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PERFORMANCE AND MEAT QUALITY OF BROILER CHICKEN FED GRADED LEVELS OF BLACK COFFEE SEED (*COFFEE ARABICA*) POWDER SUPPLEMENTED DIET

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ABSTRACT

Oxidative degradation is a deleterious condition that affects quality of meat, thereby poses a threat to poultry industry. However, this can be mitigated through feeding with antioxidants, therefore the need for inclusion of high antioxidant compound, such as coffee, in poultry diet. One-day old Ross 308 chicks (n = 200; r = 5) were randomly allotted to four diets containing black coffee seed powder (BCSP): 0.0 g/kg; 1.25 g/kg; 2.50 g/kg and 3.75 g/kg. Birds were fed *ad libitum* with free access to water for 6 weeks. Total weight gain (TWG) (g/b), total feed intake (TFI) (g), feed conversion ratio (FCR), haematology, oxidative markers, gut microbiology (x10^5cfu/mL) of birds, sensory characteristics (9-point hedonic scale) and total volatile nitrogen base (TVB-N) (mg/100 g) of broiler meats were assessed. Data were analyzed using ANOVA at P = 0.05. No significant differences in TWG (1873.12 – 1927.58) and FCR (1.55 – 1.71) were observed. However, TFI at 3.75 g/kg of BCSP (3216) was higher (P < 0.05) than at 0 g/kg (3090), 1.25 g/kg 2967 and 2.50 g/kg of BCSP (3072) in the bird diet. Haemoglobin (12.60) at 1.25 g/kg BCSP was higher (P < 0.05) than at 0 g/kg BCSP (10.83), but similar to the groups of 2.50 g/kg (10.97) and 3.75 g/kg (12.13) BCSP in the diet. Creatinine (0.97mg/dL) in the 1.25 g/kg BCSP group was higher (P < 0.05) than those in the 0 g/kg (0.68 mg/dL) and 3.75 g/kg (0.60 mg/dL) groups but similar to the 2.50 g/kg group (0.88 mg/dL). No significant differences in almost all the oxidative markers were recorded. Gut microbiology showed that heterophilic (11.60), *Coliform* (8.30) and *E.coli* (13.70) contents in the 0/kg BCSP group were higher (P < 0.05) than those in the 1.25 g/kg group (9.00, 5.58 and 4.48), the 2.50 g/kg group (3.55, 1.83 and 1.28) and 3.75 g/kg group (3.30, 0.53 and 0.25) for heterophilic, *coliform* and *E. coli* respectively. Lactobacillus in the 3.75 g/kg group (7.53) was higher (P < 0.05) than that in the 2.50 g/kg (5.63), the 1.25 g/kg (5.70) and 0 g/kg (1.58) groups. With the increase of BCSP inclusion, the TVB-N decreased; aroma and taste of BCSP meat were best and well accepted. Low pathogenic gut microorganisms and total volatile nitrogen base coupled with high sensory acceptability of meat confirm the antioxidant potential of black coffee in broiler chicken diet.

Key words: growth performance; oxidative markers; total volatile nitrogen base; black coffee seed; sensory acceptability

INTRODUCTION

Physiochemical and microbial changes are the main causes of quality deterioration in meat, and most physicochemical changes are a result of oxidative degradation (Kirmizikaya *et al.*, 2021). It is a deleterious condition and a major threat to poultry industry (Estévez, 2015) and also one of the important factors influencing consumer choice or acceptability of food (Lennernäs *et al.*, 1997). Therefore, the improvement in the quality of meat by preventing or inhibiting oxidative processes is essential. Qualitative characteristics of meat are usually achieved through supplementation of anti-oxidant in feed, which is one of the strategies used to improve oxidative status in chicken meat (Fotina *et al.*, 2013).

Antioxidants play an important role in nutrient and production performance of poultry by allowing its management for optimal quality as well as prevent and/or delay several oxidation processes that led to dete-

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rioration of meat. Therefore, inclusion of antioxidants, as additives in broiler chicken diet, is essential for optimum growth rate and production of meat of high quality (Fotina *et al.*, 2013). The high preference for natural foods by consumers has prompted the poultry industry to explore natural sources of antioxidants to ensure safety and quality of the meat for human consumption. This implies feeding poultry with available edible natural materials that possess antioxidant properties, which are usually found in natural plants such as herbs, spices and seeds.

Coffee is a rich non-enzymatic source drink popularly consumed by the public (Qosimah *et al.*, 2021). It is one of the most valuable plants with high nutrients and bioactive components such as chlorogenic acid, cafestol and kahweol (Hosseini-Vashan *et al.*, 2012). These bioactive components act as free radical scavengers at varying inclusion levels (Liang and David, 2014). It also contains caffeic acid and trigonelline, which have been reported to have inhibitory effect against the growth of microorganisms (Martinez-Tome *et al.*, 2011). However, limited evidence is available on the utilization of coffee seed as an anti-oxidant source in broilers chicken to improve meat quality.

The objective of this study was to evaluate growth performance, oxidative status, meat quality indices and sensory characteristics of broiler chickens fed black coffee seed powder as an additive during the fattening stage.

MATERIAL AND METHODS

Location of study

The experiment was carried out at the Teaching and Research Farm, University of Ibadan and Department of Animal Science, Animal Product and Processing Unit, University of Ibadan, Nigeria.

Sourcing and preparation of experimental material and diet

Black coffee seeds (*Coffea arabica*) were procured from Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. The coffee seeds were cleaned from extraneous materials, milled and poured inside air tight container for subsequent use. The basal diet was formulated to meet the nutrient requirement of broiler chicken (Table 1; Awodoyin *et al.*, 2023). The black coffee seed powder (BCSP) was included into the basal diet at 0, 1.25, 2.50 and 3.75 g of diets, respectively, as at the first day of feeding.

