemoglobin methods, respectively. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and differential counts were calculated as described by Jain (1993). The serum biochemical indices that were investigated included serum proteins, urea, creatinine, glucose, cholesterol, calcium, phosphorus, serum enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase) and serum electrolytes (potassium and sodium) and were determined using an automatic analyser (Kodak Ektachem, Eastman Kodak Company, Rochester, New York).

2.5 Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) via the SPSS Analytical Package (2012). When significant, differences among the groups were subjected to multiple comparisons using the Duncan multiple range test of the same software. All differences were considered to be statistically significant when $p < 0.05$.

3 Results

3.1 Production performance

The performance results of broilers subjected to a 42-day feeding trial with charcoal-supplemented diets are presented in Table 2. The live body weights (LBW) ($p = 0.055$), body weight gains (BWG) ($p = 0.055$), average daily weight gain (ADWG) ($p = 0.055$), average daily feed intake (ADFI) ($p = 0.610$) and feed conversion ratio (FCR) ($p = 0.492$) were unaffected by the AC and AC+APS supplementation in the diets during the first half (0–21 days) of the experimental period. By the end of the second half (22–42 days) of the feeding period, the superior growth performance of the 0.50 % AC+APS-treated broilers compared to the other treatment groups became much more evident. However, in the A1 (0.50 % AC) group, the improved performance indices were significantly ($p < 0.05$) greater than those in

Table 2: *Growth performance of broilers fed activated charcoal (AC) and AC + aged palm sap (APS) supplemented diets (n=48 animals per treatment).*

	treatments						
Parameters	*A0*	*A1*	*A2*	*A3*	*A4*	*SEM*	*P-value*
LBW (g)							
0 day	40.2	40.1	40.0	40.2	39.9	0.33	0.901
0-21 days	754.7	770.7	716.8	795.9	709.7	44.23	0.055
22-42 days	2003.6^{c}	2148.4^{b}	2061.8^{bc}	2269.0^{a}	2041.5^{bc}	28.36	0.002
BWG (g)							
0-21 days	714.5	730.6	676.8	755.7	669.8	11.44	0.055
22-42 days	1248.9^{b}	1377.8^{ab}	1345.0^{ab}	1473.1^{a}	1331.8^{b}	24.49	0.030
0-42 days	1963.4^{c}	2108.3^{b}	2021.8^{bc}	2228.8^{a}	2001.5^{c}	28.34	0.002
ADWG (g)							
0-21 days	34.0	34.8	32.2	36.0	31.9	0.55	0.055
22-42 days	59.5^{b}	65.6^{ab}	64.1^{ab}	70.1^{a}	63.4^{b}	1.17	0.033
0-42 days	46.8^{c}	50.2^{b}	48.1^{bc}	53.1^{a}	47.7^{bc}	0.67	0.002
ADFI (g)							
0-21 days	54.0	52.1	51.1	54.4	50.6	0.86	0.610
22-42 days	141.2^{b}	142.2^{b}	147.5^{a}	146.8^{a}	148.6^{a}	0.96	0.012
0-42 days	97.6	97.1	99.3	100.6	99.6	0.58	0.301
FCR							
0-21 days	1.59	1.50	1.59	1.51	1.68	0.02	0.492
22-42 days	2.38	2.17	2.31	2.10	2.35	0.04	0.086
0-42 days	2.09^{a}	1.94^{bc}	2.06^{ab}	1.90^{c}	2.09^{a}	0.03	0.028

A0: control, A1: 0.50 % AC, A2: 1.00 % AC; A3: 0.50 % AC+APS, A4: 1.00 % AC+APS; Means with different superscripts in the same horizontal row are significantly different at $p < 0.05$; LBW = live body weight, BWG = body weight gain, ADFI = average daily feed intake, FCR = feed conversion ratio.

