Growth performance and blood biochemistry of broiler chickens fed dietary white and cayenne pepper powders as additives

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Submission: 1 June 2022 Revised: 3 August 2022 Accepted: 13 August 2022

ABSTRACT

Strategies to improve performance of broiler chickens have often focused on the rapid conversion of feed to flesh and health status (blood biochemistry profile) of broiler chickens raised under optimal management. Recent findings have further explored ways dietary additives can increase growth and profitability of farmers. Hence, a study was conducted to investigate the impact of white and cayenne pepper powders fed as additives on growth performance and blood biochemistry profile of broiler chickens. A total of three hundred and thirty-six (336) 2-weeks old Cobb broiler chickens were randomly allotted into seven experimental groups of 48 birds each and four replicates of twelve birds each outlaid in a completely randomized design and reared intensively. Three levels (0, 200, and 250 g) of white pepper (WP) and cayenne pepper (CP) powders per 100 kg of compounded feed were formulated into the basal (B), B+200WP, B+250WP, B+200CP, B+250CP, B+100WP+100CP and B+125WP+125CP diets offered in two phases: starter (14 days) and finisher (18 days). At starter phase, results obtained reveal birds fed B+125WP+125CP diet had lower ($P < 0.05$) feed intake (1,119.56 g) than B+200WP (1,193.88 g) group. Feeding chickens with B+125WP+125CP diet resulted in better ($P < 0.05$) feed conversion ratio (1.62) than B+100WP+100CP (1.76). White blood cell (WBC) and lymphocyte counts were affected ($P < 0.05$) at the starter phase. Birds fed the basal diet had elevated WBC than groups given B+200CP diet and diets containing combination of additives. Increasing sole WP inclusion resulted in elevated lymphocyte count. At the finisher phase, chickens offered dietary B+250CP had a better neutrophil: lymphocyte ratio than B+250WP group. Creatinine was low ($P < 0.05$) when the basal, B+200WP, B+200CP, and B+250CP diets were supplied. Findings have demonstrated that feeding broiler chickens B+125WP+125CP diet should be adopted to improve growth performance without deleterious impact on blood profile and health status.

Keywords: Growth performance, blood biochemistry, white pepper powder, cayenne pepper powder, broiler chickens

Thai J. Agric. Sci. (2022) Vol. 55(2): 84−101

INTRODUCTION

Understanding the economic impact of rearing operations along with the technical inputs associated with broiler production have prompted carefully investigated strategies to efficiently convert diet fed into a significant gain for farmers. An excerpt from Ravindran (2013) revealed practices employed to improve performance incorporate updated knowledge. Nowadays, the additive application is embraced for its beneficial outcomes. The synergism between additives and the diet fed subject to good management results in better health and improved feed utilization. Farmers identify these areas and improve on such strategies to scale or grow the poultry business. Profitability in broiler production takes into account growth performance as a reflection of nutrition, feed utilization, and the management of flock health. By placing the findings of Tallentire *et al*. (2019) in perspective, nutrition alone does not impact eventual growth performance. The health management of birds must likewise be assessed.

Novel studies focus on the benefits poultry derive from the application of phytogenic additives such as anti-oxidative protection and efficient absorption facilitated by the improved activity of the endogenous enzyme (Pirgozliev *et al*., 2019). Phytogenic application comprises substances from diverse sources (plant-based additives, probiotics, and prebiotics) that can be applied as additives or supplements (Vukic-Vranjes *et al*., 2013; Sugiharto *et al*., 2021). Despite these opportunities, Kirkpinar *et al*. (2011) pointed out that the use of phytogenic additives does not always facilitate positive responses on production, while Yang *et al*. (2009) declared that potential outcomes hinge on additive-diet interaction.

White pepper is the fruit of black pepper (*Piper nigrum*) after the black husk has been removed. White pepper has a lower inhibitory concentration (IC50), yielding higher antioxidant capacity than black pepper (Zhang and Xu, 2015). Reported optimized aqueous yields of 5.64 and 8.72 w/w % piperine in black and white pepper respectively, as well as the compositional analyzed result of 23 and 31 bioactive compounds respectively contained in black and white pepper (Olalere *et al*., 2018) point to its potency as a possible beneficial feed additive to increase feed utilization. Red pepper (*Capsicum anuum*) is known to be harmful when consumed in excess (Nwaopara *et al*., 2007); further proven by the significantly reduced white blood cell count in Arbor Acres birds fed dietary cayenne pepper (*Capsicum frutescens*) powder (Adegoke *et al*., 2018). Dkhil and Al-Quraishy (2010) likewise alarmed that red pepper contains some chemical and pharmacological properties similar to the classes of drugs capable of inducing liver damage in rabbits. On the other hand, the beneficial activity of capsaicinoids (predominantly capsaicin) in red peppers has been positively attributed to enhance feed digestion and utilization (Al-Kassie *et al*., 2011a), especially when incorporated at low dosages alongside other additives (Adegoke *et al*., 2018). Since Chikezie *et al*. (2015) explained that the combination of plant principles increases its efficiency and a scarcity exists on the influence of white pepper as a feed additive, this experiment was therefore designed to investigate the impact of cayenne pepper and white pepper powders fed as additives on growth performance and blood biochemistry profile of broiler chickens.

MATERIALS AND METHODS

Experimental Site

The site of the feeding trial was located at the rainforest vegetation zone of South-Western Nigeria on latitude 7° 10’ 07° N, longitude 3° 21’ 57° E (Google Earth, 2020), and 100 m altitude above sea level. The birds were reared at the Poultry Unit of the Directorate of University Farms (DUFARMS).