Experimental animals, feeding and management procedures

Two hundred (200) one-day-old Ross 308 strain broiler chicks with similar hatching weight (40 g ± 3) were obtained from a commercial hatchery. On arrival, all birds were wing banded, individually weighed, using a digital scale, and randomly distributed into dietary treatments. All experimental birds were reared in deep litter pens and well-ventilated shed. Birds were weighed weekly in the morning, before feeding and watering. The experimental test feed mixtures and drinking water were supplied to the birds *ad libitum* for 42 days (experimental period). Vaccination and other routine poultry management practices were carried out neatly. All chickens appeared healthy and less than 5 % mortality was recorded throughout the entire experimental period.

Experimental design

The birds were randomly allotted to four dietary treatments in a completely randomized design. Each dietary treatment has five replicates of 10 birds per replicate.

PARAMETERS MEASURED

Phytochemical characterization and quantitative analysis of black coffee seed powder

Preparation of BCSP samples for analysis

A total of 5 g of BCSP were mixed in 70 % methanol (100 mL), stirred for 3 hours and filtered (filter paper Whatman No. 2, Maidstone, UK). The methanol was removed under vacuum from the extract in a BüCHI-rotary evaporator at 45 ˚C, this is followed by lyophilization using the Heto-Power Dry LL 300 Freeze-Dryer, Waltham, MA, USA. The resulting extract was kept at 20 °C until further analyses (Mir *et al.*, 2016).

Total Phenolic Compounds Estimation

The total phenolic compounds (TPCs) of BSCP were obtained using the methanolic extract (1 mg/mL) process and then estimated by Folin-Ciocalteu assay. Standard curve was obtained by diluting gallic acid in a distilled water at several concentrations (10 – 500 g/mL) to obtain a standard curve. The gallic acid calibration equation was $y = 0.001x + 0.0563$ ($R2 = 0.9792$), where x and y are the gallic acid concentration and absorbance in g/mL, respectively. The reaction mixture (standard solution or extract 1 mL + 3 mL diluted Folin-Ciocalteu

with distilled water 1:10, V/V + sodium carbonate 7.5 % (2 mL)) was first stirred for 60 s and then kept at room temperature in the dark for 30 minutes. The mixture absorbance at 765 nm was recorded using a spectrophotometer (JENWAY, 6405 UV/Vis, UK). The results obtained were expressed as Gallic Acid Equivalents (GAE) mg/g of extract (Abdel-Shafi *et al.*, 2019).

Determination of total flavonoids

The methanolic extract of total flavonoids (1 mg/mL) contained in BCSP was estimated following the procedure of Chen & Chen (2011) with slight modifications. Quercetin was diluted in ethanol at several concentrations (10 – 500 g/mL) to obtain a standard curve. Total flavonoid content, stated as quercetin equivalent (QE), was calculated based on the calibration curve: $Y = 0.0012x + 0.008$ ($R2 = 0.944$). The absorbance denotes y and x represent quercetin concentration (g/mL). The reaction mixture (500 L) from standard solution or extract + 1000 L of ethanolic AlCl3 solution). The colour absorbance was at 420 nm, as recorded by spectrophotometer (JENWAY, 6405 UV/Vis, UK).

Determination of saponin

The powdered sample (20 g) of BCSP was first treated with 100 mL of 20 % aqueous ethanol and heated over a hot water bath for 4 hours at 55 °C with continuous stirring. The mixture was filtered, residue re-extracted and combined extracts reduced to 40 mL over water bath at 90 °C ± 1 °C. The concentrate was transferred into a separating funnel and diethyl ether (20 mL) was added, shaken vigorously and the aqueous layer recovered. The purification process was repeated and 60 mL of n-butanol were added to the combined extract and washed twice with 10 mL of 5 % aqueous NaCl solution. The remaining solution was heated in a water bath and then dried to a constant weight in an oven and the saponin content was calculated in percentages (Sutharsingh *et al.*, 2011).

Total alkaloid estimation

This was carried out following the procedure of Harbone (1973), with slight modification by Sutharsingh *et al.* (2011). Five grams of BCSP were weighed into a 250 mL beaker, 10 % acetic acid in ethanol (200 mL) was added, covered and allowed to stand for 4 hours. Then it was filtered and the extract concentrated in water bath to one-quarter of the original volume. A drop-wise step of concentrated ammonium hydroxide was added to the extract until complete precipitation was achieved. The solution was allowed to settle, the precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The alkaloid residue was dried and weighed.

Total tannin estimation

Tannin was determined by Folin-Ciocalteu method (Galvão *et al.*, 2018). The sample solution (0.1 mL) was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35 % Na_2CO_3 solution and diluted to 10 mL with distilled water. The mixture was shaken vigorously and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) was prepared in the same manner as described above. Using a spectrophotometer (Spectrumlab 752s UV/Visible), absorbances for test and standard solutions were measured against the blank at 725 nm. The tannin content was expressed as mg of GAE /g of extract.

Assay of scavenging capability of coffee using 1-diphenyl-2-picrylhydrazyl (DPPH)

In order to evaluate the oxidative ability of BCSP, change in optical density of DPPH radicals was monitored. The sample extract (1 mL) was diluted in 1 mL of DPPH solution (0.3 mM). After 30 minutes, the absorbance was measured at 517 nm and the percentage of DPPH radical scavenging was calculated using the equation below (Lee *et al.*, 1998):

% inhibition of DPPH radical = $(Abr - Aar) \div Abr \times 100$

Abr = absorbance control

Aar = absorbance of the sample reaction has taken place

Proximate composition of black coffee seed

Proximate analyses (moisture, crude protein, ether extract, ash, crude fibre and carbohydrate) of coffee seed was carried out using the standard procedure (AOAC 2000).