Table 3: *Carcass characteristics of broilers fed activated charcoal or biochar (AC) and AC + aged palm sap (APS) supplemented diets (n=6 animals per treatment).*

	treatments						
Parameters	*A0*	*A1*	*A2*	*A3*	*A4*	*SEM*	*P-value*
Slaughter weight (g)	2150	2205	2100	2253	2176	27.29	0.523
Dressed percentage (%)	71.6	73.3	71.6	74.4	73.0	0.47	0.268
Prime cut parts %							
Thigh	13.1	12.8	12.4	12.4	12.5	0.26	0.923
Drumstick	10.2	11.0	10.7	10.6	10.4	0.14	0.402
Breast	19.8	20.8	18.6	20.6	19.9	0.46	0.663
Wing	8.1	8.4	8.7	8.4	8.2	0.17	0.919
Back	15.7	15.8	17.0	17.7	16.9	0.35	0.379
Gizzard	1.7	1.8	1.84	2.0	1.9	0.06	0.765
Liver	2.6	2.6	2.2	2.2	2.4	0.07	0.250
Abdominal fat	0.9	1.1	1.0	1.0	1.0	0.05	0.766
Intestinal weight	3.0^{a}	2.7^{ab}	3.5^{a}	2.1^{b}	2.7^{ab}	0.15	0.034
Intestinal length (cm)	208.0	245.7	270.7	248.5	242.0	9.07	0.315

A0: control, A1: 0.50 % AC, A2: 1.00 % AC; A3: 0.50 % AC+APS, A4: 1.00 % AC+APS;
Means with different superscripts in the same horizontal row are significantly different at $p < 0.05$

the control group but similar to those in the other additive-supplemented groups (1.00 % AC and AC+APS). A similar trend was also observed between treatment groups for BWG and ADWG during the finishing period and for the entire experimental period (0–42 days). Supplementation of AC and AC+APS significantly ($p = 0.012$) increased daily feed intake in the treatments A2, A3 and A4 during the finishing period, but had no significant effect ($p = 0.301$) on average feed intake of broilers during the whole experimental period. Dietary supplementation with AC+APS at the 0.50 % level also significantly improved ($p = 0.028$) the FCR of broilers during the whole production period, while the improvement was not pronounced during the finishing period ($p = 0.086$). The beneficial effect of enriching the AC with fermented palm sap on the FCR of treated broilers was eroded when AC+APS was incorporated at 1.00 %.

3.2 Broiler carcass yield characteristics

The carcass yield characteristics of the birds fed the AC- and AC+APS-supplemented diets are presented in Table 3. The addition of various additives to the broiler diets did not influence ($p > 0.05$) the live weight, dressed weight percentage or relative weights of all the prime cuts considered. There were no significant effects of supplementation with AC or AC+APS additives on any of the broiler organ development, except for the intestinal percentage weights which was significantly reduced ($p = 0.034$) in the A3 treatment compared to the control and A2 treatments.

3.3 Haematology

The haematological data in Table 4 show that the inclusion of AC in the diets of the broilers generally resulted in significant improvements in all the parameters measured ($p < 0.05$) compared with those of the control group. The WBC differed significantly ($p < 0.05$) among the treatments and linearly increased with increasing charcoal addition, while the heterophil, lymphocyte, heterophil/lymphocyte ratio, eosinophil, monocyte, and basophil counts remained unaffected ($p > 0.05$). Blood clotting time (BCT) linearly decreased significantly with increasing charcoal supplementation. However, supplementing AC+APS additives in the broiler diets significantly ($p < 0.05$) enhanced haematopoietic processes in the treated birds compared to the AC or control treatments. The improvements were more pronounced at the 1.00 % AC+APS dietary level.

3.4 Serum biochemical parameters, liver enzymes and mineral compositions

Table 5 shows the results of the serum nutrient measurements. In general, supplementation of broiler diets with AC or AC+APS improved the serum biochemistry of the treated birds ($p < 0.05$). The serum protein profiles of the A2–A4 groups were significantly higher ($p < 0.05$) than those of the control or A1 groups. The A4 group had the highest total serum protein, albumin, globulin, and glucose levels. The serum urea concentrations decreased ($p < 0.05$) progressively with increasing AC and AC+APS supplementation levels. Conversely, the serum glucose levels were progressively increased ($p < 0.05$) in broilers fed supplemented diets, while the serum cholesterol levels remained unaffected ($p = 0.091$). Treatments A4 and A3 caused a reduction in the serum creatinine levels (10.71 and 7.14 % respectively), which was lower than in birds fed the control diet. However, the reduction in creatinine levels was not significant ($p = 0.252$).

Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels decreased significantly below control levels ($p < 0.05$) with increased inclusion of AC and AC+APS in the diets. Conversely, the serum electrolyte concentrations of Ca, P, and K increased

Table 4: *Haematological indices of broilers fed activated charcoal or biochar (AC) and AC + aged palm sap (APS) supplemented diets (n=6 animals per treatment).*

	treatments						
Parameters	*A0*	*A1*	*A2*	*A3*	*A4*	*SEM*	*P-value*
Hb ($g\,dl^{-1}$)	13.10^c	14.00^b	14.30^b	14.27^b	14.80^a	0.15	0.000
PCV (%)	33.93^d	42.00^c	42.77^b	43.00^b	44.40^a	0.45	0.000
RBC ($\times 10^6\,\mu l$)	7.10^d	7.50^c	8.00^b	8.20^a	8.30^a	0.12	0.000
MCV (fl)	128.00^b	129.60^b	134.40^a	136.00^a	136.00^a	0.96	0.000
MCH (pg)	25.43^d	26.56^c	27.68^b	28.00^b	28.80^a	0.33	0.000
MCHC (pg)	30.00^e	32.10^d	33.00^c	34.00^b	34.20^a	0.41	0.000
BCT (sec)	41.00^a	38.00^b	36.00^c	33.00^d	30.00^e	1.05	0.000
WBC ($\times 10^3\,\mu l$)	5.00^d	5.50^c	5.50^c	5.70^b	6.00^a	0.09	0.000
Heterophils (%)	24.67	25.00	25.67	25.00	26.00	0.18	0.092
Lymphocyte (%)	66.00	65.00	66.30	66.00	66.00	0.26	0.598
Eosinophil (%)	3.00	3.00	1.33	2.00	2.00	0.30	0.356
Monocyte (%)	6.33	7.00	6.67	7.00	6.00	0.32	0.876
H/L ratio	0.37	0.38	0.39	0.38	0.39	0.003	0.291

A0: control, A1: 0.50 % AC, A2: 1.00 % AC; A3: 0.50 % AC+APS, A4: 1.00 % AC+APS; Means with different superscripts in the same horizontal row are significantly different at $p < 0.05$; Hb: Haemoglobin concentration; PCV: Packed cell volume; RBC: Red blood cell count; MCV: Mean cell volume; MCH: Mean cell haemoglobin; MCHC: Mean cell haemoglobin concentration; BCT: Blood clotting time; WBC: White blood cell count; H/L: Heterophil/lymphocyte ratio.

Table 5: *Serum biochemical indices of broilers fed activated charcoal or biochar (AC) and AC + aged palm sap (APS) supplemented diets (n=6 animals per treatment).*

	treatments						
Parameters	*A0*	*A1*	*A2*	*A3*	*A4*	*SEM*	*P-value*
TSP ($g\,dl^{-1}$)	8.15^b	8.25^b	8.52^a	8.60^a	8.60^a	0.05	0.000
Albumin ($g\,dl^{-1}$)	4.37^b	4.40^b	4.50^a	4.50^a	4.54^a	0.02	0.000
Globulin ($g\,dl^{-1}$)	3.78^b	3.92^{ab}	4.02^a	4.10^a	4.06^a	0.04	0.017
Alb/Glob ratio	1.15	1.12	1.12	1.10	1.12	0.01	0.400
Urea ($mg\,dl^{-1}$)	25.60^a	24.60^b	24.10^c	24.00^c	23.33^d	0.20	0.000
Glucose ($mg\,dl^{-1}$)	156.40^d	158.00^c	165.00^b	165.20^b	166.00^a	1.09	0.000
Cholesterol ($mg\,dl^{-1}$)	128.00	128.30	130.00	131.00	132.00	0.57	0.091
Creatinine ($pg\,dl^{-1}$)	1.40	1.40	1.30	1.30	1.25	0.03	0.252
ALP (IU/L)	67.00^a	66.00^{ab}	65.00^{abc}	64.00^{bc}	63.50^c	0.44	0.038
AST (IU/L)	12.20^a	12.00^a	10.80^b	10.30^c	10.00^c	0.24	0.000
ALT (IU/L)	9.00^a	8.70^b	8.00^c	8.00^c	8.00^c	0.12	0.000
Calcium ($mg\,dl^{-1}$)	10.40^c	10.50^c	11.00^b	11.30^a	11.40^a	0.11	0.000
Phosphorus ($mg\,dl^{-1}$)	3.17^c	3.30^c	3.60^b	3.97^a	4.00^a	0.09	0.000
Sodium ($mmol\,l^{-1}$)	140.00^a	140.20^a	139.00^a	136.00^b	136.00^{bc}	0.57	0.000
Potassium ($mmol\,l^{-1}$)	2.70^d	2.93^c	3.00^c	3.20^b	3.40^a	0.07	0.000