Sourcing and Processing of Experimental Materials (Test Ingredients)

Whole dried peppers (white and cayenne) were sourced from a reputable spice market and separately ground using an attrition mill, then sealed and stored in coloured vials before incorporation into the basal diet.

Determination of Principal Active Constituents in Cayenne and White Pepper Powders

Capsaicinoid content was analyzed using high-performance liquid chromatography (HPLC) following the methodology described by Al Othman *et al*. (2011), while piperine in white pepper was determined following the specification detailed by Weaver *et al*. (1998). Analysis of the chemical composition of active ingredients in cayenne and white pepper powders (Table 1) revealed that the capsaicin composition was highest at 57.2%, followed by dihydrocapsaicin content at 30.3%, while nordihydrocapsaicin was least at 2.5%. White pepper powder had 7.1% piperine constituent, which is the principal antioxidant and alkaloid in white pepper.

Table 1 Compositional (%) analysis of active ingredients in white and cayenne pepper powders

Cayenne pepper powder	Composition (%)	White pepper powder	Composition (%)
Capsaicin	57.20	Piperine	7.10
Dihydrocapsaicin	30.30		
Nordihydrocapsaicin	2.50		

Research Policy

The guideline of the Animal Ethics Committee of the Department of Animal Production and Health and College of Animal Science and Livestock Production (FUNAAB-APH20-03), Federal University of Agriculture, Abeokuta (FUNAAB, 2014) was adhered to before the commencement of the experiment.

Experimental Birds, Management and Layout

360-day-old mixed-sex broiler chicks of Cobb-500 strain were sourced from a reputable hatchery and were collectively floor-brooded for the first two weeks in a deep litter pen. Post-brooding, three hundred and thirty-six (336) birds outlaid in completely randomized design were randomly allotted to seven treatment groups comprising 48 birds per treatment, further replicated four times - each consisting of 12 birds per replicate. The experiment had starter (14 days) and finisher (18 days) phases respectively, with a diet assigned to each treatment group offered *ad libitum*. Clean water was adequately provided daily while vaccinations and medication required were administered accordingly. Experimental diets are presented in Tables 2 and 3.

Diets were formulated following the nutrient requirement standard established by NRC (1994) with additives incorporated per 100 kg of the basal diet.

Treatment (T) 1: Basal diet (B) (no additive added)

T2: Basal diet + 200 g of white pepper (WP) (B+200WP)

T3: Basal diet + 250 g of white pepper (B+250WP)

T4: Basal diet + 200 g of cayenne pepper (CP) (B+200CP)

T5: Basal diet + 250 g of cayenne pepper (B+250CP)

T6: Basal diet + 100 g of white pepper + 100 g of cayenne pepper (B+100WP+100CP)

T7: Basal diet + 125 g of white pepper + 125 g of cayenne pepper (B+125WP+125CP)

The proximate composition (crude protein, crude fibre, moisture, and ash) of test diets was determined by AOAC methods (2005).

Table 2 Composition of experimental diets at starter phase

Composition	Basal (B)	B +200WP	B +250WP	B +200CP	B +250CP	B +100WP +100CP	B +125WP +125CP
Ingredients (kg)							
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	5.50	5.30	5.25	5.30	5.25	5.30	5.25
Soybean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Groundnut cake	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Fish meal (72%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral and vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cayenne pepper	0.00	0.00	0.00	0.20	0.25	0.10	0.125
White pepper	0.00	0.20	0.25	0.00	0.00	0.10	0.125
Total	100.00	100.20	100.25	100.20	100.25	100.20	100.25
Determined analysis (%)							
Crude protein	23.59	23.62	23.63	23.61	23.62	23.62	23.62
Metabolizable energy (MJ)	12.06	12.01	12.01	12.01	12.01	12.01	12.01
Ether extract	3.97	3.99	3.99	3.98	3.99	3.98	9.99
Crude fibre	3.83	3.89	3.90	3.87	3.88	3.88	3.89
Calcium	1.29	1.29	1.29	1.29	1.29	1.29	1.29
Phosphorus	0.69	0.70	0.70	0.70	0.70	0.70	0.70
Lysine	1.66	1.66	1.66	1.66	1.66	1.66	1.66
Methionine	0.57	0.57	0.57	0.57	0.57	0.57	0.57

Note: * Premix composition per kg diet: vitamin A 400,000 IU, vitamin D3 800,000 IU, vitamin E 9,200 mg, vitamin K 800 mg, vitamin B1 1,000 mg, vitamin B6 500 mg, vitamin B12 25 mg, niacin 6,000 mg, pantothenic acid 2,000 mg, folic acid 200 mg, biotin 8 mg, Mn 300,000 g, Zn 20,000 g, cobalt 80 mg, I 40 mg, choline 80,000 g, antioxidants 800 mg. WP = white pepper powder, CP = cayenne pepper powder.