Productive performance parameters

Live body weight (LBW) at 42 days of age and total feed consumption (FC) were recorded. The mortality rate was noted and recorded daily, and the productive performance parameters were adjusted for mortality. Feed conversion ratio (FCR), body weight gain (BWG) and average daily weight gain were calculated. The birds were weighed using a digital weighing scale with high sensitivity.

$$\text{Average daily feed intake}\left(\frac{\text{g}}{\text{bird}}/\text{day}\right) = \frac{\frac{\text{total feed consumed}}{\text{number of live birds}}}{\text{seven (7 days)}}$$

$$\text{Feed conversion ratio} = \frac{\text{average daily feed intake}}{\text{average daily weight gain}}$$

Blood sample analysis

At the 42nd day of life, one broiler chicken (one bird per replicate, five birds per treatment) with a body weight (BW) similar to the mean BW of the full replicate were randomly selected for blood sample collection. The blood was taken from the wing vein into two separate heparinised tubes to determine haematological and biochemical indices. The haematological parameters measured were: red blood cells (RBCs), packed cell volume (PCV %), haemoglobin concentration (Hb %), white blood cells, lymphocytes (%) and heterophils. For blood biochemical analysis, blood samples were centrifuged at 3000 rpm for 15 minutes; plasma was kept at -80 °C until spectrophotometer analysis of liver function, lipid profile and kidney function using commercial kits (Bio-diagnostic Company, Giza, Egypt). The effect of the diet on the liver was accessed through total protein, albumin and globulin, while the effect of the diet on the kidney was determined through creatinine assessment. For the lipid profile assessment, the levels of cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL were determined. The serum glutathione, glutathione peroxidase and glutathione S- transferase activities were determined using a commercial enzyme kit (Ransel, RANDOX/RS-504 by Randox Laboratories, Crumlin, United Kingdom). Superoxide dismutase (SOD), vitamin C and hydrogen peroxide activity were determined according to Osman *et al.* (2014) using an enzyme kit (Ransod, RANDOX/SD-125 by Randox Laboratories, Crumlin, United Kingdom). Malondialdehyde (MDA) and myeloperoxidase (MPO) levels were determined using MDA colorimetric assay kit (TBA method) (Elabscience, USA) and T-AOC – using auto analyser kit (Bio-Med Diagnostics, Egypt; De Gobba *et al.*, 2014; Abdel-Hamid *et al.*, 2017).

Gut microbiology

Fresh *Caecum digesta* samples were taken from each broiler chicken (three birds/replicate). Digesta was collected from the junction between duodenum and jejunum region of the gut. This was placed into transparent bottles and the digesta released into the bottles and transported to the laboratory to estimate bacterial counts in the gut of the broiler. The digesta was cultured using appropriate nutrient agar, stored and subsequently identified. Bacteria colonies were identified based on morphological and biochemical characteristics after staining. Bacteria colonies on the plates were counted and expressed as colony forming units, which were expressed as log cfu/mL. The microorganism parameters determined were: total heterotrophic, *Staphylococci*, *Pseudomonas*, Coliform *Echerichia coli* and lactobacilli counts (Brugger *et al.*, 2012; Alagawany *et al.*, 2019).

Determination of total volatile nitrogen base (TVB-N) of chicken meat

This was determined according to the Food Safety and Standards Authority of India (2012) with slight modification. Hundred (100 g) grams of fresh meat samples were weighed and blended with 300 mL of 5 % trichloroacetic acid. The mixture was then centrifuged at 3000 rpm for 1 hour to obtain a clear extract. The extract (5 mL) was pipetted into the Markhan apparatus and 5 mL of 2 M sodium hydroxide (NaOH) was added. This was then steam distilled into 15 mL of standard 0.01 M hydrochloric acid (HCl) containing 0.1 mL rosolic indicator. After distillation, excess acid was titrated into the receiving flask using standard 0.01 M NaOH to a pale pink end point. A procedural blank was obtained using 5 mL of trichloroacetic acid. The concentration of TVBN (mg/100 g sample) was calculated as follows:

$$\text{TVBN}\left(\frac{\text{mgN}}{\text{100 g}}/\text{sample}\right) = \frac{(\text{M})\,(\text{VB}-\text{VS})\,(14)\,(300+\text{W})}{\text{weight of sample}}$$

Where: VB = NaOH (mL) used for blank titration
W = water content of sample in g/100 g
M = molarity of NaOH standard solution
VS = mL of NaOH used for sample titration

Sensory characteristics assessment

The sensory evaluation was carried out on breast muscle meat of chicken from different dietary treatments after cooking (at 100 °C) for 20 minutes. Doneness was ascertained when internal core temperature of cooked meat reached 72 °C. An untrained sensory panel of 40 people was formed comprising of staff and students from the Department of Animal Science, University of Ibadan. The sensory panel had no prior training because the aim was to obtain a general acceptance evaluation. The meat was coded with 3-digit number and randomly presented to the panellists. Aroma, taste, juiciness, flavour and overall acceptance of the

cooked breast muscle meat were evaluated. Each panellist evaluated 4 samples (one per treatment) and qualified the acceptance of the above-mentioned properties, through a 9-point hedonic scale: 1 – "I extremely dislike", and 9 – "I extremely like". To neutralize the taste of one sample before the other, the panellists were provided with crackers and water, which were taken in-between samples.

STATISTICAL ANALYSIS

Data obtained from the study were subjected to one-way analysis of variance (ANOVA). Duncan multiple range test at $P \leq 0.05$ was used to compare the means using the SAS 12.0 version statistical package (2020).