A0: control, A1: 0.50 % AC, A2: 1.00 % AC; A3: 0.50 % AC+APS, A4: 1.00 % AC+APS; Means with different superscripts in the same horizontal row are significantly different at $p < 0.05$; TSP: Total serum protein; Alb/Glob: albumin/globulin ratio; ALP: Alkaline phosphate; ALT: Alanine amino transferase; AST: Aspartate amino transferase.

significantly ($p < 0.05$) with increasing AC supplementation in the diet. Again, the combined effect of AC and aged palm sap further increased ($p < 0.05$) the serum concentrations of these minerals while decreasing their Na concentrations compared to those in the control or AC groups.

4 Discussion

4.1 Productive performance

Growth performance data showed improved growth indices (LBW, BWG, ADWG and FCR) in broilers fed 0.50 % AC+APS (A3) when compared to the other groups during the finishing period (22–42 days) and throughout the entire growth period (0–42 days). These results suggest that the incorporation of aged palm sap into AC and supplemented at 0.50 % level in the diet potentiated the effects of AC inclusion on growth performance. Similar findings of improved performance due to the synergistic effect of dietary supplementation of AC and organic acid blends in poultry diets have been reported (Sittiya *et al*., 2021; Rattanawut *et al*., 2021). The beneficial effects of aged palm wine inclusion in the diet may be linked to the amplification of enzymes that enhance ingested feed metabolism and nutrient retention (Vengadaramana *et al*., 2016; Ohanaka *et al*., 2023), gut ecology and pH modifications and increased solubility of nutrients (Watarai & Tana, 2005; Dittoe *et al*., 2018; Rattanawut *et al*., 2019). The nutrients and metabolic products of palm sap fermentation have been associated with improved performance, antimicrobial activity, and immuno-modulatory functions (Ohanaka *et al*., 2023). However, the benefits of AC+APS in broiler diets appear to be dose-dependent, as its inclusion at 1.00 % (A4) did not elicit similar growth performance responses as the A3 group, despite consuming a similar amount of feed. This may possibly be due to nutrient dilution or binding, or a reduction in energy and protein absorption by the birds, resulting in poor performance when higher dietary levels of AC were fed to broilers (Wang *et al*., 2006; Evans *et al*., 2015; Rashidi *et al*., 2020; Goiri *et al*., 2021).

4.2 Carcass yield characteristics

Supplementation of the broiler diets with AC or AC+APS in this study had no deleterious effect on the carcass or organ characteristics of the broilers. This finding is similar to the findings of Majewska *et al*. (2011) and Jiya *et al*. (2014), who also reported no deleterious effects on the carcass or organ characteristics of broilers. All the treatments showed dressed weights ranging from 71.60 to 74.42 %. Supplementation of broiler diets with 0.50 % AC resulted in a 2.43 % increase in the dressed yield percentage of treated broilers, which was further increased (3.94 %) by supplementation with 0.50 % AC+APS. However, a 1.00 % increase in additive supplementation reduced the observed gains. El-Ghalid *et al*. (2022) reported significant improvements in carcass percentage weights resulting with biochar supplementation while liver, gizzard and pancreas weights were similar to the control. Farghly *et al.* (2023) also reported significantly higher dressed percentage weights in ducks fed 1.50 and 2.00 % AC, while organ weights remained similar to those of the control. Similarly, Nnaemeka & Chikezie (2022) reported improved dressing percentages in broilers fed activated charcoal derived from pig dung-oil palm waste-at 0.5–1.00 g kg^{-1} liveweight.