Table 3 Composition of experimental diets at finisher phase

Composition	Basal (B)	B +200WP	B +250WP	B +200CP	B +250CP	B +100WP +100CP	B +125WP +125CP
Ingredients (kg)							
Maize	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Wheat offal	10.50	10.30	10.25	10.30	10.25	10.30	10.25
Soybean meal	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Groundnut cake	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral and vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Cayenne pepper	0.00	0.00	0.00	0.20	0.25	0.10	0.125
White pepper	0.00	0.20	0.25	0.00	0.00	0.10	0.125
Total	100.00	100.20	100.25	100.20	100.25	100.20	100.25
Determined analysis (%)							
Crude protein	20.50	20.53	20.54	20.52	20.53	20.53	20.53
Metabolizable energy (MJ)	11.93	11.93	11.93	11.93	11.93	11.93	11.93
Ether extract	3.94	3.95	3.96	3.95	3.95	3.95	3.95
Crude fibre	3.68	3.74	3.76	3.73	3.74	3.73	3.74
Calcium	1.26	1.26	1.26	1.27	1.27	1.26	1.26
Phosphorus	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Lysine	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Methionine	0.47	0.47	0.47	0.47	0.47	0.47	0.47

Note: * Premix composition per kg diet: vitamin A 400,000 IU, vitamin D3 800,000 IU, vitamin E 20,000 IU, vitamin K 800 mg, vitamin B1 1,000 mg, vitamin B6 500 mg, vitamin B12 25 mg, niacin 6,000 mg, pantothenic acid 7,500 mg, folic acid 200 mg, biotin 8 mg, Mn 300,000 g, Zn 20,000 g, cobalt 80 mg, I 40 mg, choline 80,000 g, antioxidants 125 mg. WP = white pepper powder, CP = cayenne pepper powder.

Performance Data

Records of initial weight, final weight, feed intake, and weight gain were taken, while feed conversion ratio (FCR) was calculated following the procedure detailed by Galli *et al.* (2020).

Total feed consumed (g)

$$= \text{Feed offered (g)} - \text{Leftover feed (g)}$$

Average feed intake (g/bird)

$$= \frac{\text{Total feed consumed (g)}}{\text{Number of birds}}$$

Total weight gain (g)
$$= \text{Final weight (g)} - \text{Initial weight (g)}$$

Feed conversion ratio
$$= \frac{\text{Total feed consumed (g)}}{\text{Total weight gain (g)}}$$

Mortality (%)
$$= \frac{\text{Number of dead birds}}{\text{Total number of birds stocked}} \times 100$$

Blood Collection and Indices

On days 14 and 32 of the trial, 3 and 5 mL of blood were respectively collected from the wing vein into test tubes containing anticoagulant (ethylene diamine tetra-acetate, EDTA) and plain vials for haematological and serum indices. One bird was selected per replicate for blood profile analysis. Haemoglobin concentration and packed cell volume were determined using Van Slyke and Hackley haematocrit centrifuge (UK) apparatuses, respectively. White blood cell was obtained using the Neubauer count chamber following the process employed by Cray and Zaias (2004) while serum count was colorimetrically measured with a Jenway 6405 UV/VIS spectrophotometer (UK). The blood neutrophil-lymphocyte ratio was calculated as N: L indicator. Blood protein and serum albumin were obtained according to the method described by Tietz and Norbert (1995) and Bromo Cresol Green (BCG) application by Doumas *et al*. (1971), respectively. Serum alanine transaminase (ALT), aspartate transaminase (AST) (Bergmeyer *et al*., 1986a; 1986b), and Cholesterol (Gordon and Amer, 1977) values were obtained, while creatinine content was measured using a beam spectrophotometer (492 nm). Blood low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were determined using the protocol described by Burtis and Ashwood (1999). Blood analysis was determined at the Molecular Microbiology Laboratory Unit of the Veterinary Teaching Hospital, College of Veterinary Medicine (COLVET) at the Federal University of Agriculture, Abeokuta.

Statistical Analysis

Data obtained were subjected to a one-way analysis of variance using the general linear model procedure of SPSS (2012) version 21. Significantly different ($P < 0.05$) means were separated using Duncan's new multiple range test of the same statistical package.

RESULTS AND DISCUSSION

Growth Performance

Starter phase

At the end of the starter phase (14–28 days), growth performance was examined (Table 4), and data obtained revealed no impact ($P < 0.05$) was observed for the final weight, weight gain, and daily weight gain parameters by the diets fed. On the contrary, feed intake and daily feed intake significantly differed ($P < 0.05$) between B+125WP+125CP and B+200WP groups while FCR was different between B+125WP+125CP and B+100WP+100CP groups ($P < 0.05$). Chickens given B+125WP+125CP diet had lower feed intake than B+200WP group as well as better FCR than B+100WP+100CP group. B+125WP+125CP diet offered chickens lowered feed intake than B+200WP at the starter phase. Observation from this study on cayenne pepper disagrees with the report of Yoshioka *et al*. (1999) who identified capsaicin in cayenne pepper as responsible for increased appetite in experimental subjects but agrees with the outcome of the investigation of Adegoke *et al*. (2018) who found that feeding dietary red pepper lowered appetite. Ghrelin (sometimes called lenomorelin), a fast-acting hormone produced in the digestive pathway, also termed hunger hormone functionally regulates appetite (Klok *et al*., 2007), was influenced by capsaicin produced. Richards *et al*. (2006) report on 3-week-old male broiler chickens subjected to dietary trial further alludes to this with identified autocrine or paracrine modification of avian ghrelin and ghrelin receptor genes were identified in appetite regulation for birds. Therefore, intake, regulated by B+125WP+125CP diet, could be attributed to the repressed activity of ghrelin by combined feeding of capsaicin and piperine. Increased feed intake among groups offered B+200WP diet suggests concentration of piperine was insufficient to limit retention time as well as suppress ghrelin production. The FCR among chickens fed B+125WP+125CP was better than B+100WP+100CP. Munglang and Vidyarthi (2019) highlighted the capacity of red pepper to potentiate the activities of pancreatic and intestinal enzymes. At sufficient quantities of both peppers, the consequent increase in bile acid secretion and translation of feed into weight gain ensued as the transit time of feed was regulated. Al-Kassie *et al*. (2011b) further explained that the high activity of piperazine citrate, a bioactive content in *Piper nigrum*, improved the flow of digestive juices during digestion. These beneficial activities contributed to improved digestion by supporting the proliferation of beneficial microbes in the gut.