Table 1. Composition and calculated analysis (g/100 g DM) of experimental basal diets (as fed basis)

Ingredients	Starter	Finisher
Maize	54.50	64.40
Soya bean meal (46 %)	38.00	28.00
Meat and Bone meal (50 %)	3.00	2.50
Soya oil	1.00	1.50
Limestone	2.00	2.00
Dicalcium Phosphate (18 % P)	0.40	0.40
*Broiler premix	0.25	0.25
DL-Methionine	0.22	0.20
L-Lysine	0.18	0.10
Salt	0.25	0.25
Toxin binder	0.20	0.25
Total	100.00	100
Calculated Nutrient		
Crude protein (%)	23.74	19.45
Crude fat (%)	5.16	4.37
Crude fibre (%)	3.84	3.42
Calcium (%)	1.14	1.07
Avail. Phosphorus (%)	0.43	0.41
ME (Kcal/kg)	2914.00	3021.96

Source: Awodoyin *et al.*, 2023

*Composition of premix per kg of the diet: Vitamin A (IU): 10,000,000; Vitamin D3 (IU): 2,500,000; Vitamin E (IU): 40,000; Vitamin K (mg): 2,250; Vitamin B1 (mg): 1,750; Vitamin B2 (mg): 5,000; Vitamin B6 (mg): 4,000; Vitamin B12 (mg): 15; Niacin (mg): 40,000; Panth. Acid (mg): 10,000; Folic Acid (mg): 3000; Biotin (mg): 100; Choline Chloride (mg): 400,000; Manganese (mg): 80,000; Zinc (mg): 60,000; Iron (mg): 40,000; Copper (mg): 8000; Iodine (mg): 1200; Selenium (mg): 200; Cobalt (mg): 300; Antioxidant (g): 125.

RESULTS

The black coffee seed powder contained 8.99 % moisture, 2.59 % ash, 9.20 % crude fibre, 2.81 % fat content, 9.49 % protein and 66.93 % carbohydrate (Table 2).

Table 2. Proximate composition of black coffee seed powder

Parameters (%)	Mean ± standard deviation
Moisture	8.99 ± 0.07
Ash	2.59 ± 0.03
Crude fibre	9.20 ± 0.04
Fat	2.81 ± 0.03
Crude protein	9.49 ± 0.07
Carbohydrate	66.93 ± 0.19

The quantities of the phytochemicals present in BCSP are shown in the Table 3.

Table 3. Quantitative analysis of some phytochemicals contained in black coffee seed

Parameters (%)	Mean ± standard deviation
Saponin (%)	3.55 ± 0.08
Alkaloid (%)	2.85 ± 0.10
Flavonoid (mg QE/g)	5.98 ± 0.14
Phenol (mg GAE/g)	8.42 ± 0.02
Tannin (mg GAE/g)	0.31 ± 0.001

The qualitative phytochemical analysis of coffee seed powder showed that the black coffee seeds, used in this study, contained saponin, alkaloid, flavonoid, tannin, coumarin, steroid, terpenoid, cardiac glycosides, quinines, phytosteriods and phenols, while glycosides and anthocyanin were absent Table 4.

The DPPH radical scavenging activities of BCSP at different concentrations are presented in Table 5. The inhibition were 30.43 %, 40.85 %, 62.11 %, 86.11 % and 88.82 % at 15.625 %, 31.25 %, 62.50 %, 125.00 % and 250.00 % µg/L concentrations respectively.

Table 4. Qualitative phytochemicals of black coffee seed powder

Parameters	Sample
Saponin (Froth's Test)	+
Alkaloid (Hager's Test)	+
Flavonoid (Lead acetate Test)	+
Tannin (Braymer's Test)	+
Coumarin (Reaction with 10 % NaOH)	+
Steroid (Salkowaski's Test)	+
Terpenoid (Salkowaski's test)	+
Cardiac Glycosides (Legal's Test)	+
Glycosides	-
Quinones	+
Anthocyanin	-
Phytosteroids	+
Phenols (Ferric Chloride Test)	+

Table 5. Di-phenyl picrylhydrazil radical scavenging activities of black coffee seed

Concentration (µg/L)	% Inhibition (Mean ± standard deviation)
15.625	30.43 ± 0.12
31.250	40.85 ± 0.02
62.500	62.11 ± 0.05
125.000	86.11 ± 0.07
250.000	88.82 ± 0.06

The growth performance of broiler chicken fed graded levels of BCSP showed no significant difference ($p > 0.05$) in the initial weight (39.90 – 41.60 g/b), final weight (1914.22 – 1967.48 g/b), total weight gain (1873.12 – 1927.58 g/b), average daily weight gain (44.60 – 45.89 g/b/d), average daily feed intake (70.64 – 76.57 g/b/d) and feed conversion ratio (1.55 – 1.71) among all the treatments. Total feed intake of birds fed 3.75 g/kg BCSP was significantly higher ($p < 0.05$) than recorded for birds fed 0.00 g/kg, 1.25 g/kg and 2.50 g/kg BSCP (Table 6).

The haematological indices showed that PCV (37.67) of birds fed 2.50 g/kg of BCSP was significantly higher ($P < 0.05$) than those fed 1.25 g/kg and 0 g/kg, but similar with those fed 3.75 g/kg of BCSP. The haemoglobin of the bird fed 2.50 g/kg of BCSP was significantly higher than those fed 0 g/kg BCSP but similar with those fed 1.25 g/kg and 3.75 g/kg. No significant differences in RBC (3.32 – 3.78), WBC (16.45 – 18.28), lymphocytes (62.33 – 68.67), neutrophils (23.33 – 31.00) and platelets (14.20 – 16.67) were observed among the groups (Table 7).