Generally, supplementation with AC or AC+APS in broiler diets had no effect on the gizzard weight percentage, abdominal fat pad, weight of liver and the intestinal length. Other workers reported decreases in liver weights and increased gizzard weight when plant ash or AC was added to broiler diets (Nwogu *et al.*, 2014; Ohanaka *et al.*, 2022; Nnaemeka and Chikezie, 2022), which indicates that the feeding of AC to broilers did not cause any hepatotoxicity or excess liver weight.

The inclusion of 0.50 % AC+APS significantly (p=0.034) lowered the small intestinal weight of the broilers compared to that of the other groups. Lower small intestinal weights in broilers have been suggested to be a sign of improved digestive function due to reduced intestinal burden following functional additive feeding in animals (Kim *et al.*, 2006; Sarker *et al.*, 2016). Other studies reported heavier intestinal tracts in broilers with inferior growth performance and digestive efficiency (Van Eerden *et al.*, 2004; de Verdal *et al.*, 2010). AC and AC+APS did not significantly affect intestinal length, although treated birds had longer intestines than found for the control birds. Similarly, Nnaemeka & Chikezie (2022) reported significantly longer intestinal lengths in AC-treated broilers than in control broilers. Longer intestines are often associated with efficient feed digestion and the provision of a larger surface area for nutrient absorption (Mabelebele *et al.*, 2014; Ohanaka *et al.*, 2022).

4.3 Haematological indices

Haematological indices are reliable indicators for examining the nutritional, health, management and physiological status of broilers (Onasanya *et al.*, 2015). Blood parameters improved progressively with charcoal addition. Similar findings of improved Hb, PCV, and RBC concentrations resulting from increased charcoal addition in broiler diets have been reported (El-Ghalid *et al.*, 2022). The hematopoietic processes further increased with the dietary supplementation of AC+APS at different dietary levels. These findings indicate that these additives support better blood formation than the control, possibly due to improved bioavailability and uptake of minerals from the digestive tract (Ohanaka *et al.*, 2018). Dim *et al.* (2018) also reported improvements in the haematological parameters of broilers fed corn stover biochar even at 4-6 % dietary inclusion, while no significant effects on haematological indices were observed with biochar feeding in turkeys and broilers (Majewska *et al.*, 2009; Kana *et al.*, 2014). In contrast to our findings, Nnaemeka & Chikezie (2022) suggested that AC feeding in broilers significantly reduces RBC, PCV and Hb concentrations with increased dietary inclusion level (1.5 g kg-1) at 28 and 42 days of age.

The significant decrease in blood coagulation time (BCT) with increasing levels of AC and AC+APS in the diet suggest that these additives contain minerals such as calcium, which has been reported to enhance blood clotting ability (Ohanaka, 2016; Salama *et al.*, 2023), in the form of reducing BCT. The improvements in white blood cell (WBC) counts observed in the AC+APS- and AC-treated broilers indicate that these groups had a superior immunological status and a better disease fighting ability than the control group (Guyton & Hall, 2006). WBCs defend the body against microbial subjugation and thus provide sufficient antibodies for the immune response. Previous reports indicate that animals with moderately high WBC counts are able to produce antibodies and exhibit greater disease resistance and adaptability to the local environment (Soetan *et al.*, 2013).

4.4 Biochemical parameters, liver enzymes and serum mineral concentrations

Serum biochemical indices can reflect the health, nutritional metabolism, and physiological functions of the body. Compared with those in the control group, the serum protein profiles (TSP, ALB, and Globulin) were significantly enhanced with increased inclusion of AC and AC+APS. This could be due to increased synthesis of protein caused by improved liver functions, increased protein absorption from the small intestine and decreased losses from the renal system, all of which indicate improvements in protein handling by the birds (Zantop, 1997). Total serum protein and albumin are associated with dietary protein availability and decrease due to dietary protein deficiency, while increases in globulin suggest an improved immune response (Dzoyem *et al.*, 2014; Ohanaka, 2016). El-Ghalid *et al.* (2022) reported similar responses of improved TSP and globulin concentrations resulting from dietary charcoal supplementation. These results imply that the protein levels in the treated diets were sufficient to sustain or support the normal level of serum protein.