Finisher phase

All parameters measured excluding FCR were not influenced by diet (P > 0.05) as shown in Table 5. Feeding dietary pepper powders resulted in FCR range of 2.33–2.67. However, FCR of 2.33 was the best for chickens supplied B+125WP+125CP diet. At the finisher phase, chickens fed B+125WP+125CP diet had the best FCR. The efficient activity of phytochemicals can also be attributed to the antioxidant, antimicrobial and digestive properties known to regulate and modify/stabilize the intestinal microbiota which subsequently facilitates the expulsion of by-products of cellular metabolism, a relief from an intestinal challenge and immune stress, with performance optimized (Kim *et al*., 2015). Bajad *et al*. (2001) expressed the benefits of piperine and *Piper nigrum* activity on gastrointestinal system function, anti-diarrhea system function, such as its anti-diarrhea system function, such as its anti-diarrhea properties and healthier ultrastructure of the intestinal villi. This promotes better absorption capacity. In turn, the larger absorptive surface area stimulates the expression of brush border enzymes and the nutrient transport system (Cardoso *et al*., 2012). On the other hand, capsaicin, a potent bioactive principle associated with negative energy balance bind with high affinity transient receptor potential cation receptor, resulting in regulated motility and higher absorption efficiency in the upper intestinal tract (Ludy and Mattes, 2011). Additionally, a detailed review by Kaiya *et al*. (2013) showed that central ghrelin in avian species acts as an anorexigenic neuropeptide that can suppress intake, mediated by urocortin which is a member of the corticotropin-releasing factor family. Such combined activity of both peppers may have contributed to the outcomes observed.

Table 4 Effect of dietary pepper powders on growth performance (mean ± standard error) of broiler chickens at starter phase

Parameters	Basal (B)	B+200WP	B+250WP	B+200CP	B+250CP	B+100WP +100CP	B+125WP +125CP
Initial weight (g)	318.63 ± 4.74	323.42 ± 0.35	323.56 ± 0.11	323.69 ± 0.10	323.29 ± 0.28	322.40 ± 1.30	318.10 ± 5.26
FI (g)	1,183.52 ± 8.22[ab]	1,193.88 ± 19.43[a]	1,141.48 ± 30.65[ab]	1,125.29 ± 28.87[ab]	1,191.29 ± 6.68[ab]	1,191.32 ± 11.28[ab]	1,119.56 ± 32.68[b]
DFI (g)	84.54 ± 0.59[ab]	85.28 ± 1.32[a]	81.53 ± 2.19[ab]	80.38 ± 2.06[ab]	85.09 ± 0.48[ab]	85.09 ± 0.81[ab]	79.97 ± 2.33[b]
Final weight (g)	1,008.83 ± 21.98	1,020.06 ± 12.79	1,014.19 ± 5.63	1,009.56 ± 9.08	1,037.98 ± 2.46	998.52 ±14.07	1,008.20 ± 19.29
Weight gain (g)	690.21 ± 19.05	696.65 ± 12.91	690.63 ± 5.66	685.88 ± 8.99	714.69 ± 2.61	676.13 ± 14.04	690.09 ± 15.43
DWG (g)	49.30 ± 1.36	49.76 ± 0.92	49.33 ± 0.40	48.98 ± 0.64	51.05 ± 0.19	48.29 ± 1.00	49.29 ± 1.10
FCR	1.72 ± 0.05[ab]	1.71 ± 0.02[ab]	1.65 ± 0.03[ab]	1.64 ± 0.04[ab]	1.67 ± 0.01[ab]	1.76 ± 0.03[b]	1.62 ± 0.06[a]

Note: [a,b] Means in the same row with different superscripts differ significantly ($P < 0.05$). WP = white pepper powder, CP = cayenne pepper powder, FI = feed intake, DFI = daily feed intake, DWG = daily weight gain, FCR = feed conversion ratio

Table 5 Effect of dietary pepper powders on growth performance (mean ± standard error) of broiler chickens at finisher phase

Parameters	Basal (B)	B+200WP	B+250WP	B+200CP	B+250CP	B+100WP +100CP	B+125WP +125CP
Initial weight (g)	1,008.83 ± 21.98	1,020.06 ± 12.79	1,014.19 ± 5.63	1,009.56 ± 9.08	1,037.98 ± 2.46	998.52 ± 14.07	1,008.20 ± 19.29
FI (g)	2,674.52 ± 23.05	2,668.00 ± 18.39	2,550.94 ± 45.28	2,604.17 ± 21.75	2,627.56 ± 28.64	2,576.34 ± 61.81	2,510.57 ± 127.58
DFI (g)	148.58 ± 1.28	148.22 ± 1.02	141.72 ± 2.52	144.68 ± 1.21	145.98 ± 1.59	143.13 ± 3.43	139.48 ± 7.09
Final weight (g)	2,053.13 ± 47.27	2,019.79 ± 19.64	2,014.06 ± 9.75	2,021.88 ± 26.97	2,062.88 ± 30.90	2,003.59 ± 22.94	2,083.10 ± 52.18
Weight gain (g)	1,044.29 ± 25.51	999.73 ± 7.62	999.88 ± 15.19	1,012.31 ± 32.87	1,024.90 ± 30.10	1,005.07 ± 9.52	1,074.90 ± 32.90
DWG (g)	58.02 ± 1.42	55.54 ± 0.42	55.55 ± 0.84	56.24 ± 1.83	56.94 ± 1.67	55.84 ± 0.53	59.72 ± 1.83
FCR	2.57 ± 0.07[b]	2.67 ± 0.02[b]	2.55 ± 0.08[b]	2.58 ± 0.08[b]	2.57 ± 0.06[b]	2.56 ± 0.06[b]	2.33 ± 0.05[a]
Mortality (%)*	0	2.08	0	2.08	4.17	0	0