The serum analysis showed no significant differences ($P > 0.05$) in total protein (3.12 – 3.69) albumin (1.67 – 1.75), globulin (1.44 – 2.02), cholesterol (132.74 – 166.76), HDL 95.58 – 110.81) and LDL (28.19 – 32.18), while creatinine (0.97) of birds fed 1.25 g/kg BCSP in the diet was significantly higher than in birds fed 3.75 g/kg of BCSP but similar with those fed 2.50 g/kg of BCSP (Table 8).

The oxidative markers of the birds showed no significant differences ($P > 0.05$) in total protein (6.10 – 8.94),

Table 6. Effect of various inclusion levels of black coffee seed powder on growth performance of broiler chicken

	Various inclusion levels (BCSP g/kg)					
Parameters	0.00	1.25	2.50	3.75	SEM	P-Value
Initial Weight (g/b)	41.10	41.60	39.90	41.10	0.43	0.57
Final Weight (g/b)	1955.44	1957.61	1967.48	1914.22	24.15	0.12
T.W.G (g/b)	1914.34	1916.01	1927.58	1873.12	24.17	0.13
A.D.W.G(g/b/d)	45.58	45.62	45.89	44.60	0.58	0.89
T.F.I (g)	3090.00^{b}	2967.00^{d}	3072.00^{c}	3216.00^{a}	20.30	<.0001
A.D.F.I (g/b/d)	73.57	70.64	73.14	76.57	0.48	0.12
FCR	1.61	1.55	1.59	1.71	0.03	0.35

^{abcd}Means on the same rows with different superscripts are statistically different ($P < 0.05$). T.W.G = Total Weight Gain; A.D.W.G = Average Daily Weight Gain; T.F.I = Total Feed Intake; A.D.F.I = Average Daily Feed Intake; FCR = Feed Conversion Ratio; g/d/b = g/day/bird.

Table 7. Haematological indices of broiler chicken fed diets supplemented with various inclusion levels of black coffee seed powder

	Various inclusion levels (BCSP g/kg)					
Parameters	0.00	1.25	2.50	3.75	SEM	P-Value
PCV (%)	32.00^{b}	32.33^{b}	37.67^{a}	36.33^{ab}	1.00	0.03
Haemoglobin (g/dL)	10.83^{b}	10.97^{ab}	12.60^{a}	12.13^{ab}	0.38	0.01
RBC (10^6ul)	3.32	3.68	3.43	3.78	0.11	0.18
WBC (10^3ul)	16.45	18.23	17.23	18.28	0.73	0.50
Lymphocytes (%)	62.33	68.67	66.67	67.33	1.52	0.20
Neutrophils (%)	31.00	23.33	27.00	25.00	2.08	0.28
Platelet (10^4ul)	14.57	16.17	16.67	14.20	1.07	0.77

^{ab}Means on the same rows with different superscripts are statistically different (P < 0.05). PCV = Packed cell volume; RBC = Red Blood Cell; WBC = White Blood Cell.

Table 8. Blood total protein, its fractions and lipid profile of broiler chicken fed diets supplemented with various inclusion levels of black coffee seed powder

	Various inclusion levels (BCSP g/kg)					
Parameters (mg/dL)	0.00	1.25	2.50	3.75	SEM	P-Value
Total protein (g/dL)	3.16	3.69	3.69	3.12	0.23	0.17
Albumin (g/dL)	1.73	1.67	1.75	1.68	0.07	0.83
Globulin (g/dL)	1.44	2.02	1.94	1.44	0.21	0.11
Cholesterol	166.76	160.86	144.44	132.74	11.69	0.20
HDL	95.58	110.81	105.56	105.96	8.36	0.63
LDL	32.18	29.40	28.19	31.20	1.78	0.41
Creatinine	0.68^{bc}	0.97^{a}	0.88^{ab}	0.60^{c}	0.09	0.03

^{abc}Means on the same rows with different superscripts are statistically different (P < 0.05). HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein.

H_2O_2 (125.12 – 144.01), glutathione (68.26 – 88.54), GPX (53.17 – 62.68), NP-THIOL (34.00 – 42.79), vitamin C (0.30 – 0.38), malonaldehyde (0.95 – 1.40), SOD (46.67 – 57.48), GST (1.76 – 2.58) and myelo-peroxidase (15.28 – 23.30). Thiol level of the birds fed 3.75 g/kg of BCSP was significantly higher than those fed 2.50 g/kg but similar with the birds fed 1.25 g/kg of BCSP and 0 g/kg of BCSP (Table 9).

The gut ecology of broiler chicken fed varying levels of BCSP indicated that THC and TEC of birds fed 2.50 g/kg and 3.75 g/kg of BCSP were similar (P > 0.05) but significantly lower (P < 0.05) than in birds fed 1.25 g/kg and 0 g/kg of BCSP. The TCC (0.53) of 3.75 g/kg BCSP fed birds was significantly lower than in birds fed 2.50 g/kg, 1.25 g/kg and 0 g/kg. The TLC of birds fed 3.75 g/kg BCSP was significantly higher than those of birds fed 2.50 g/kg, 1.25 g/kg and 0 g/kg of BCSP (Table 10).

The total volatile nitrogen base (pooled over 21 days of storage) of meat from broiler chicken fed varying inclusion levels of BCSP is represented in Figure 1. The TVB-N of the meat from the birds fed 3.75 g/kg was significantly lower than the values recorded in the meat of broiler chicken fed 0.00, 1.25 and 2.50 g/kg of BCSP.