Plasma urea nitrogen is an accurate indicator of amino acid utilisation in swine diets (Guzik *et al.*, 2005). Therefore, the significant decrease in the urea values of AC- and AC+APS-treated broilers suggested that these additives improved the synthesis of proteins and amino acid utilisation compared to those of the control (Denli *et al.*, 2009). The linear and significant increase in the serum glucose concentration in broilers following AC and AC+APS supplementation suggested increased energy absorption from the feed by supplemented broilers (Gerlach & Schmidt, 2012) and adequate synthesis of glucose from its major precursor (propionate) in the liver (Van Houtert, 1993). This was also evidenced by the superior LBW and BWG of the treated broilers compared to the control. Similar reports of increased serum glucose in broilers fed biochar-supplemented diets have been published (El-Ghalid *et al.*, 2022). Serum enzymes are produced in the liver and other organs, and clinical assays of these enzymes constitute so-called liver function tests. AST, ALT, and ALP are enzymes that are present mostly in liver cells and can leak into the blood during hepatic injury or inflammation. The presence of significant quantities of these liver enzymes in the blood indicates increased liver tissue damage, toxicity, or metabolic disturbances. The higher AST and ALT values recorded in the birds fed the control diet may be due to increased metabolic activity in the liver (Szabo *et al.*, 2005), increased liver lesions because of their lower feed ingredient utilisation, or a slow growth rate. The significantly lower AST and ALT levels observed in supplemented birds indicate that AC and AC+APS did not compromise the hepatocellular membrane integrity of the birds. Ohanaka (2016) also reported lower ALT and ALP values but similar AST values in broilers fed palm kernel shell ash-supplemented diets. Serum ALT activity is usually low in chicken tissue but may increase due to tissue damage (Zantop, 1997). The decreased ALP activity in the AC- and AC+APS-treated broilers compared to that in the control group indicates a reduced expression of this enzyme resulting from increased mineral availability, especially phosphorus availability, in supplemented broilers (Huff *et al.*, 1998). Reports have shown that the incorporation of dietary organic acids in poultry diets increases mineral retention and phytate phosphorus utilization (Park *et al.*, 2009; Khan and Iqbal, 2016).

The significantly ($p < 0.05$) greater serum Ca, P and K concentrations recorded in the AC+APS group than in the control or AC groups may be linked to the organic acid effect of aged palm wine, which has a decreasing effect on gut pH values, thus increasing the absorption of these minerals from the gut into the bloodstream. Kishi *et al.* (1999) reported improved Ca solubility and absorption in rats fed acetic acid in their diet. The dietary inclusion of citric acid in the broiler chick diet significantly increased the serum mineral (Ca and P) concentrations of treated broilers (Abdel-Fattah *et al.*, 2008; Ebrahimnezhad *et al.*, 2008). The improved absorption of these minerals in the serum may partly explain the enhanced growth performance observed in the treated broiler groups compared to the control group. Although supplemental AC+APS in broiler diets reduced the serum Na concentration to below the level in the control or AC-supplemented boilers, the values obtained were within the normal ranges for broilers (Jain, 1993). A normal range indicates that all the groups received sufficient dietary Na capable of maintaining glucose and amino acid absorption (Bröer, 2014). Generally, the feeding of AC and AC+APS to broilers seems to exert major effects on the gastrointestinal tract (GIT), where it causes better uptake of essential minerals; improved digestion; and absorption of proteins, lipids, and carbohydrates in birds. These effects are reflected by the higher serum nutrients observed in these groups of birds.

5 Conclusion

Based on the results of this research, 0.50 % AC+APS dietary supplementation maintained superior growth performance in broilers more than in the control or AC groups. Haematopoietic processes improved with charcoal addition and were further enhanced by the addition of fermented sap-enriched activated charcoal coproducts (AC+APS). Moreover, the AC+APS additive improved the serum protein and glucose synthesis and decreased the serum enzyme activity while increasing the mineral retention in broilers compared to those in the control or AC-supplemented broilers. The effects of AC+APS supplementation as a feed additive in broiler diets were dose dependent and therefore should not exceed 0.50 %, beyond which it becomes less tolerable and has an impact on broiler performance. Thus, 0.50 % AC+APS may serve as a viable substitute for antibiotic additives in poultry production. Further research however is required to elucidate its mechanism of action and influence on gut microbial ecology, faecal characteristics and immune modulatory-related gene expressions in broilers.

Conflict of interest

The authors declare that they have no conflict of interest.

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