Note: [a,b] Means in the same row with different superscripts differ significantly ($P < 0.05$). WP = white pepper powder, CP = cayenne pepper powder, FI = feed intake, DFI = daily feed intake, DWG = daily weight gain, FCR = feed conversion ratio, * mortality was not analysed

Haematological Indices

Starter phase

Blood haematological indices of birds fed experimental diets at the starter phase are presented in Table 6. All indices measured were not significant ($P > 0.05$) except WBC and lymphocyte counts ($P < 0.05$). Chickens on the basal diet had significantly higher WBC count than B+200CP, B+100WP+100CP, and B+125WP+125CP groups. Lymphocyte was significantly elevated and reduced among groups supplied dietary B+250WP and B+200WP, respectively. The $10.5 \times 10^3/mm^3$ (or $10.5 \times 10^3/mL$) WBC count of the basal group was elevated than the highest value of 8.35 published by Adegoke *et al*. (2018). Such increased WBC count may indicate an underlying condition. White blood cell was within the range declared by Onunkwo *et al*. (2018) at 6.29–9.85 × $10^3/mL$, excluding groups fed B+200CP, B+100WP+100CP, and B+125WP+125CP diets with lower values than the basal group. According to Akimoto *et al*. (2009), dihydrocapsaicin and capsaicin positively contributed to increased WBC in rats by significantly altering the lymphocyte and neutrophil content, with limited impact on eosinophil and basophil counts. Notably, these 3 groups had cayenne pepper in common with the impact of white pepper beneficial below 200 g. With no observable decline in WBC count among groups fed B+125WP+125CP diet at the finisher phase, it is safe to allude that the combination of additives [Cayenne – White (1:1)] had an altering activity on WBC production as chickens adjusted to pepper principles or alkaloids at the levels of combination feeding. Additionally, these 3 groups had higher WBC than the 4.07–4.32 × $10^3/mL$ and $5.5 \times 10^3/mL$ declared by Campbell (1994) and by Merck (2012) respectively, excluding B+100WP+100CP group at $3.10 \times 10^3/mm^3$ with a lower value than the $4.25 \times 10^3/mm^3$ published by Medugu *et al*. (2010), though close to the $3.15 \times 10^3/mm^3$ WBC count documented by Adegoke *et al*. (2018), yet slightly above normal range of 1.2–3.0 × 10^3 published by Jain (1993). Notably, very low WBC increases the risk of developing a potentially threatening infection. Reduced WBC in B+100WP+100CP could be a result of no disease condition or low production from bone marrow as high WBC can indicate infection (Medugu *et al*., 2010; Egbewande, 2018).

Age-specific reference ranges must be considered alongside significant lymphocyte count at the chick phase (Inoue, 2020). A temporarily high lymphocyte count is reported as a normal response by the body's immune system. Demonstrated existence of daily rhythms with diurnal acrophases in blood indices of broiler chickens kept under natural photoperiods and tropical conditions was established by Makeri *et al*. (2017). A significant rise in lymphocyte counts among chickens fed 250 g/100 kg of dietary white pepper but not 200 g/100 kg implies chicks conveniently adapted to higher alkaloids and antinutritional substances in the diet. Notably, lymphocyte counts reported in the study of Makeri *et al*. (2017) fluctuated between 78–90%, which is higher than the 50–62% range recorded for broiler chickens fed control diets in Nigeria (Onibi *et al*., 2011; Adeyemo and Sani, 2013). A dietary trial in a similar tropical climate as the two authors cited above published a range of 53.00–66.50% at starter phase, though strain and management could have contributed to such outcomes. Significantly, the 56.50–70.50% range across the treatment groups in this study falls within the normal range for lymphocyte count in the tropical region, with strain, diet, and management impacting. Considering that this study experimented on Cobb broiler chickens, not Arbor Acres chicks that were experimented on by Adegoke *et al*. (2018) leads to such inference.

Finisher phase

Haematological indices of chickens offered diets incorporated with pepper powders at finisher phase are shown in Table 7. Blood eosinophil and neutrophil: lymphocyte (N: L) ratio were influenced ($P < 0.05$) by diet. Birds on the basal diet had increased eosinophil count than chickens given B+200WP diet ($P < 0.05$). Offering B+250CP diet resulted in lower N: L ratio as opposed to elevated N: L ratio among chickens supplied B+250WP diet, though similar ($P > 0.05$) as other groups.