Sensory evaluation of the meat of broiler chickens fed varying levels of BCSP showed that aroma of cooked meat from chickens fed 2.50 g/kg BCSP was significantly higher than that of chicken fed 0 g/kg, but similar with those fed 1.25 g/kg and 3.75 g/kg of BCSP. The tastes

Table 9. Serum oxidative markers at 42 day of broiler chicken fed various levels of black coffee seed powder supplemented diets

	Various inclusion levels (BCSP g/kg)					
Parameters	0.00	1.25	2.50	3.75	SEM	P-Value
Total protein (mg/dL)	7.17	8.94	6.45	6.10	0.97	0.43
H_2O_2 (mmol)	125.12	131.23	130.75	144.01	8.41	0.67
Glutathione (U/mL)	81.59	78.04	68.26	88.54	6.46	0.43
GPX (U/mL)	61.90	53.17	62.68	56.58	7.47	0.88
NP-THIOL (U/mL)	41.66	34.00	42.79	41.23	4.07	0.64
Thiol (U/mL)	141.87^{ab}	174.50^{ab}	122.23^{b}	213.36^{a}	19.38	0.13
Vitamin C (mg/dL)	0.30	0.30	0.38	0.34	0.05	0.82
Malondialdehyde (x 10^5 nmol/mL)	1.12	1.40	1.01	0.95	1.51	0.43
SOD (U/mL)	57.48	49.78	54.96	46.67	6.61	0.80
GST (U/mL)	2.07	2.58	1.86	1.76	0.28	0.43
Myeloperoxidase (mol/L)	18.47	23.30	17.70	15.28	4.83	0.83

^{ab}Means on the same rows with different superscripts are statistically different (P < 0.05). H_2O_2 = Hydrogen peroxide; GPX = Glutathione Peroxide; NP-Thiol = Non protein Thiol; SOD = Superoxide Dismutase; GST = Glutathione S-Transferase.

Table 10. Gut ecology of broiler chickens fed various levels of black coffee seed powder

	Various inclusion levels (BCSP g/kg)					
Parameters (10^5cfu/mL)	0.00	1.25	2.50	3.75	SEM	P-Value
THC	11.60^{a}	9.00^{b}	3.55^{c}	3.30^{c}	2.91	0.02
TCC	8.30^{a}	5.58^{b}	1.83^{c}	0.53^{d}	0.22	0.01
TEC	13.70^{a}	4.48^{b}	1.28^{c}	0.25^{c}	0.42	<.0001
TLC	1.58^{c}	5.70^{b}	5.63^{b}	7.53^{a}	0.78	0.02
TSS	NG	NG	NG	NG	-	-
TPC	NG	NG	NG	NG	-	-

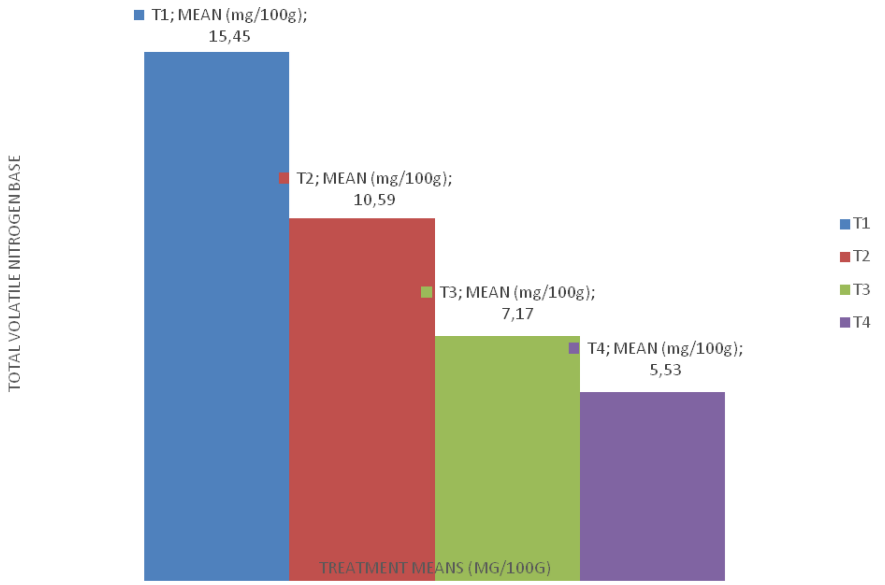
^{abcd}Means on the same rows with different superscripts are statistically different (P < 0.05). THC = Total Heterotrophic counts; TCC = Total *Coliform* counts; TLC = Total *Lactobacillus* counts; TEC = Total *E. coli* counts; TSS = Total *Staphylococci* counts; TPC = Total *Pseudomonas* counts; NG = No Growth.

of the meat from the chicken fed 1.25 g/kg, 2.50 g/kg and 3.75 g/kg of BCSP were similar among each other but they were significantly higher when compared to 0 g/kg of BCSP. The overall acceptability of all cooked meat from birds fed BCSP, rated by the panellist, were higher (P < 0.05) compared to the birds fed 0 g/kg of BCSP (Table 11).

DISCUSSION

The flavonoid content of coffee, used in this study, was lower than those found in green coffee seed powder (Ashour *et al.*, 2020), while the alkaloid content was higher than it was measured in caffeinated (2.57 %) and decaffeinated (1.43 %) coffee (Adetunji *et al.*, 2021). Differences in results among different studies suggest that the composition and effectiveness of most phytogenic additives depends on many factors such as the botanical specificity, animal management etc. (Abdel-Shafi *et al.*, 2019). Evidently, BCSP had a strong free radical scavenging ability in 2,2-diphenyl-1-picrylhydrazyl (DDPH), which could make it a potential source of natural antioxidants.