Table 6 Effect of dietary pepper powders on haematological indices (mean ± standard error) of broiler chickens at starter phase

Parameters	Basal (B)	B+200WP	B+250WP	B+200CP	B+250CP	B+100WP +100CP	B+125WP +125CP
PCV (%)	28.50 ± 0.50	28.00 ± 0.00	29.50 ± 5.50	26.00 ± 2.00	28.50 ± 3.50	38.50 ± 8.50	33.00 ± 2.00
Hb (g/dl)	9.62 ± 0.38	9.32 ± 0.02	9.70 ± 1.60	8.66 ± 0.56	9.90 ± 1.10	12.85 ± 2.85	10.85 ± 0.55
RBC (10^{12}/L)	2.43 ± 0.05	2.41 ± 0.01	2.50 ± 0.30	2.27 ± 0.09	2.47 ± 0.24	3.27 ± 0.77	2.76 ± 0.10
WBC ($10^3/mm^3$)	10.50 ± 0.60^a	6.46 ± 1.67^{ab}	6.47 ± 2.05^{ab}	4.08 ± 1.24^b	7.76 ± 1.44^{ab}	3.10 ± 0.10^b	4.90 ± 1.50^b
Neutrophil (%)	36.00 ± 6.00	39.00 ± 0.00	25.00 ± 2.00	26.00 ± 0.00	32.50 ± 2.50	45.50 ± 14.50	29.00 ± 5.00
Lymphocyte (%)	61.50 ± 6.50^{ab}	56.50 ± 0.50^b	70.50 ± 1.50^a	69.00 ± 1.00^{ab}	63.50 ± 2.50^{ab}	62.50 ± 2.50^{ab}	67.50 ± 5.50^{ab}
Monocyte (%)	0.00 ± 0.00	2.00 ± 1.00	2.50 ± 1.50	2.50 ± 0.50	1.50 ± 0.50	1.50 ±0.50	2.00 ± 0.50
Eosinophil (%)	1.50 ± 0.05	1.50 ± 0.05	1.00 ± 0.00	1.00 ± 0.00	1.50 ± 1.50	1.00 ± 0.00	0.50 ± 0.50
Basophil (%)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 1.00	2.00 ± 0.00	1.00 ± 1.00	0.50 ± 0.50	1.00 ± 1.00
Neutrophil: Lymphocyte	0.60 ± 0.16	0.69 ± 0.01	0.30 ± 0.04	0.38 ± 0.01	0.51 ± 0.06	0.74 ± 0.26	0.44 ± 0.11

Note: [a,b] Means in the same row with different superscripts differ significantly ($P < 0.05$). WP = white pepper powder, CP = cayenne pepper powder, PCV = packed cell volume, Hb = haemoglobin, RBC = red blood cell, WBC = white blood cell

Table 7 Effect of dietary pepper powders on haematological indices (mean ± standard error) of broiler chickens at finisher phase

Parameters	Basal (B)	B+200WP	B+250WP	B+200CP	B+250CP	B+100WP +100CP	B+125WP +125CP
PCV (%)	40.00 ± 4.62	33.00 ± 2.89	38.00 ± 0.58	35.00 ± 2.89	30.00 ± 1.16	41.00 ± 5.77	38.00 ± 4.62
Hb (g/dl)	13.50 ± 1.79	12.70 ± 1.96	12.90 ± 0.29	11.80 ± 0.98	10.20 ± 0.69	13.80 ± 2.13	12.40 ± 1.27
RBC (10^{12}/L)	4.89 ± 0.51	4.11 ± 0.61	4.82 ± 0.18	4.39 ± 0.56	3.32 ± 0.08	5.01 ± 0.87	4.73 ± 0.73
WBC ($10^3/mm^3$)	14.80 ± 2.42	19.70 ± 5.08	17.40 ± 0.00	14.30 ± 2.94	11.40 ± 3.46	12.30 ± 0.87	21.20 ± 6.41
Neutrophil (%)	29.00 ± 10.97	28.00 ± 4.62	44.00 ± 15.69	28.00 ± 4.62	16.00 ± 1.73	37.00 ± 11.55	31.00 ± 8.63
Lymphocyte (%)	85.00 ± 12.70	75.00 ± 4.62	77.00 ± 15.59	75.00 ± 3.46	82.00 ± 1.73	77.00 ± 9.24	80.00 ± 9.24
Monocyte (%)	1.00 ± 1.00	3.50 ± 0.50	3.50 ± 0.50	2.50 ± 0.50	4.00 ± 2.00	1.00 ± 0.00	1.00 ± 1.00
Eosinophil (%)	5.00 ± 0.00^a	1.00 ± 0.58^b	3.00 ± 0.58^{ab}	2.00 ± 0.58^{ab}	2.00 ± 1.16^{ab}	4.00 ± 2.31^{ab}	4.00 ± 1.16^{ab}
Basophil (%)	0.50 ± 0.50	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.50	1.00 ± 1.00
Neutrophil: Lymphocyte	0.34 ± 0.11^{ab}	0.37 ± 0.05^{ab}	0.59 ± 0.19^a	0.37 ± 0.05^{ab}	0.19 ± 0.02^b	0.48 ± 0.09^{ab}	0.39 ± 0.04^{ab}

Note: [a,b] Means in the same row with different superscripts differ significantly ($P < 0.05$). WP = white pepper powder, CP = cayenne pepper powder, PCV = packed cell volume, Hb = haemoglobin, RBC = red blood cell, WBC = white blood cell

At finisher phase, eosinophil percentage was highest among birds offered the basal diet, though suppressed upon offering B+200WP diet. Piperine, an amide alkaloid has been identified as a potent leishmanicidal agent (Ferreira *et al*., 2011). Pund and Joshi (2017) established mannosylated piperine to be effective liposomes in the elimination of intracellular amastigotes of *Leishmania donovani* in splenic macrophages, with reduced spleen parasite load reported. The authors further identified localized piperine at a single dose of 5 mg/kg effective in *L. donovani*-infected BALB/c mice with a significant reduction in the liver and splenic parasite load. The addition of 200 g of white pepper to 100 kg of the basal diet likely led to the repressed growth of internal parasites that subsequently lowered eosinophil production.

The specific interpretation of the N: L ratio helps depict overall stress levels imposed either by the external or internal environment. White blood cells such as neutrophils and lymphocytes are essentials to combat infections. Neutrophils function in phagocytosis and activation of bactericidal mechanisms (Janeway *et al*., 2001). Unchanged N: L ratio exists when immune function is raised as a result of the overall changes from possible variation in cell numbers, while increased neutrophil counts rise independent of lymphocyte counts that subsequently indicate an ongoing immune response to a challenged system (Govori, 2019). From this research, the N: L ratio for groups given B+250WP supports the posit above, as revealed by the indicative numerically high values of neutrophil. For B+200WP and B+200CP groups, the identical impact was recorded, but not upon sole 250 g inclusion of both peppers to the basal diet. Apparently, an increase in capsaicinoid, primarily capsaicin facilitates the release of substance P (SP). Substance P has been reported to affect the activation of immune system cells. It facilitates permeability of the vascular endothelium as well as stimulates leukocyte chemotaxis (Vigna, 2004). Cayenne pepper inclusion at 250 g/100 kg of basal diet suggests mediated neurotransmission, resulting in suppressed blood production by the bone marrow. Lowered production of SP and its preferred neurokinin 1 (NK1) receptor affected production of red and white blood cells, though elevated lymphocyte count (Graefe and Mohiuddin, 2020) likely lowered neutrophil (%) production in B+250CP group. With SP and NK1 implicated in a variety of physiological and pathophysiological processes (Ebner and Singewald, 2006), increased cayenne pepper inclusion, led to elevated N: L ratio.

Serum Biochemical Indices

Starter phase

Serum biochemical indices were measured at the starter phase (Table 8) and globulin and high-density lipoprotein were affected by diet. Globulin level increased when chickens were fed B+200CP diet when compared to the group offered B+200WP diet. Serum HDL of birds supplied B+200WP, B+200CP, B+100WP+100CP, and B+125WP+125CP diets were lower than B+250CP group ($P < 0.05$). Blood serum at the starter phase had globulin values of 2.29–2.90 g/dL similar to the range of 2.33–3.33 g/dL declared by Muhammad *et al*. (2015) with the exclusion of B+200WP group. Globulin was higher at 200 g of white cayenne pepper inclusion but reduced upon the addition of 200 g of white pepper to the basal diet. Globulins are the main sites of immunoglobulin (Peters *et al*., 1982), and an increase in immunoglobin generally arises from an increase in globulin, but there can be an increase due to characteristic pathological states. Busher (1990) explained that three-fourth of the immunoglobin level in normal serum belongs to the gamma immunoglobin (IgG) type that acts as antibodies to viruses and bacteria. Hashemipour *et al*. (2014) also posited that increased plasma protein, albumin, and globulins concentrations occur as a consequence of better digestion and absorption of nutrients. An inference from the findings above is that cayenne pepper at lower dosage specifically boosted the IgG as a result of better digestion and absorption of feed. Also, higher HDL-cholesterol for chickens offered B+250WP diet signifies improved activity of pepper on liver cholesterol production. TRPV1 (Transient receptor potential cation channel subfamily V member 1), also known as the capsaicin receptor is expressed by the vascular endothelial cells and hepatocytes (Gunthorpe and Szallai, 2008) that can potentially modulate the function of cells by boosting intracellular-free calcium levels. Cayenne pepper act on the liver by preventing cholesterol gallstones and protecting the structural integrity of erythrocytes under conditions of hypercholesterolemia. Dihydrocapsaicin was reportedly more effective than capsaicin in modifying HDL-cholesterol production (Srinivasan, 2016). Capsaicin lowered risk factors of coronary heart disease (CHD) in subjects with low HDL-cholesterol; suggesting it can contribute to the prevention and treatment of CHD (Qin, 2017).

Finisher phase

All serum indices measured were not influenced ($P > 0.05$) by diet except creatinine (Table 9). Serum creatinine was higher ($P < 0.05$) upon feeding B+125WP+125CP diet than others, but comparable to B+250WP and B+100WP+100CP count in both groups. Creatinine excreted through the kidney, a sensitive biochemical marker employed in the diagnosis of renal (Akande *et al*., 2013) was impacted. Any increase in serum creatinine can be applied as a marker for kidney health, though measurement of plasma creatinine alone is inconclusive in renal function assessment (Adinoyi, 2020). The creatinine level of 0.70–1.20 mg/dL in this study was lower than the 1.08–1.45 mg/dL documented by Adeniran *et al*. (2017) but higher than the 0.20–0.45 mg/dL reported by Adegoke *et al*. (2018). The previous author revealed increased creatinine levels as dietary levels of fermented castor oil seed meal given with different methionine sources varied. Similarly, the latter author declared increased creatinine as cayenne pepper powder inclusion was raised. Ebegbulem (2018) likewise observed that creatinine level was raised simultaneously with increased ginger inclusion. Though Begum *et al*. (2018) alluded to nicotine, a major alkaloid that acts as drug-specific present in leaves, significantly correlated with increased creatinine excreted, therapeutic phytochemicals have no negative impact when used within safe dosage levels (Adinoyi, 2020). White and cayenne peppers contain alkaloids and flavonoids, which when incorporated within safe levels present a non-toxic effect on kidney health.