The crude protein and ash contents of the black coffee, used in this study, were lower than 17.14 %



T1: meat from broiler fed 0 g/kg BCSP; T2: meat from broiler fed 1.25 g/kg BCSP;
T3: meat from broiler fed 2.50 g/kg BCSP; T4: meat from broiler fed 3.75 g/kg BCSP

Figure 1. Total volatile nitrogen base (mg/100 g) of meat broiler chicken fed varying levels of BCSP

Table 11. Sensory evaluation of cooked meat samples from broiler chicken fed diets supplemented with various inclusion levels of black coffee seed powder

	Various inclusion levels (BCSP g/kg)					
Parameters	0.00	1.25	2.50	3.75	SEM	P-Value
Aroma	2.33^{b}	5.00^{ab}	6.67^{a}	4.33^{ab}	0.28	0.03
Flavour	4.67	6.00	4.33	4.33	0.36	0.24
Taste	3.33^{b}	5.33^{a}	6.33^{a}	6.00^{a}	0.20	0.03
Juiciness	5.00	5.00	6.67	4.00	0.27	0.19
Overall acceptability	4.00^{b}	6.67^{a}	6.67^{a}	7.67^{a}	0.22	0.004

^{ab}Means on the same rows with different superscripts are statistically different ($P < 0.05$).

(CP) and 4.38 % (ash), while moisture content was higher than 5.4 % measured in green coffee (Hosseini-Vashan *et al.*, 2012). These differences in values could be due to differences in genetic species, breed and geographical location of the coffee used (Farah and Donangelo, 2006). The low ash content, found in the BCSP showed that the coffee seed powder contained less minerals.

This study elucidates, that those chicks, who received different levels of BCSP in the diet, consumed more feed than the control throughout the experimental period. The increased feed consumption may be since BCSP has a positive effect on the palatability of feed, which inspires appetite. It could also be due to the presence of some bioactive compounds in coffee, which might have aided digestion (Ashour *et al.*, 2020) consequently increasing the appetite and, thus, resulting in more feed consumption. This agreed with Peric *et al.* (2009) and Scheuermann *et al.* (2009) reports, who opined that phytogenic additive supplementation

could improve the feed consumption in broilers. It would have been expected that the FCR and TWG of BCSP fed broiler chickens should improve because the amount of feed consumed is closely associated with the growth performance in meat-type poultry (Ferket & Gernat, 2006). Also, chlorogenic acid, which is in abundance in BCSP, have been reported to aid nutrient absorption (Ashour *et al*., 2020), which is expected to improve the weight gain and FCR. Again, coffee powder is reported to have a stimulating effect on water consumption, which further stimulates food consumption and weight gain (Skinner-Noble and Titter, 2004). However, no significant increase in weight or improvement in FCR was observed with BCSP inclusion into the broiler's diet. This implied that the organic acid content in the black coffee, used in this study, did not positively affect weight gain. This might probably be due to the caffeine presence in a coffee, which has a fat-burning effect thereby reducing fat retention in tissues (Garg, 2016) and consequently causing a decrease in weight gain despite more food consumption. This result affirmed the report of Cho *et al.* (2014) that phyto-additives in broiler diets had no significant effect on FCR. The total weight gained by the birds in this study were lower than 2295.68 g, 2319.98 g, 2300.88 g and 2285.54 g weights attained by broiler birds fed with green coffee, green tea, cinnamon and rosemary extracts, respectively (Abo-Ghanima *et al.*, 2021). Haematological indices are one of the markers for assessing the nutritional and health status of animals. The PCV is correlated with the nutritional status of animals (Iheukwumere, 2008), and higher values indicate improved blood quality.

The present study revealed that addition of BCSP at 2.50 and 3.75 g/kg into broiler feed increased the PCV percentage and Hb concentrations during the experimental period. The increased PCV indicated that BCSP presence in the broiler diet improved the quality of the broiler blood. The increased PCV implied that the bird's blood will have higher iron contents, which may result in high RBC and Hb concentrations (Ashour *et al.*, 2020). But in this study, only the Hb concentrations in the bird blood were significantly increased with BCSP inclusion. Total blood haemoglobin concentration is a reflection of the potential of a bird to satisfy its oxygen requirements (Minias, 2015), while the red blood cells function in the transportation of oxygen and carbon dioxide in the blood. Thus, the higher concentrations of Hb provided that the aerobic capacity of the birds was improved by the inclusion of BCSP into their diets. The PCV values of BCSP fed broilers in this study are higher than 24.33 – 28.33 % recorded when broilers birds were fed Gmelina arborea seed meal (Fatokun *et al.* 2013) and 29.40 – 34.20 % reported by Ayeni *et al.* (2022). Furthermore, the PCV of birds fed 2.50 and 3.75 g/kg fell within the range of 33.57 – 37.27 % obtained in broiler chickens fed graded levels of Roselle seed (Onunkwo *et al.*, 2019) and 35.9 – 41.0 % reported by Odunitan-Wayas *et al.* (2018), who opined that PCV within this range may indicate that the birds were not anaemic. The Hb of all the birds fall within the normal range of 8.00 – 13.00 g/dL for domestic chickens, while the RBC of the birds were slightly lower than 4.10 – 4.16, reported by Onunkwo *et al.* (2019).

The presence of BCSP in the broiler diet is expected to increase the haematological indices, especially the RBC and WBC of the chickens, because natural feed supplements act as immunity enhancer for broiler chickens (Geetha and Chakravarthula, 2018) but these remained unchanged. This implied that similar levels of antibodies (which protected the birds from infectious agents) were produced by the WBC irrespective of the feed. Again, the high Hb concentration should have resulted in high RBC concentration, but surprisingly, the results showed that presence of BCSP in feed had no discernible impact on the broiler RBC and some other haematological indices measured at 6 weeks of age in this study.