Table 8 Effect of dietary pepper powders on serum biochemical indices (mean ± standard error) of broiler chickens at starter phase

Parameters	Basal (B)	B+200WP	B+250WP	B+200CP	B+250CP	B+100WP +100CP	B+125WP +125CP
Protein (g/dL)	6.76 ± 0.07	6.21 ± 0.67	5.96 ± 0.16	6.65 ± 0.27	6.15 ± 0.20	6.09 ± 0.70	6.15 ± 0.45
Albumin (g/dL)	3.93 ± 0.17	3.93 ± 0.58	3.09 ± 0.04	3.75 ± 0.17	3.66 ± 0.26	3.48 ± 0.43	3.60 ± 0.16
Globulin (g/dL)	2.83 ± 0.10^{ab}	2.29 ± 0.10^{b}	2.87 ± 0.12^{ab}	2.90 ± 0.10^{a}	2.50 ± 0.06^{ab}	2.62 ± 0.27^{ab}	2.50 ± 0.29^{ab}
AST (mg/dL)	139.45 ± 6.85	123.75 ± 28.45	125.70 ± 11.80	135.05 ± 1.75	122.50 ± 1.00	111.85 ± 5.05	139.95 ± 0.35
ALT (mg/dL)	22.65 ± 12.35	18.20 ± 6.10	18.00 ± 3.00	23.75 ± 5.25	31.85 ± 9.45	21.70 ± 1.70	34.00 ± 11.00
Creatinine (mg/dL)	0.75 ± 0.15	0.75 ± 0.05	1.10 ± 0.10	0.90 ± 0.10	0.75 ± 0.25	1.05 ± 0.05	0.75 ± 0.05
Cholesterol (mg/dL)	149.85 ± 12.75	157.15 ± 24.75	127.40 ± 5.40	140.75 ± 33.15	131.10 ± 17.60	146.45 ± 5.45	133.65 ± 12.05
HDL (mg/dL)	59.30 ± 38.20^{ab}	28.05 ± 0.05^{b}	58.50 ± 7.50^{ab}	30.60 ± 9.40^{b}	93.35 ± 7.15^{a}	20.85 ± 1.85^{b}	34.50 ± 11.50^{b}
LDL (mg/dL)	29.80 ± 9.10	26.55 ± 7.65	34.10 ± 0.70	26.25 ± 4.85	31.70 ± 4.20	33.25 ± 10.05	25.35 ± 3.75
Triglyceride (mg/dL)	89.20 ± 10.50	99.50 ± 7.30	81.60 ± 7.60	92.95 ± 7.55	83.25 ± 12.05	88.65 ± 16.20	80.65 ± 2.55

Note: [a,b] Means on the same row with different superscripts differ significantly ($P < 0.05$). CP = cayenne pepper powder, WP = white pepper powder, HDL = high-density lipoprotein, LDL = low-density lipoprotein, g/dL = gram/decilitre, mg/dL = milligram/decilitre

Table 9 Effect of dietary pepper powders on serum biochemical indices (mean ± standard error) of broiler chickens at finisher phase

Parameters	Basal (B)	B+200WP	B+250WP	B+200CP	B+250CP	B+100WP +100CP	B+125WP +125CP
Protein (g/dL)	7.27 ± 0.24	7.49 ± 0.96	6.93 ± 0.09	7.01 ± 0.06	6.83 ± 0.44	6.82 ± 0.54	7.37 ± 0.21
Albumin (g/dL)	4.18 ± 0.18	4.49 ± 0.85	4.17 ± 0.01	4.16 ± 0.99	4.01 ± 0.10	3.66 ± 0.13	4.46 ± 0.25
Globulin (g/dL)	3.09 ± 0.06	3.00 ± 0.11	2.76 ± 0.09	3.06 ± 0.12	3.08 ± 0.58	3.26 ± 0.47	2.98 ± 0.04
AST (mg/dL)	82.60 ± 36.27	134.80 ± 39.03	96.80 ± 11.55	102.13 ± 24.19	129.53 ± 33.08	62.40 ± 7.04	143.90 ± 18.01
ALT (mg/dL)	34.10 ± 13.57	45.20 ± 17.90	32.33 ± 10.63	20.40 ± 1.85	44.80 ± 18.13	22.00 ± 2.42	52.90 ± 2.71
Creatinine (mg/dL)	0.80 ± 0.00^{b}	0.70 ± 0.00^{b}	1.00 ± 0.00^{ab}	0.80 ± 0.12^{b}	0.80 ± 0.12^{b}	0.90 ± 0.06^{ab}	1.20 ± 0.23^{a}
Cholesterol (mg/dL)	139.70 ± 6.35	156.60 ± 28.98	143.10 ± 11.72	130.00 ± 11.43	151.80 ± 15.76	137.90 ± 11.36	141.90 ± 7.97
HDL (mg/dL)	70.20 ± 3.46	77.30 ± 18.65	76.20 ± 19.92	66.53 ± 9.01	76.50 ± 14.32	64.50 ± 7.97	66.80 ± 0.35
LDL (mg/dL)	51.50 ± 3.93	58.10 ± 6.76	62.90 ± 8.31	51.00 ± 5.39	58.27 ± 2.19	56.80 ± 1.50	55.70 ± 5.48
Triglyceride (mg/dL)	98.90 ± 5.14	105.50 ± 17.90	92.17 ± 3.12	90.40 ± 17.10	91.80 ± 4.21	28.90 ± 10.10	97.20 ± 10.91

Note: [a,b] Means on the same row with different superscripts differ significantly ($P < 0.05$). CP = cayenne pepper powder, WP = white pepper powder, HDL = high density lipoprotein, LDL = low density lipoprotein, g/dl = gram/decilitre, mg/dl = milligram/decilitre

CONCLUSION

Combination of white and cayenne pepper powders at 125: 125 g (B+125WP+125CP) should be incorporated into the basal diet to improve growth performance. The diet significantly lowered intake and improved feed utilization without significant deleterious action on the blood profile and health status of broiler chickens.

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