Serum blood parameters are usually used to assess the nutritional, pathological and physiological status of the chicks, thus, reflecting the impact of diets supplemented with feed additives. The inclusion of coffee in the diet of the chicken did not have any significant effect on the serum blood parameters (except creatinine). This is opposite to Ashour *et al.* (2020), who reported no significant differences in creatinine, when broiler diet was supplemented with green coffee seed. Although no significant differences existed in almost all the parameters measured but the values were slightly different. For instance, the globulin and HDL levels increased, while cholesterol and LDL levels decreased with BCSP inclusion. This similar trend was observed by Abo Ghanima *et al.* (2021), who reported a reduced serum cholesterol in heat stressed broilers fed 1.25 and 2.50 g/kg of green coffee in their diet. This reduction is attributed to the lipogenesis effect of coffee (Ding *et al.*, 2020).

Chlorogenic acid in coffee is expected to participates in scavenging free radicals and activates endogenous antioxidants against free radicals (Liang and Kilts, 2016),

which will leads to increased SOD activity and reduced MDA content (Chen *et al.*, 2018). However, this study observed insignificant fluctuations in the values (except the thiol levels) in almost all the oxidative parameters measured irrespective of the feed. The similar trend was observed by Ashour *et al.* (2020), when green coffee powder was fed to broiler chicken.

Results of the present study showed a reduction in the gut pathogenic microbe counts in birds fed BCSP in their diets. This confirmed the inhibitory effect of caffeic acid and trigonelline contained in coffee against bacteria (Martínez-Tomé, *et al.*, 2011). It also confirms the efficacy of the caffeine component in coffee, which was reported to play a critical role in developing immune resistance to pathogenic bacterial invaders (Ashour *et al.*, 2020). Similar trend was also observed by Nishitsuji *et al.* (2018), who opined that coffee and its components have a positive effect on the gut microbial load. It is assumed, that coffee seed can increase the concentration of specific immune competent cells and improve lysozyme activity (Rangari, 2004). The results of this study show that dietary supplementation of BCSP in broiler feed could be used to decrease the population of pathogenic micro-organisms and, at the same time, increase the beneficial (lactic acid bacteria) microorganisms in the broiler's gut. The effect of this increment is observed in the increased feed intake of birds as inclusion levels of BCSP increased. Lactic acid aids in the breakdown of food components and the increased population in the gut of BCSP fed birds is assumed to have increased their rate of food breakdown. This in turn is assumed to have increased the birds' appetite thus the pronounced/ increased feed intake observed in birds fed BCSP diets.

As the consumer demand for safety and quality criteria, such as meat freshness, are increasing (Li *et al.*, 2019), the need to ascertain the quality of meat in terms of freshness arises. Total volatile basic nitrogen (TVB-N) is a physicochemical index used to assess the freshness and monitor the quality and safety of meat (Ozogul and Ozogul, 2009; Ma *et al.*, 2013). It is the sum of primary, secondary (dimethylamine) and tertiary (trimethylamine), ammonia and other constituents in form of volatile amines and toxic nitrogen compounds. These compounds are responsible for the microbial enzyme activity for protein and non-protein nitrogenous compound degradation in meat (Zhao *et al.*, 2019).

This study revealed that as the inclusion levels of BCSP increased, the TVB-N of the meat decreased indicating that the freshness of the meat is improved. This implied that BCSP was able to reduce the activities of microbes and proteolytic enzymes that aids in the breakdown of the meat protein, which would contribute to spoilage of the meat. This confirmed the high scavenging activities of coffee, as shown by the DPPH scavenging activities at different concentration (Table 2). This can be attributed to the polyphenol aggregation (Acidri *et al.*, 2020) in coffee. The result obtained here is similar to the findings of Ashour *et al.* (2020), who reported a decrease in TVB-N values with increasing level of green coffee powder in broiler diet.

The panellist sensory assessment of the broiler cooked meat revealed that addition of BCSP to broilers' diet will not have any negative impact on sensory qualities of the broiler chicken meat. This confirmed the report of Gok *et al.* (2008), who opined that natural constituents in food do not usually have any negative implication. The effect of varying inclusion levels of BCSP were more pronounced in the aroma and taste, where the meat from coffee fed broiler were rated high. The panellist acceptance score of aroma, flavour, taste and general overall acceptability of chicken meat from BCSP fed broilers was between 4.33 (indifferent) and 7 (I like it). Furthermore, the panellists in this study were indifferent about the flavour and juiciness of the meat. This is opposite to Ashour *et al.* (2020), who reported reduction in juiciness and no significant changes in the taste and aroma in meat from broiler fed basal diet and those fed green coffee powder diet.

CONCLUSION

The present study shows that black coffee seeds contain a number of phytochemicals at significant amounts. Black coffee seed powder did not show any negative effect on the health status of broiler chicken, as revealed by the haematological indices and gut ecology, thus, indicating positive effect of black coffee on the bird health.

The addition of black coffee seed powder to the broilers' diet improved the meat quality, which explains the reduced TVB-B recorded in the meat from coffee-fed broilers, when compared to the control and the continuous decrease as inclusion level increased. The sensory characteristics of the cooked meat from coffee-fed broilers were also not compromised as its inclusion did not confer any noticeable negative effect on the sensorial attributes, but rather improved it, which was reflected in the high taste ratings of the

meat. It is concluded that coffee seed powder could be offered to broiler chickens up to 3.75 g/kg of the diet, as additive, without any deleterious effect both on the birds and eating quality of the meat.

ETHICAL APPROVAL

All procedures were approved by the Animal Care and Use Committee of the University of Ibadan, Ibadan, Oyo State.

AUTHOR'S CONTRIBUTIONS

Conceptualization: AWODOYIN, O. R.

Methodology: AWODOYIN, O. R., OBAFEMI, A. I., AGAMUGAGA, I. B.

Investigation: AWODOYIN, O. R.

Data curation: OBAFEMI, A. I., AGAMUGAGA, I. B.

Writing-original draft preparation: AWODOYIN, O. R.

Writing-review and editing: AWODOYIN, O. R., OBAFEMI, A. I.

Project administration: AWODOYIN, O. R.

All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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